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The Philippines stingless bee propolis promotes hair growth through activation of Wnt/β-catenin signaling pathway

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Abstract: Although hair loss is not a horrible disease, it sometimes reduces the patients' quality of life (QOL) and increases their mental stress. Currently, there is no effective treatment for hair loss. It is known that honeybee propolis has various biological activities, including stimulating the proliferation of hair matrix keratinocytes. However, little is known with the hair promoting activity of stingless bee propolis. Hence, this study investigates the hair growth-promoting activity of Philippines stingless bee propolis extract and the underlying a molecular mechanism of promoting hair growth. For the evaluation of hair growth stimulating activity, 99.5% ethanolic extract of Philippines stingless bee propolis is examined using the simple shaving model in C57BL/6N mice. Melaninization of dorsal skin and histological analysis of hair follicles (HFs) revealed that propolis promotes hair growth by stimulating HFs development. The expression of mRNA (*Wnt3a, Ctnnb1/β-catenin, Lef1,* and *Bmp2*) and protein (WNT3A and β-catenin) of selected Wnt/β-catenin associated genes explains Philippines stingless bee propolis promoting HFs development by activating Wnt/β-catenin signaling pathway. These results suggest that the treatment of propolis strongly promotes hair growth by stimulating the development of HFs via activation of Wnt/β-catenin signaling pathway. This further indicates the potential of Philippines stingless bee propolis as a novel promising agricultural product for hair growth.
Key words: hair follicles, hair growth, stingless bee propolis, Wnt/β-catenin signaling pathway

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Introduction

Hair loss is a disorder that reduces the patients' quality of life (QOL) and increases their mental stress. It may be caused by numerous factors including genetic predisposition, hormonal imbalance, auto-immune condition, medication, etc. Some of these causes may induce hair loss by affecting the development of hair follicles (HFs) [1]. At present, the market potential for hair loss treatments is great. The global expenditure for hair loss therapy reached an astounding amount of $4.2 billion in 2020, and this is projected to increase further at a compound annual growth rate (CAGR) of 15.4% from 2021 to 2028 [2].

Currently, only Finasteride and Minoxidil are approved by the US FDA as effective medications for treating hair loss. Finasteride increases the stemness of the dermal papilla cells and decreases the level of dihydrotestosterone to promote hair growth. Minoxidil, on the other hand, promotes hair growth by stimulating HFs in the telogen stage to enter the anagen stage. However, Finasteride has only been restricted for use in cases of androgenic hair loss while Minoxidil has been largely exploited for treatment of temporary hair loss [3]. Therefore, it is desired to explore other alternative therapies for managing hair loss such as the use of natural products and its derived compounds.

Propolis is a natural resinous product that is produced by bees through the mixing of saliva and beeswax gathered from tree buds, sap flows, or other botanical sources. Propolis has been used as a medicine for hundreds of years. It is recognized to have diverse biological activities, which are determined by the source of plants and bee species. However, the biological activity of propolis derived from the indigenous population of *Tetragonula biroi* Friese or Philippines stingless bees is relatively unknown. Our previous study reported that the Philippines stingless bee propolis exerts a tumor-suppressing potential on differentiated-type of gastric adenocarcinoma. In the same study, several 60-week-old *A4gnt* KO mice showing a moderate degree of hair loss were incidentally observed to exhibit pronounced hair growth development following the 30-day oral administration of Philippines stingless bee propolis [4]. The effect of stingless bee propolis on the development of HFs has not yet been investigated. Therefore, we focus on the functional evaluation of the hair growth stimulating activity of the Philippines stingless bee propolis.

Materials and Methods

Propolis sample preparation

Propolis flake samples from Philippines indigenous stingless bee, *Tetragonula biroi* Friese, were obtained from the UPLB Bee Program Meliponary, Institute of Biological Sciences, University of the Philippines Los Baños, Laguna, Philippines [4]. The crude extract was prepared by adding 250 ml of 99.5% ethanol to 67.6 g of the crude propolis, followed by shaking for 30 min by ultrasonic cleaning. The ethanolic extract of propolis was then stored at 4°C.

Animals

Six weeks old female C57BL/6N mice were purchased from Japan SLC company and were maintained at 22–25°C, 40–50% humidity and 12 h light/dark cycle. Rodent chow (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and water were given *ad libitum*. All experimental procedures were performed in accordance with the guidelines and approval of the Institutional Animal Care and Use Committee, Graduate School of Agriculture and Life Sciences, The University of Tokyo (Approval No. P18-096, P20-062).

