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Hair-Loss Preventing Effect of *Grateloupia elliptica*

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Abstract

This study was conducted to evaluate the effect of *Grateloupia elliptica*, a seaweed native to Jeju Island, Korea, on the prevention of hair loss. When immortalized rat vibrissa dermal papilla cells were treated with extract of *G. elliptica*, the proliferation of dermal papilla cells significantly increased. In addition, the *G. elliptica* extract significantly inhibited the activity of 5α-reductase, which converts testosterone to dihydrotestosterone (DHT), a main cause of androgenetic alopecia. On the other hand, the *G. elliptica* extract promoted PGE_2 production in HaCaT cells in a dose-dependent manner. The *G. elliptica* extract exhibited particularly high inhibitory effect on LPS-stimulated IL-12, IL-6, and TNF-α production in lipopolysaccharide (LPS)-stimulated bone marrow-derived dendritic cells. The *G. elliptica* extract also showed inhibitory activity against *Pityrosporum ovale*, a main cause of dandruff. These results suggest that *G. elliptica* extract has the potential to treat alopecia via the proliferation of dermal papilla, 5α-reductase inhibition, increase of PGE_2 production, decrease of LPS-stimulated pro-inflammatory cytokines and inhibitory activity against *Pityrosporum ovale*.

Key Words: Prevention of hair loss, *Grateloupia elliptica*, Dermal papilla cell, 5α-reductase, PGE_2, LPS-stimulated pro-inflammatory cytokine, *Pityrosporum ovale*

INTRODUCTION

Alopecia is a distressing condition for an increasing number of men and women and includes androgrenetic alopecia (AGA), alopecia areata (AA), telogen effluvium and so on (Kaufman *et al.*, 1998). Hair loss is emerged from psychological and physical stress, and dandruff. However, the underlying causes of baldness are poorly understood and only two FDA-approved drugs (finasteride and minoxidil) have been available for nearly 50 years (Burton and Marshall, 1979; Kaufman *et al.*, 1998). Finasteride, a type II 5α-reductase inhibitor, was initially used for curing prostatic hypertrophy (Gormley, 1995), but later found to stimulate hair growth in men with AGA, which is the most common type of alopecia (Van Neste *et al.*, 2000; Kaufman *et al.*, 2008a; Kaufman *et al.*, 2008b). Nevertheless, its use is limited because of potential side effects, especially in women (Whiting *et al.*, 1999). Minoxidil, an anti-hypertensive, has been reported to stimulate hair growth by the opening of ATP-sensitive K^+-channel (Hamaoka *et al.*, 1997; Shorter *et al.*, 2008), the up-regulation of vascular endothelial growth factor (VEGF) (Lachgar *et al.*, 1998) and the activation of β-catenin pathway (Kwack *et al.*, 2011) in dermal papilla cells (DPCs). Han *et al.* reported that minoxidil has proliferative and anti-apoptotic effects on DPCs (Han *et al.*, 2004). The DPCs consists of a cluster of specialized fibroblasts that play important roles in the regulation of the hair cycle through the secretion of diffusible proteins such as insulin-like growth factor-1 (IGF-1) (Itami *et al.*, 1995), hepatocyte growth factor (HGF) (Shimaoka *et al.*, 1994), VEGF (Lachgar *et al.*, 1996) and transforming growth factor-β (TGF-β) (Soma *et al.*, 2002; Soma *et al.*, 2003). Several reports have also described implication of PG pathway in hair growth and PGE_2 is also described as a possible modulator of hair growth (Coleman *et al.*, 1994b; Colombe *et al.*, 2007; Colombe *et al.*, 2008).