Experimental design

Using a model of gentle anagen induction [5], mice were anesthetized at 8 weeks of age (telogen stage) and a 12cm^2 area (horizontal length, 3 cm; longitudinal length, 4 cm) on the skin aspect of the back was shaved using an electric shaver. Next, 200 μl of respective treatments namely, (i) 50% ethanol (control group), (ii) 99.5% ethanolic extract of propolis diluted two-fold in distilled water (EEP group) and (iii) 1% minoxidil (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) dissolved in 50% ethanol (minoxidil group) were topically administered onto the shaved area for a consecutive period of 28 days starting at day 2 post-induction (*pi*) up to day 30 *pi*. The effect of each treatment was observed for an extended period of 22 days (day 52 *pi*). Mice were sacrificed at two different time points (n=6/group/time point): day 20 *pi* and day 52 *pi* (Fig. 1a).

Macroscopic assessment

Macroscopic assessment of the hair growth-stimulating effect of each treatment was carried out by determining the degree of melanization of the shaved area that is indicative of middle anagen induction [5]. Additionally, 6 hair samples were collected in areas showing prominent hair growth and their corresponding length (in millimeters) were measured using a ruler. Sampling was performed on days 20, 23, 32 and 43 *pi*.

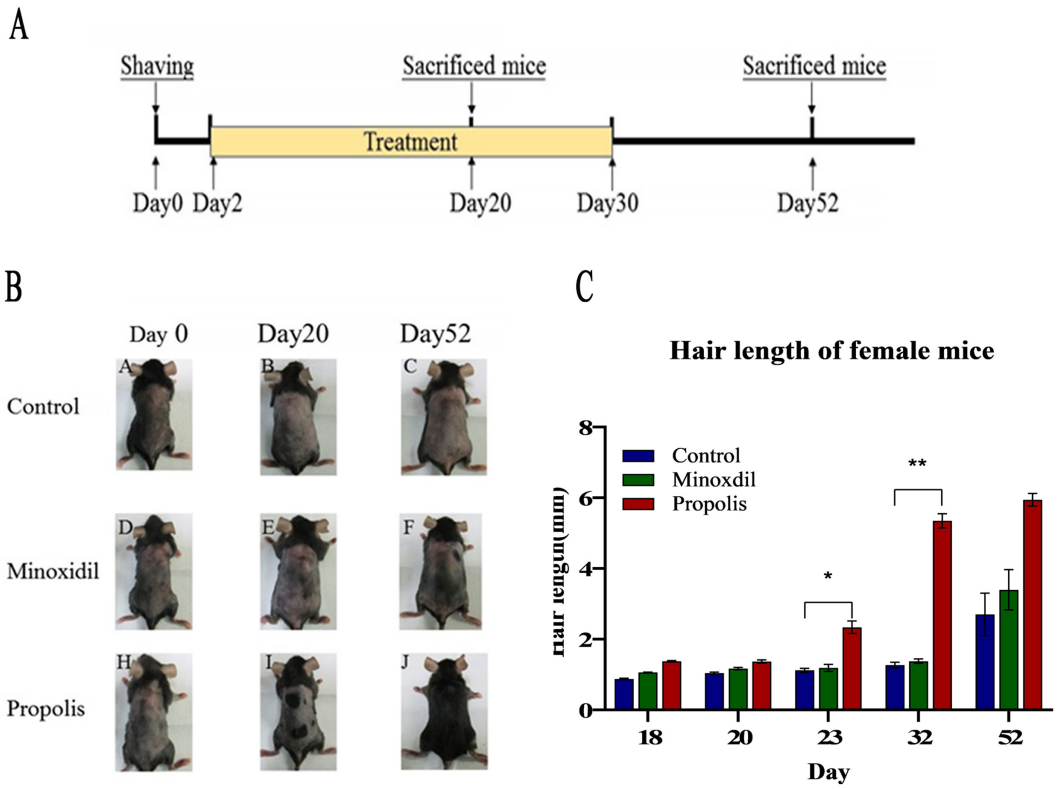

Fig. 1. The Philippines Stingless bee propolis induces C57BL/6N female mice skin hair follicles (HFs) into the anagen stage. (A) Experimental time course. (B) Representative images of mice applied with various treatment at the 0 day, 20th day, and 52nd day respectively. (C) Hair length of the dorsal skin. Values are mean ± SD (*P<0.05 and **P<0.01).

Histopathology

The harvested skin samples were fixed in 10% neutral buffered formalin and processed with routine paraffin technique. Paraffinized tissue samples were cut into 4-μm thick sections, and stained with H&E. The number of HFs in a defined 4.8 cm^2 area of the skin sections at a magnification of 100× was counted.