On the other hand, AA is one of the most common autoimmune diseases which is characterized by autoimmune assault on the hair follicle resulting in hair loss (Alkhalifah *et al.*, 2010). Many reports suggest that AA is mediated by T-lymphocytes with type-1 helper T-cell (Th1) cytokine profile. TNF-α, IL-6 and IL-12, pro-inflammatory cytokines, are involved in Th1-mediated inflammation as part of the normal immune response, as well as inflammatory diseases, including AA (Gately *et al.*,

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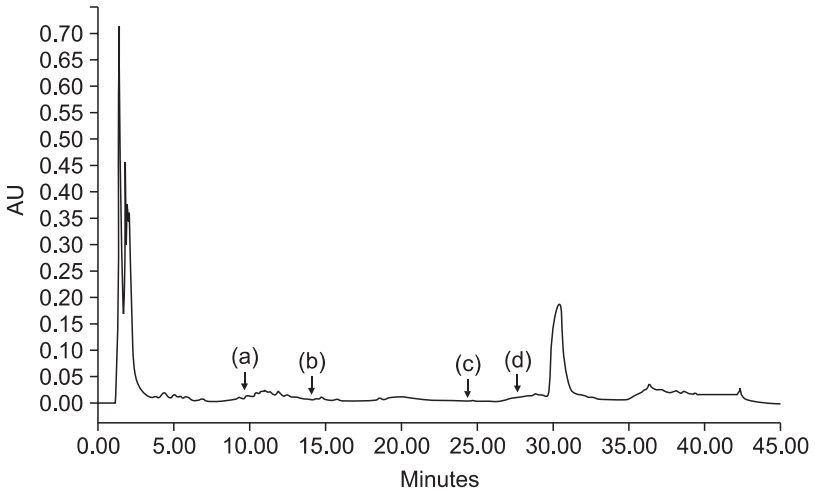

Fig. 1. HPLC profile of *G. elliptica* extract. HPLC analysis was performed on the HPLC (Alliance 2695 system with PDA, Waters Co., Milford, MA, USA); Used column was XTerra® C_{18} (100×4.6 mm, i.d. 3.5 um; Waters Co. Ltd. USA), mobile phase, linear-gradient mixture of A (aqueous 0.5% acetic acid) and B (0.5% acetic acid in acetonitrile) for 0-45 min; injection volume 10 ul; flow rate, 1 ml/min; and detection, UV at 254 nm. Each arrow indicates catechin (a), rutin (b), quercetin (c) and flavone (d).

1998; Gilhar and Kalish, 2006).

The association between hair loss and dandruff has been reported (Nematian *et al.*, 2006; Pierard-Franchimont *et al.*, 2006). Hair shedding increases with cutaneous infection of *Pityrosporum ovale* (Nematian *et al.*, 2006).

To develop new therapies to enhance hair growth, we screened the extracts of Jeju seaweeds and discovered that *Grateloupia elliptica* (*Grateloupiaceae*) has the potential to promote hair growth. *G. elliptica*, a red seaweed, was reported to have anti-inflammatory effects by the decrease of production of pro-inflammatory mediators (Yang *et al.*, 2010). Bromophenols of *G. elliptica* showed high α-glucosidae activity and seem to have potential to prevent diabetes mellitus (Kim *et al.*, 2008). However, the effect of *G. elliptica* on the prevention of hair loss has not yet been reported. Therefore, the present study was carried out to investigate the preventing effect of *G. elliptica* extact on the hair loss.

MATERIALS AND METHODS

Extract and HPLC analysis

G. elliptica were collected along the coast of Sungsanpo in Jeju Island, Korea, between March and June 2009. The seaweed was washed three times with tap water to remove the salt, epiphytes, and sand attached to the surface, then carefully rinsed with fresh water and maintained in a medical refrigerator at −20°C. Thereafter, the frozen whole body was lyophilized and homogenized with a grinder prior to extraction. The seaweed sample was pulverized into powder using a grinder. The powder (1 g) was extracted with 70% aqueous ethanol (100 ml) at room temperature for 24 h and filtrated. After filtration, the ethanol extract was evaporated to dryness under vacuum.

The amount of polyphenols in the ethanol extract of *G. elliptica* measured by the Folin-Ciocalteu colorimetric method was 13.5%. HPLC analysis was performed on the HPLC (Alliance 2695 system with PDA, Waters Co., Milford, MA, USA); Used column was XTerra® C_{18} (100×4.6 mm, i.d. 3.5 um; Waters Co., Milford, MA, Ltd. USA), mobile phase, linear-gradient mixture of A (aqueous 0.5 % acetic acid) and B (0.5% acetic acid in acetonitrile) for 0-45 min; injection volume 10 ul; flow rate, 1 ml/min; and detection, UV at 254 nm (Fig. 1). The *G. elliptica* extract used in the study was not identified to have antioxidant compounds such as catechin, rutin, quercetin and flavone (Fig. 1).

This extract was dissolved in dimethyl sulfoxide (DMSO) (Sigma, St. Louis, MO, USA) for subsequent treatment; the final concentration of DMSO was adjusted to 0.2% (v/v) in the following experiments.

Assay for the proliferation of dermal papilla cells

Rat vibrissa immortalized dermal papilla cell line (Filsell *et al.*, 1994) was donated by the Skin Research Institute, Amore Pacific Corporation R&D Center, Korea. The dermal papilla cells were cultured in DMEM (Hyclone Inc, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco BRL, Rockville, NY, USA) and penicillin/streptomycin (100 unit/ml and 100 μg/ml, respectively) at 37°C in a humidified atmosphere under 5% CO_2.

The proliferation of dermal papilla cells was evaluated by measuring the metabolic activity using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay (Carmichael *et al.*, 1987). Briefly, dermal papilla at 1.0×10^4 cells/ml were seeded into 96-well plate, cultured 24h in serum-free DMEM, and then treated with vehicle (DMSO diluted 1:500 in serum-free DMEM) as a control and *G. elliptica* extract of 0.1, 1, 10 and 100 μg/ml for 4 days. After incubation, 0.1 mg (50 μl of a 2 mg/ml solution) of MTT (Sigma, St. Louis, MO, USA) was added to each well, and the cells were then incubated at 37°C for 4 h. Next, the plates were centrifuged at 1,000 rpm for 5 min at room temperature and the media was then carefully aspirated. DMSO 200 μl was then added to each well to dissolve the formazan crystals and the absorbance of the plate at 540 nm was then read immediately on a microplate reader (BioTek Instrument, Inc., Winooski, VT, USA). All experiments were performed three times and the mean absorbance values were calculated. The results are expressed as the percentage in the absorbance caused by treatment with *G. elliptica* extract compared to that of the untreated controls. Minoxidil (Sigma, St. Louise, CA, USA) was used as a positive control.

Assay for prostatic 5α-reductase activity

Male Spargue-Dawley (SD) rats (8 wk old) were purchased from Dae-Han Biolink (Eumseong, South Korea), and given a standard laboratory diet with water *ad libitum*. All animals

were cared for by using protocols (20100031) approved by the IACUC of Jeju National University. Male SD rats (8 wk old) were sacrificed with CO_2. The rat prostates were removed from their capsules, washed with saline, and stored at −80°C. Frozen tissues were thawed on ice and procedures were carried out at 4°C. The tissues were homogenized with a Polytron homogenizer (Brinkman Instruments, Westbury, NY, USA) in 5-6 tissue volumes of medium A (0.32 M sucrose, 1 mM dithiothreitol (DTT), 0.2 mM phenylmethylsulfonylfluoride (PMSF); and 20 mM potassium phosphate buffer, pH 6.6). The homogenates were centrifuged at 1,500 g for 20 min. The pellets were recovered, washed with three tissue volumes of medium A, and centrifuged two additional times at 400 g for 10 min. The washed pellets were suspended in medium A and stored at −80°C until use. The suspension (2.5 mg protein/ml as determined by the Bradford assay using Bio-Rad reagents) was used as source of 5α-reductase. 5α-reductase activities were analyzed as previously described (Hirosumi, *et al.* 1995). The reaction mixture had a final volume of 500 μl and contained 1 mM DTT, 40 mM potassium phosphate buffer, pH 6.6, 2 mM NADPH, and 120 nCi [1,2,6,7-^{3}H] testosterone. Triplicate reactions were initiated when the reaction mixture was added to the rat prostatic enzyme fraction (250 μg of protein) containing 0.2% DMSO (as a control), or *G. elliptica* extract (0.1, 1 and 10 μg/ml). Finasteride 2 nM (Merck-Sharpe-Dohme, Whitehouse Station, NJ, USA) was used as a positive control. The mixture was incubated at 37°C for 60 min, and then stopped by adding 1 ml of ethyl acetate and mixing for 1 min. After centrifugation at 1,000 g for 5 mim, the organic phase was removed, dried under a heating plate, dissolved in 50 μl of ethyl acetate containing 500 μg/ml of testosterone and 500 μg/ml dihydrotestosterone (DHT), and applied to a silica gel 60 F254 TLC plate (Merck). The plate was developed in a solvent system consisting of an ethyl acetate:cyclohexane (1:1) solution, and the plate was air dried. Testosterone was visualized under UV light (254 nm) and DHT was detected using a 10% H_2SO_4 solution and posteriorly heating the plate. Under these conditions, DHT develops a classical dark yellow color. Areas containing androgen were removed and the strips were soaked in 5 ml of ULTIMA GOLD™ Cocktail (PerkinElmer, Waltham, MA, USA) and radioactivity was measured by a liquid scintillation counter (Packard Bioscience, Meriden, CT, USA). The activity of 5α-reductase was expressed as a ratio calculated by the equation [DHT/(T+DHT)]×100.