Immunohistochemistry

The protein expression of β-catenin and Ki67 markers was accomplished through immunohistochemistry. Formalin-fixed and paraffin-embedded (FFPE) tissue sections were deparaffinized, rehydrated, and heated in sodium citrate buffer (pH 6.0) prior to blocking for endogenous peroxidase and non-specific background staining. Afterwards, tissue sections were incubated overnight at 4°C using the following primary antibodies: anti-β-catenin (polyclonal, Dako Cytomation, Santa Clara, USA; 1:1,000 dilution), and anti-Ki67 (polyclonal; Dako Cytomation; 1:200 dilution). HRP-conjugated streptavidin (Dako Cytomation; 1:200 dilution) for labeling β-catenin and Ki67 was used as secondary antibodies. Finally, immunoreaction was visualized using the diaminobenzidine tetrahydrochloride-H_2O_2 method.

Immunofluorescence

The protein expression of WNT3A was accomplished through Immuno-fluorescence. FFPE tissue sections were deparaffinized, rehydrated, treated in citrate buffer, pH 6.0 for antigen retrieval, and blocked in milk (Morinaga & Co., Ltd., Tokyo, Japan). The sections were incubated overnight at 4°C with the primary antibody, anti-Wnt3a (ab19925; polyclonal, Abcam, Boston, MA, USA; 1:300 dilution). Alexa Fluor™ 594-conjugated goat anti-Rabbit IgG (H+L) (Code No. A-11012, Thermo Fisher; 1:1,000 dilution, Waltham, MA, USA) was used as secondary antibody. Finally, sections were mounted using Vectashield® Antifade mounting medium with DAPI (Vector Laboratories, Inc., Newark, CA, USA).

Western blotting

Skin samples (120 mg) were homogenized in RIPA lysis buffer (1 ml) with protease inhibitor. The total protein was quantified using the BCA method (Pierce TM BCA protein assay kit, Thermo Fisher) and 60 μg proteins were fractionated on 10% SDS-PAGE gels and transferred to a polyvinylidene difluoride membrane. The membrane was incubated in anti-WNT3A (ab19925, Abcam; 1:200 dilution) at 4°C overnight after blocking

with 5% milk (Morinaga & Co., Ltd.). HRP-conjugated antibody (Goat anti-Rabbit IgG (H+L) (Code No. 65-6120, ThermoFisher; 1:1,000 dilution) was used as the secondary antibody to incubate the membrane at room temperature for 1h. The membranes were analyzed using β-actin (Cell signaling, Danvers, MA, USA; 1:1,000 dilution) as the reference primary antibody.

Quantitative real-time PCR (q-PCR)

The harvested dorsal skin of mice was kept in RNA later™ solution (Invitrogen, Vilnius, Lithuania) and homogenized using a Shake Master Auto BMS-A20T-Pver.2.0 (BMS, Tokyo, Japan). Total RNA was extracted using TRIzol reagent (Invitrogen, Waltham, MA, USA) and synthesized to first strand cDNA by Prime Script RT reagent kit (Perfect Real Time, Takara Bio, Shiga, Japan) according to the manufacturer's protocol. Real Time PCR analysis was performed following the TB Green Premix Ex Taq II (Tli RNaseH Plus, Takara Bio) protocol and the expression levels of Wnt/β-catenin associated genes such as *Wnt3a*, *Dvl2*, *Ctnnb1* (encoding β-catenin), *Lef1*, *Bmp2* were quantified by 2-ΔΔCT method with *Actb*/β-actin as the reference gene [6]. The primer details were shown in Table 1.

Statistical analysis

Statistical analysis was performed by Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and the unpaired Student's *t*-test was used to examine the differences. A *P*-value of 0.05 was considered judged significant, while a *P*-value of 0.01 was regarded highly significant. These *P*-values are shown by the symbols * and **, respectively.

Table 1. Primers details of the genes

Mouse genes		5'-> 3'
Actb	Forward	AAGTGTGACGTTGACATCCG
	Reverse	GATCCACATCTGCTGGAAGG
Wnt3a	Forward	CAGGAACTACGTGGAGATCATGC
	Reverse	CGTGTCACTGCGAAAGCTACT
Ctnnb1	Forward	AGTGAGCCGGACAGAAAAGC
	Reverse	CTTGCCACTCAGGGAAGGA
Dvl2	Forward	TGTCGTCAGATACCCCACAG
	Reverse	CTGGATACATTAGGGTGGAAGGA
Lef1	Forward	TGTTTATCCCATCACGGGTGG
	Reverse	CATGGAAGTGTCGCCTGACAG
Bmp2	Forward	GGGACCCGCTGTCTTCTAGT
	Reverse	TCAACTCAAATTCGCTAGGGAC
Bmp4	Forward	TTCCTGGTAACCGAATGCTGA
	Reverse	CCTGAATCTCGGCGACTTTTT