Assay for PGE_2 production in HaCaT keratinocyte cells

The immortalized human keratinocyte cell line, HaCaT, were cultured in RPMI-1640 (GIBCO, Grand Island, NY, USA) supplemented with 10% FBS (GIBCO, Grand Island, NY, USA) and penicillin/streptomycin (100 unit/ml and 100 μg/ml, respectively) at 37°C in a humidified CO_2 incubator.

HaCaT cells (2.0×10^5 cells/ml) were pre-incubated for 18 h and treated with various concentrations of *G. elliptica* extract (50, 25 and 12.5 μg/ml) for 24 h. PGE_2 amount in the culture supernatant was measured with human PGE_2 enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Briefly, PGE_2 antibodies and cell culture supernatants were added to each well and incubated for 2 h at room temperature on an orbital shaker. After washing, para-nitrophenylphosphate (*p*NPP) solution was added to each well. The plates were incubated for 1 h at room temperature on an orbital shaker. The optical density of each well was measured at 450 nm using an ELISA reader.

Measurement of cytokine production

Bone marrow-derived dendritic cells (BMDCs) were grown from wild-type C57BL/6 mice (Taconic Farm, Germantown, NY, USA) as described previously (Koh *et al.,* 2010). Briefly, the mouse tibia and femur was obtained by flushing with DMEM to yield bone marrow cells. The cells were cultured in RPMI 1640 medium containing 10% FBS (Gibco, Rockville, NY, USA), 50 μM β-mercaptoethanol, 2 mM glutamine supplemented with 3% J558L hybridoma cell culture supernatant containing granulocyte-macrophage colony-stimulating factor. The culture medium was replaced with fresh medium every second day. At day 6 of culture, non-adherent cells and loosely adherent DC aggregates were harvested, washed, and resuspended in RPMI 1640 supplemented with 5% FBS.

The BMDCs were incubated in 48-well plates in 0.5 ml containing 1×10^5 cells per well treated with *G. elliptica* extract of 0 to 50 μg/ml for 1 h before stimulation with 10 ng/ml LPS from *Salmonella minnesota* (Alexis, NY, USA). Supernatants were harvested 16 h after stimulation. Concentrations of murine TNF-α, IL-6 and IL-12 p40 in the culture supernatant were determined by ELISA (Pharmingen, San Diego, CA, USA) according to the manufacture's instructions. The inhibitory activity was expressed as the inhibition rate (%), which was calculated from the following formula: Inhibitory activity (%)=[(cytokine production in DMSO-treated DC−cytokine production in compound-treated DC)/ cytokine production in DMSO-treated DC] ×100

Culture of *Pityrosporum ovale* and determination of the antifungal activity

P. ovale was purchased from the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea). The strain was cultured in modified Dixon's broth (1.5% Malt extract, 2% Oxbile, 1% Tween 40, 0.25% Glycerol) at 37°C. To assess the antifungal activity of extracts, the agar-well diffusion method was used (Anesini and Perez, 1993). The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation. Inoculums suspensions were adjusted by spectrophotometer (SpectraMAX 190, Molecular devices, Sunnyvale, CA, USA) to an absorbance 0.6 of at 550 nm. Two-layer plates were prepared with 20 ml of lower agar followed by, 10 ml of upper agar inoculated with 200 μl of *P. ovale* (5×10^5 CFU/ml) in Petri dishes. Forty five microliter aliquots of 40 mg/ml were individually dispensed onto 6-mm-diameter sterile filter paper discs (Adventec, Tokyo, Japan). Each disc was placed on nutrient agar that had been previously seeded with the target *P. ovale* and incubated for 2 days at 37°C. Inhibition zones (including the disc diameter) were measured using a ruler. The values were the average (mm) of 3 measurements per disk, taken at 3 different directions. Zinc pyrithione was used as a positive control, and DMSO was used as negative control. The experiment was performed in triplicate.