Results

EEP promotes hair growth significantly in the mice model of anagen induction

The hair growth stimulatory activity of EEP was initially investigated *in vivo* using the mouse model of gentle anagen induction. Female C57BL/6N mice were preferred over male ones primarily because the result of our pilot study has disclosed that male C57BL/6N mice exemplify a prominent hair cycle progression during the 32-day observation period even in the absence of any intervention or stimulation with propolis (Supplementary Fig. 1). Additionally, previous studies have shown that female C57BL/6N mice may serve as a good model of gentle anagen induction [5, 7, 8]. In this model, the phase of the hair growth cycle is determined grossly by examining the degree of melanization at the caudal back skin. After shaving, mice belonging to all treatment groups demonstrated a pinkish-colored skin indicating that the HFs are in the telogen stage of the hair cycle [9]. At the onset of the 20th day *pi* period, however, the entire shaved region in the EEP-treated mice had noticeably turned into grayish color, thereby signifying that the HFs are no longer in the telogen stage but already shifted to the anagen phase of the hair cycle. Worth noting also, several small to medium patches of fully-grown hairs about 140 mm in length were significantly observed during this period. By 52nd day *pi*, these fully-grown hairs were found to almost completely cover the shaved region completely (Fig. 1).

Microscopically, the anagen phase of the hair cycle can be ascertained through observation of a higher magnitude of proliferating HFs [10]. As shown in Figs. 2A and C, an obvious increase in the number of developing HFs (59 ± 22) was evidently noted in mice given EEP at 20th day *pi*. This was confirmed by Ki67 staining which reveals an intense nuclear immunoreactivity of the matrix cells of these anagen HFs (Figs. 2B and C). Conversely, a greatly reduced number of HFs (20 ± 6) that are distributed along the dermis was documented in the ethanol control group (Figs. 2A–C), hence validating the existence of the hair growth cycle at the telogen stage in this group. The results suggest that EEP promotes hair growth by inducing transition of HFs into anagen stage and enhancing the development of HFs.

EEP enhances the development of HFs via activation of Wnt/β-catenin signaling pathway in the model of anagen induction

Wnt signaling plays a key regulatory role in the development of HFs. To assess whether this signaling pathway is critically involved in the hair growth-stimu-

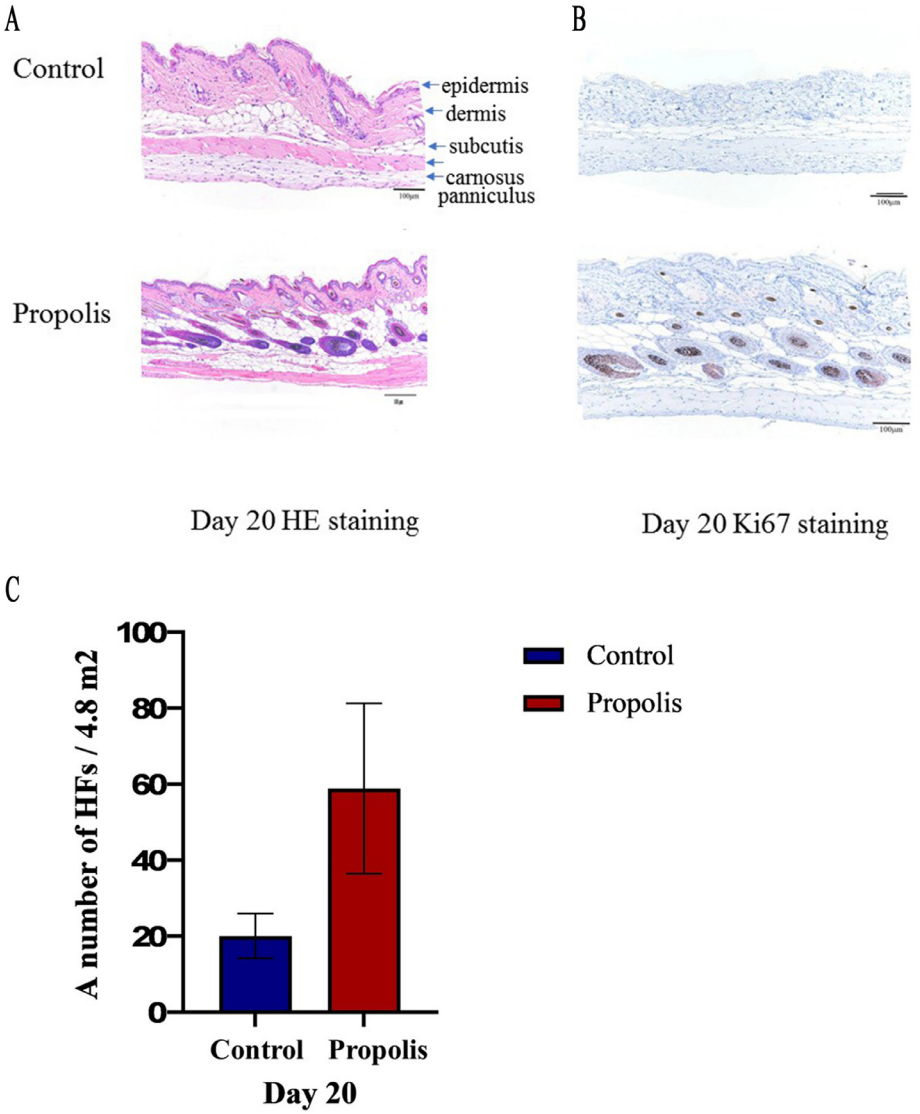

Fig. 2. Histological analysis of hair follicles (HFs) in the model of induction anagen. (A) H&E stain of dorsal skin at the 20th day. (B) Immunohistochemistry of Ki67 (brown) in the dorsal skin at the 20th day. (C) The number of HFs of the dorsal skin, Values are mean ± SD (**P*<0.05 and ***P*<0.01).

lating activity of EEP, the transcriptional expression of selected Wnt/β-catenin associated genes was evaluated. Among these, q-PCR unveiled a significant upregulation of *Bmp2, Ctnnb1*, *Wnt3a* and *Lef1* in the EEP-treated group (Fig. 3A). Notably, the latter two genes displayed a 11-fold and 18-fold increments, respectively, as opposed to their corresponding ethanol-treated controls. At the protein level, EEP administration commensurately induced a marked overexpression of WNT3A protein as determined by immunofluorescence and western blot analyses (Figs. 3B and C). Moreover, the profound overexpression of *Ctnnb1* gene in the EEP group was efficiently translated into an increased level of β-catenin protein displaying a strong cytoplasmic immunoreaction of the matrix cell of individual HFs (Fig. 3D). It is demonstrated that EEP enhances the development of HFs via Wnt/β-catenin signaling pathway.

Discussion

HF is a dynamic miniorgan of the skin that regulates hair growth and is capable of self-renewal and cycling through stages of anagen, catagen, and telogen [11]. Abnormal circulation of HFs can lead to hair loss by triggering a slow entry into anagen from telogen stage or inducing a premature entry into catagen phase [12]. Here, we found that EEP significantly enhances the development of HFs in the shaving model in comparison to their corresponding controls. In consonance with our findings, Brazilian propolis has been previously report-