Statistical analyses

Student's t-test was used to determine the statistical significance of differences between values for the experimental and control groups. The data were presented as means ± standard deviation (SD) of at least three independent experiments per-

formed in triplicate.

RESULTS

Effect of *G. elliptica* extract on the proliferation of dermal papilla cells

To evaluate the effect of *G. elliptica* extract on cell proliferation of hair follicles, immortalized rat vibrissa dermal papilla cells were treated with various concentrations of *G. elliptica* extract, and proliferation of dermal papilla cells was examined.

G. elliptica extract promoted the proliferation of dermal papilla cells by 102.1, 104.7, 114.4 and 169.5% at the concentration of 0.1, 1, 10 and 100 μg/ml compared with the vehicle-treated control, respectively (Fig. 2). Specifically, 100 μg/ml of *G. elliptica* extract was found to induce a greater increase in proliferation of dermal papilla cells than 10 μM of minoxidil, a positive control. The results suggest that *G. elliptica* extract might have hair-growth promoting effect via the proliferation of dermal papilla cells.

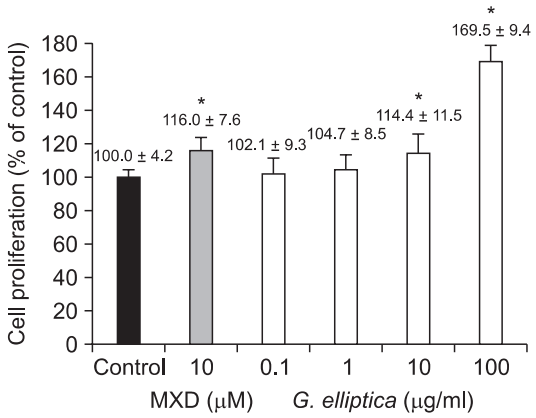

Fig. 2. Proliferation effect of *G. elliptica* extract on cultured dermal papilla cells. Immortalized dermal papilla cells (DPCs) from rat vibrissa follicles (1.0×10^4 cells/ml) were plated in 96 well plates. DPCs were treated with various concentration of *G. elliptica* extract extract or minoxidil (MXD), as indicated. Cell proliferation was measured using a MTT assay for 4 days. All experiments were performed in triplicate. Data are presented as the mean ± the S.D. **p*<0.05 vs. control.

Effect of *G. elliptica* extract on 5α-reductase activity

Conversion of testosterone to DHT is important in hair loss and, whether *G. elliptica* extract could inhibit 5α-reductase activity, we examined the 5α-reductase activity with crude enzyme from rat prostate. As shown in Fig. 3, *G. elliptica* extract inhibited 5α-reductase activities by 7.2%, 14.7% and 48.3% at the concentration of 0.1, 1 and 10 μg/ml in dose-dependent manner. Finasteride, a positive control, inhibited 5α-reductase activities by 98% at 2 nM concentration. The result suggests that *G. elliptica* extract could have the potential for the treatment of AGA via the 5α-reductase inhibition.

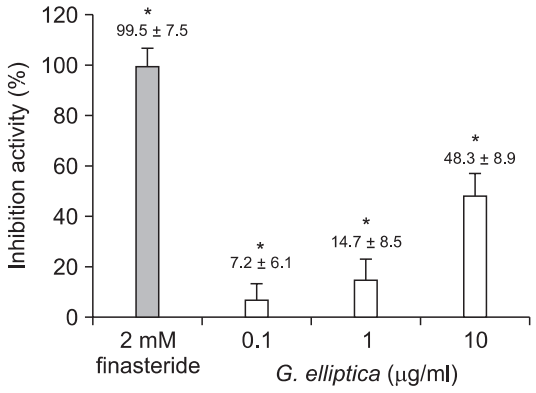

Fig. 3. The effect of *G. elliptica* extract on the inhibition of 5α-reductase. Assay of 5α-reductase inhibition was performed using crude extract of rat prostate as described in "Materials and Methods". The conversion rate of testosterone (T) to dihydrotestosterone (DHT) was calculated by the equation [DHT/(T+DHT)]×100. Inhibition activity (%) was expressed as a percentage of reduced conversion rate compared to the control. The inhibition activity of control group was regarded as 0% (not shown). Finasteride was used as a positive control. Data are presented as the mean ± the S.D of three independent experiments. **p*<0.05 vs. control.

Effect of *G. elliptica* extract on the PGE_2 production in HaCaT cells

The PGE_2 production effect of *G. elliptica* extract was measured in HaCaT human keratinocytes. *G. elliptica* extract at 12.5, 25, and 50 μg/ml increased PGE_2 production in a dose dependent manner (Fig. 4). Minoxidil, a positive control, significantly increased PGE_2 production at 100 μM. This result indicates that *G. elliptica* extract might have hair-growth promoting effect via the increase of PGE_2 production.

Inhibitory effect of *G. elliptica* extract on the production of pro-inflammatory cytokines in LPS-stimulated bone marrow-derived dendritic cells

To evaluate the *G. elliptica* extract for anti-inflammatory activity, *G. elliptica* extract was tested for the inhibitory effect on LPS-stimulated IL-12 p40 production in BMDCs (Fig. 5). *G. elliptica* extract inhibited IL-12 p40 production by LPS-stimulated BMDCs with the inhibition values of 47.8% at the concentration of 50 μg/ml. In addition, *G. elliptica* extract exhibited significantly inhibitory effect on IL-6 production in LPS-

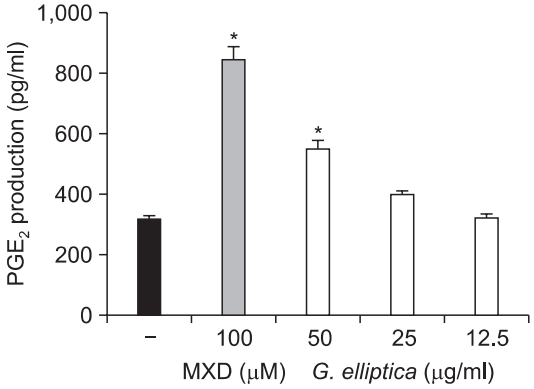

Fig. 4. Effect of *G. elliptica* extract on PGE_2 production in HaCaT human keratinocytes. HaCaT cells (2.0×10^5 cells/ml) were pre-incubated for 18 h, and cells were treated with *G. elliptica* extract for 24 h. PGE_2 amount was determined from the culture supernatant by ELISA method. Data are presented as the mean ± the S.D of three independent experiments. **p*<0.05 vs. control.