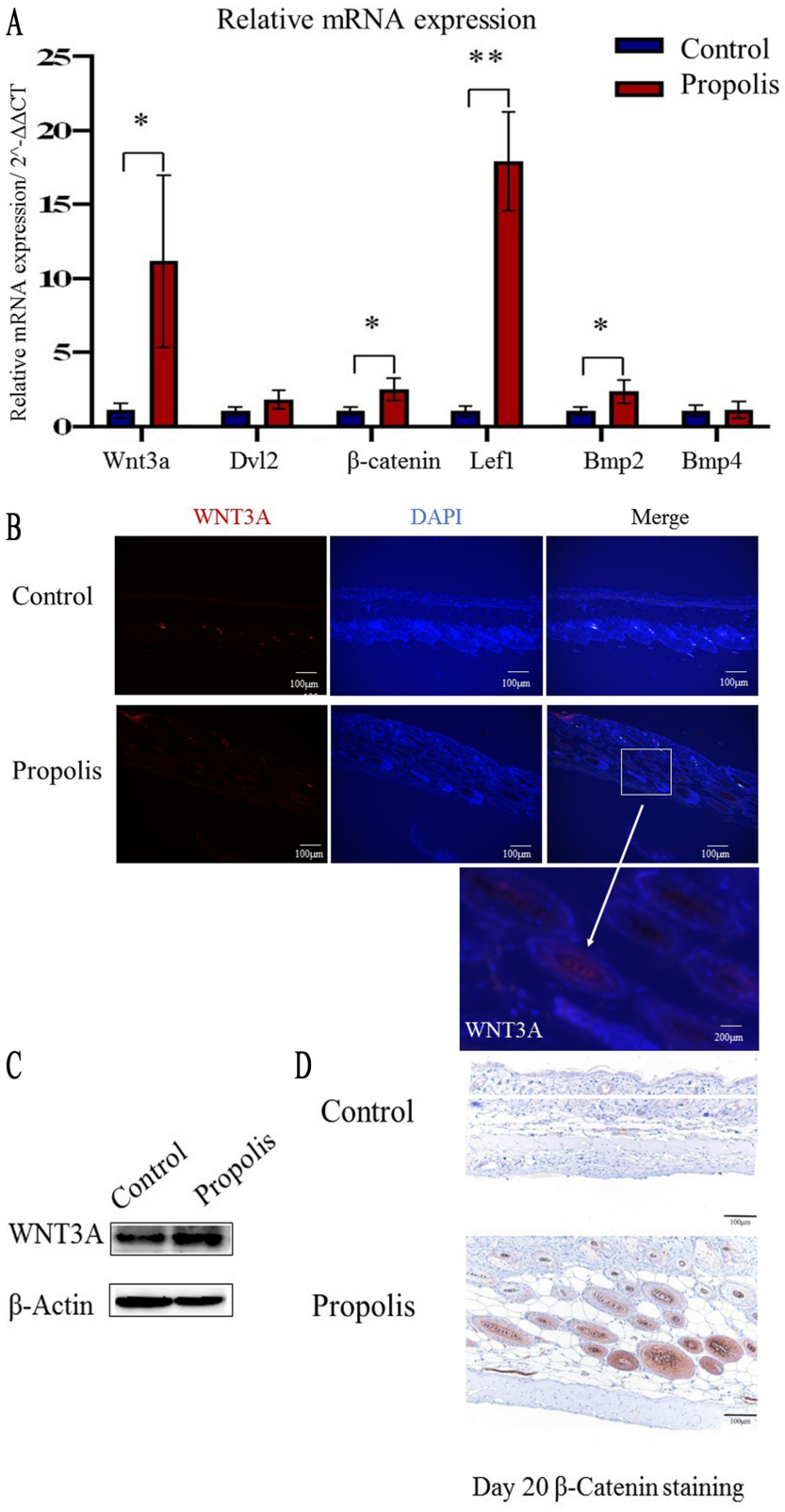

Fig. 3. The Philippines Stingless bee propolis initiates the development of hair follicles (HFs) through Wnt signaling pathway. (A) The mRNA expression of *Wnt3a*, *Dvl2*, *β-catenin/Ctnnb1, Lef1*, *Bmp2* and *Bmp4* of the dorsal skin at the 20th day (B) Immunofluorescence of WNT3A (red) in the dorsal skin at the 20th day. (C) Western blot data of WNT3A protein in the dorsal skin at the 20th day. (D) Immunohistochemistry of β-catenin (brown) in the dorsal skin at the 20th day. Values are expressed as mean ± SD (**P*<0.05 and ***P*<0.01).

ed to stimulate hair growth by stimulating the proliferation of hair matrix keratinocytes [5]. Interestingly, the accelerated hair growth observed in EEP-treated mice in the present study at the 20th and 52nd days *pi* appeared to be more superior than those of the positive control mice treated with Minoxidil, a well-known commercial drug that is already established to treat hair loss in humans. However, as opposed to our findings, a previous study reported that 1% Minoxidil promotes hair growth as early as day 12 in depilation model of C57BL/6 mice [13]. Presently, there are two mice models used to evaluate hair growth- shaving model and depilation model. Unlike depilation, which can synchronize HFs into the growth phase, shaving is gentler and induces HFs into anagen more slowly and is not as damaging to HFs, thereby serving as a good model to investigate the hair growth promoting effect of a compound, product or biological material [14].

The Wnt/ β -catenin signaling pathway is an important pathway in promoting HF development [15–18]. This signaling system regulates the proliferation of hair matrix cells and derma papilla cells which are the vital cells for hair growth. Wnt3a signaling is activated during the anagen stage of the HFs [19]. Following activation, Wnt signaling binds with phosphorylated LRP6 in the presence of Dvl [20]. On the other hand, the binding of Wnt signaling, Frizzled receptors and receptor-related protein LRP5/6 leads to accumulation of the downstream target, β-catenin which stimulates HFs morphogenesis [21]. Moreover, LEF-1 contributes to the development of HFs as the number of HFs was reported to be substantially decreased in *Lef1*$^{-/-}$ mouse [22, 23]. The association between β-catenin and LEF-1/TCF in the nucleus eventually results in the activation of the transcription process [24]. In this study, we showed that EEP treatment significantly upregulates the transcriptional expression of Wnt/β-catenin components such as *Wnt3a, Lef1* and *Ctnnb1*, and consistently induced a marked overexpression of WNT3A protein. Additionally, we have demonstrated that EEP can positively regulate the mRNA levels of *Bmp2* gene, thus reinforcing the notion that Wnt/β-catenin signaling pathway can activate BMP signaling which promotes the proliferation of postnatal HFs [25, 26]. Taken together, the present data suggest that EEP enhances the development of HFs and induces HFs into the anagen stage by promoting the synergism between Wnt/β-catenin signaling and BMP signaling pathways.