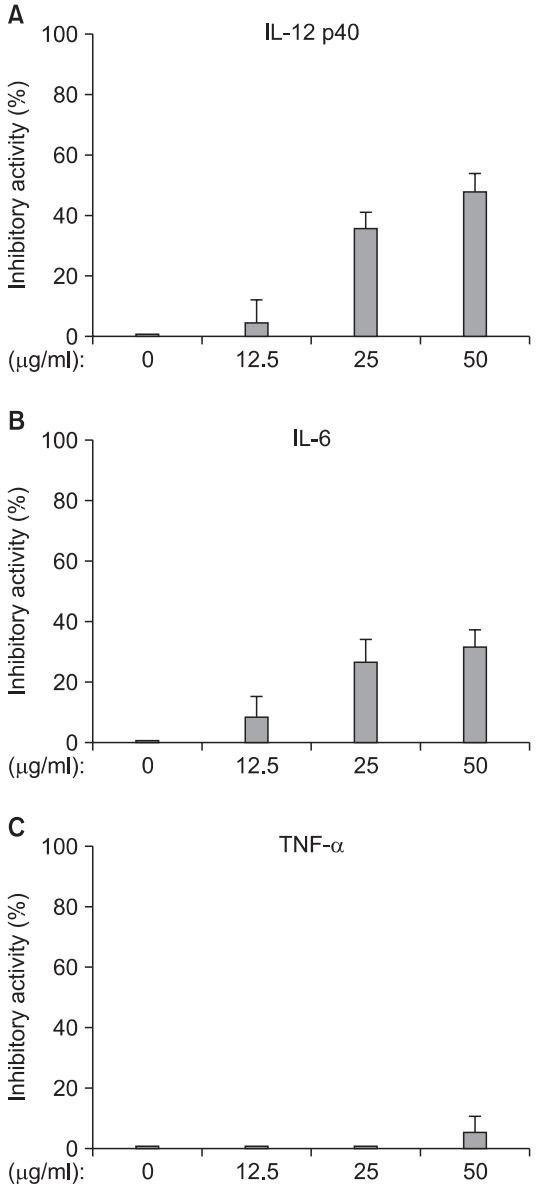

Fig. 5. Inhibitory activity of *G. elliptica* extract on IL-12 p40 (A), IL-6 (B), and TNF-α (C) production by LPS-stimulated BMDCs. DCs were treated with the indicated concentration of the extract for 1 h before stimulation with LPS (10 ng/ml). Supernatants were harvested 16 h after stimulation. Concentrations of murine IL-12 p40, IL-6, and TNF-α in the culture supernatants were determined by ELISA. The data were presented as inhibition activity (%) compared to the value of vehicle-treated BMDCs.

stimulated DC with the inhibition value of 31.4%. In order to confirm the anti-inflammatory activity of *G. elliptica* extract, cell viability was simultaneously determined by using the colorimetric MTT assay and as a result, the extract had little or no effect at the concentration tested (data not shown). These results suggest that *G. elliptica* extract could have the potential for the treatment of AA via the decrease of pro-inflammatory cytokine production.

Table 1. Inhibitory activity of *G. elliptica* extract against *P. ovale*

Sample	Diameter (mm)
G. elliptica extract	10
DMSO	-
Zinc pyrithione	35

DMSO: Dimethyl sulfoxide.

Antifungal activity of *G. elliptica* extract

Hair shedding has been reported to increase with cutaneous infection of *P. ovale* (Nematian *et al.*, 2006). We examined whether *G. elliptica* extract could inhibit the growth of *P. ovale*, normal flora yeast in dandruff. *G. elliptica* extract and zinc pyrithione, a positive control, exhibited 10 mm and 35 mm inhibition-zone diameter, whereas inhibitory-zone was 0 mm in the negative control (Table 1). The result shows that *G. elliptica* extract may prevent hair loss via inhibitory activity against *P. ovale*.

DISCUSSION

In this study, the hair-loss preventing effects of *G. elliptica* extract were investigated. To the best of our knowledge, this study is the first to demonstrate that *G. elliptica* extract has the potential to treat alopecia via the proliferation of dermal papilla, 5α-reductase inhibition, increase of PGE_2 production, decrease of LPS-stimulated pro-inflammatory cytokines and inhibitory activity against *P. ovale*.

The mesenchyme-derived dermal papilla cells play a pivotal role in hair growth regulation. The morphology of dermal papilla cells can be altered through the hair growth cycle, being maximal in volume in the growing phase (anagen) and least in the resting phase (telogen). Evidence has shown that the size of dermal papilla cells is well correlated with hair growth, and the cell number of dermal papilla cells is increased in the growing phase of hair cycle (Jahoda *et al.*, 1984; Elliott *et al.*, 1999).

In the continuing search for new treatment of alopecia from natural sources, we examined several seaweed extracts on the proliferation of dermal papilla cells. We found that the *G. elliptica* extract significantly increased the proliferation of dermal papilla cells by 169.5% at 100 μg/ml concentration compared with the control group, whereas extracts of *Sargassum coreanum*, *Halymeni adilatata* and *Laurencia pinnata* did not show proliferation activity of dermal papilla cells (data not shown). Specifically, 100 μg/ml of *G. elliptica* extract was found to induce a greater increase in proliferation of dermal papilla cells than 10 μM of minoxidil, a positive control.