The diverse biological activity of propolis mainly depends on its chemical composition that is influenced by several factors like the plants' source and bee species. Presently, almost 300 compounds were identified in bee propolis. The main classes of bioactive compounds such as the flavonoids, terpenes, and phenolics have been identified in honeybee propolis. However, phenolic compounds and terpenes have been predominantly detected in samples of stingless bee propolis [27–29]. In our preliminary experiments, we have applied 70% EEP onto the dorsal back of the shaved mice. Surprisingly, unlike the 99.5% EEP, no significant hair growth and development of HFs were accounted (Supplementary Fig. 2). Hence, propolis extracted by solvents of different polarities would similarly dictate the nature of resulting bioactive components and constituents, which leads to differences in biological activity [30, 31]. In future studies, we will analyze the components of 99.5% EEP by GC-MS/MS and try to underpin the components which may directly promote hair growth.

In conclusion, 99.5% ethanolic extract of Philippines stingless bee propolis promoted hair growth via activation of Wnt/β-catenin signaling pathway and exerted a significant hair growth stimulating activity compared to Minoxidil in this study. Thus, the Philippines stingless bee propolis may serve as a promising agent for addressing hair loss problem.

Author Contributions

All authors equally contributed in the critical review and correction of the manuscript. Y.T, M.J.D. and S.Ka. Study conception and design. M.A.E. and C.C. Propolis sample procurement and quality control assurance. C.W., M.K.K., J.K.C. and K.U. Technical and material support. Y.K. M.J.D. and H.U. Data analysis and interpretation. Y.T. and S.Ka. Preparation and organization of tables and figures. S.Ky. and S.Ka. Study supervision.

Competing Financial Interests

The authors declare that there is no potential conflict of interests.

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References

1. Phillips TG, Slomiany WP, Allison R. Hair loss: common causes and treatment. Am Fam Physician. 2017; 96: 371–378. [Medline]
2. Global hair loss treatment products market. U.S. Verified Market Research, 2021.
3. Santos Z, Avci P, Hamblin MR. Drug discovery for alopecia: gone today, hair tomorrow. Expert Opin Drug Discov. 2015;