AGA, the most common type of alopecia, may be modulated by the inhibition of 5α-reductase, which converts testosterone to DHT (Kaufman, 1996). Finasteride is known to repress the progression of AGA through inhibition of 5α-reductase (Kaufman, 1996). We found that *G. elliptica* extract could inhibit the activity of 5α-reductase by 48% at 10 μg/ml concentration. Previous studies suggest that AGA may be caused by DHT in different ways: The miniaturization of dermal papilla and hair follicles is induced by DHT, which leads to transition from anagen to catagen (Sinclair, 1998). DHT increases the

levels of transforming growth factor-β1 (TGF-β1) and TGF-β2 in dermal papilla cells, which leads to decreased proliferation of epithelial cells (Inui *et al.*, 2002; Hibino and Nishiyama, 2004). Up-regulation of dickkopf related protein-1 (DKK-1) by DHT can cause repression of the growth of epithelial cells in hair follicles (Kwack *et al.*, 2008). In further study, we need to examine whether *G. elliptica* extract can regulate the levels of TGF-β1/β2 and DKK-1 in dermal papilla cells.

Hair follicle growth and cycle could be controlled by prostaglandins, under autocrine and paracrine loops. Minoxidil increases the activity of purified COX-1, suggesting a positive role of PG in hair growth onset (Coleman *et al.*, 1994a; Johnstone and Albert, 2002; Colombe *et al.*, 2007; Colombe *et al.*, 2008). Lantanoprost, a $PGF_{2\alpha}$ analogue, is widely used in the treatment of glaucoma and has a hypertrichotic side effect such as the increased number, length, thickness, and darkening of eyelashes hair (Johnstone and Albert, 2002; Uno *et al.*, 2002; Messenger and Rundegren, 2004). PGE_2 is described as a possible factor of hair growth (Colombe *et al.*, 2007). Indeed, viprostol, a PGE_2 analog, is an effective antihypertensive agent and was reported to increase human hair growth (Johnstone and Albert, 2002; Colombe *et al.*, 2007). *G. elliptica* extract was found to increase PGE_2 production in a dose dependent manner. By the way, there is a report that PGE_2 was found in various species of seaweed *Gracilaria*. *asiatica*, *G. lichenoides* and *G. rhodocaudata* (Bernard, 2008). We thus examined the PGE_2 amount in *G. elliptica* through the processing in a condition without HaCaT cells. PGE_2 in *G. elliptica* couldn't be detected at 50 μg/ml concentration (data not shown).

Cyclosporin A (CsA), a T cell-specific immunosuppressant, has the hair growth stimulating effect, which is observed not only in normal but also in patients with alopecia areata (Yamamoto and Kato, 1994). Recent studies suggest that CsA may induce hair growth by inhibiting calcineurin which is needed for nuclear localization of NFATc1 in the bulge region. NFATc1 is activated by bone morphogenic protein (BMP) signaling, which is required for the maintenance of stem cell quiescence (Horsley *et al.*, 2008). Therefore, when calcineurin/NFATc1 signaling is suppressed, stem cells are activated prematurely, resulting in hair follicular growth. In addition, TNF-α is required for a timely anagen-catagen transition in mouse pelage follicles (Tong and Coulombe, 2006). In the other hand, because IL-12 is a key cytokine in Th1-mediated autoimmune responses, down-regulation of IL-12 production may ameliorate the autoimmune diseases such as alopecia areata (Taki *et al.*, 1997). Fig. 5 shows that *G. elliptica* extract may have potent anti-inflammatory action and can be useful for amelioration of the autoimmune diseases such as alopecia areata.

The non-pathogenic yeast *P. ovale* can undergo transition to a pathogenic form under favorable conditions. At high concentrations, this opportunistic organism diminishes the normal protective barrier of skin and affects the body's ability to control inflammation. *P. ovale*-related diseases such as Seborrheic dermatitis, psoriasis are often difficult to treat. In particular, hair shedding is known to increase with dandruff by infection of *P. ovale* (Nematian *et al.*, 2006). *G. elliptica* extract may have a potential as a treatment for *P. ovale*-associated hair diseases including hair loss.

Overall, the results of this study demonstrated that *G. elliptica* extract is capable of preventing hair loss via the proliferation of dermal papilla, 5α-reductase inhibition, increase of PGE_2 production, decrease of pro-inflammatory cytokines and inhibitory activity against *P. ovale*. In further studies, active compounds from *G. elliptica* extract should be elucidated.

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