10: 269–292. [Medline] [CrossRef]
4. Desamero MJ, Kakuta S, Tang Y, Chambers JK, Uchida K, Estacio MA, et al. Tumor-suppressing potential of stingless bee propolis in *in vitro* and *in vivo* models of differentiated-type gastric adenocarcinoma. Sci Rep. 2019; 9: 19635. [Medline] [CrossRef]
5. Miyata S, Oda Y, Matsuo C, Kumura H, Kobayashi K. Stimulatory effect of Brazilian propolis on hair growth through proliferation of keratinocytes in mice. J Agric Food Chem. 2014; 62: 11854–11861. [Medline] [CrossRef]
6. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Δ Δ C(T)) Method. Methods. 2001; 25: 402–408. [Medline] [CrossRef]
7. Wang H, Liu G, Yang X, Song C, Zhong H, Feng L, et al. Topical application of 8DSS induces anagen hair growth in telogenic mouse skin. Int J Clin Exp Med. 2016; 9: 12584–12593.
8. Choi M, Choi SJ, Jang S, Choi HI, Kang BM, Hwang ST, et al. Shikimic acid, a mannose bioisostere, promotes hair growth with the induction of anagen hair cycle. Sci Rep. 2019; 9: 17008. [Medline] [CrossRef]
9. Müller-Röver S, Handjiski B, van der Veen C, Eichmüller S, Foitzik K, McKay IA, et al. A comprehensive guide for the accurate classification of murine hair follicles in distinct hair cycle stages. J Invest Dermatol. 2001; 117: 3–15. [Medline] [CrossRef]
10. Datta K, Singh AT, Mukherjee A, Bhat B, Ramesh B, Burman AC. *Eclipta alba* extract with potential for hair growth promoting activity. J Ethnopharmacol. 2009; 124: 450–456. [Medline] [CrossRef]
11. Schneider MR, Schmidt-Ullrich R, Paus R. The hair follicle as a dynamic miniorgan. Curr Biol. 2009; 19: R132–R142. [Medline] [CrossRef]
12. Stenn KS, Paus R. Controls of hair follicle cycling. Physiol Rev. 2001; 81: 449–494. [Medline] [CrossRef]
13. Son MJ, Jeong JK, Kwon Y, Ryu JS, Mun SJ, Kim HJ, et al. A novel and safe small molecule enhances hair follicle regeneration by facilitating metabolic reprogramming. Exp Mol Med. 2018; 50: 1–15. [Medline] [CrossRef]
14. Choi N, Shin S, Song SU, Sung JH. Minoxidil promotes hair growth through stimulation of growth factor release from adipose-derived stem cells. Int J Mol Sci. 2018; 19: 691–706. [Medline] [CrossRef]
15. Huelsken J, Vogel R, Erdmann B, Cotsarelis G, Birchmeier W. β-Catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. Cell. 2001; 105: 533–545. [Medline] [CrossRef]
16. Liu B, Chen X, Yi H, Han L, Ji B, Chen H, et al. β-Catenin is involved in oleanolic acid-dependent promotion of proliferation in human hair matrix cells in an *in vitro* organ culture model. Fitoterapia. 2017; 121: 136–140. [Medline] [CrossRef]
17. Enshell-Seijffers D, Lindon C, Kashiwagi M, Morgan BA. β-catenin activity in the dermal papilla regulates morphogenesis and regeneration of hair. Dev Cell. 2010; 18: 633–642. [Medline] [CrossRef]
18. Andl T, Reddy ST, Gaddapara T, Millar SE. WNT signals are required for the initiation of hair follicle development. Dev Cell. 2002; 2: 643–653. [Medline] [CrossRef]
19. Guo H, Yang K, Deng F, Ye J, Xing Y, Li Y, et al. Wnt3a promotes melanin synthesis of mouse hair follicle melanocytes. Biochem Biophys Res Commun. 2012; 420: 799–804. [Medline] [CrossRef]
20. Zeng X, Huang H, Tamai K, Zhang X, Harada Y, Yokota C, et al. Initiation of Wnt signaling: control of Wnt coreceptor Lrp6 phosphorylation/activation via frizzled, dishevelled and axin functions. Development. 2008; 135: 367–375. [Medline] [CrossRef]
21. Bilić J, Huang YL, Davidson G, Zimmermann T, Cruciat CM, Bienz M, et al. Wnt induces LRP6 signalosomes and promotes dishevelled-dependent LRP6 phosphorylation. Science. 2007; 316: 1619–1622. [Medline] [CrossRef]
22. Eastman Q, Grosschedl R. Regulation of LEF-1/TCF transcription factors by Wnt and other signals. Curr Opin Cell Biol. 1999; 11: 233–240. [Medline] [CrossRef]
23. van Genderen C, Okamura RM, Fariñas I, Quo RG, Parslow TG, Bruhn L, et al. Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in *LEF-1*-deficient mice. Genes Dev. 1994; 8: 2691–2703. [Medline] [CrossRef]
24. MacDonald BT, Tamai K, He X. Wnt/β-catenin signaling: components, mechanisms, and diseases. Dev Cell. 2009; 17: 9–26. [Medline] [CrossRef]
25. Kulessa H, Turk G, Hogan BL. Inhibition of Bmp signaling affects growth and differentiation in the anagen hair follicle. EMBO J. 2000; 19: 6664–6674. [Medline] [CrossRef]
26. Zhang R, Oyajobi BO, Harris SE, Chen D, Tsao C, Deng HW, et al. Wnt/β-catenin signaling activates bone morphogenetic protein 2 expression in osteoblasts. Bone. 2013; 52: 145–156. [Medline] [CrossRef]
27. Liberio SA, Pereira ALA, Dutra RP, Reis AS, Araújo MJA, Mattar NS, et al. Antimicrobial activity against oral pathogens and immunomodulatory effects and toxicity of geopropolis produced by the stingless bee *Melipona fasciculata* Smith. BMC Complement Altern Med. 2011; 11: 108–118. [Medline] [CrossRef]
28. Umthong S, Puthong S, Chanchao C. *Trigona laeviceps* propolis from Thailand: antimicrobial, antiproliferative and cytotoxic activities. Am J Chin Med. 2009; 37: 855–865. [Medline] [CrossRef]
29. Teerasripreecha D, Phuwapraisirisan P, Puthong S, Kimura K, Okuyama M, Mori H, et al. *In vitro* antiproliferative/cytotoxic activity on cancer cell lines of a cardanol and a cardol enriched from Thai *Apis mellifera* propolis. BMC Complement Altern Med. 2012; 12: 27–44. [Medline] [CrossRef]
30. Huang S, Zhang CP, Wang K, Li GQ, Hu FL. Recent advances in the chemical composition of propolis. Molecules. 2014; 19: 19610–19632. [Medline] [CrossRef]
31. Pereira FAN, Barboza JR, Vasconcelos CC, Lopes AJO, Ribeiro MNS. Use of Stingless Bee Propolis and Geopropolis against Cancer-A Literature Review of Preclinical Studies. Pharmaceuticals (Basel). 2021; 14: 1161–1183. [Medline] [CrossRef]