

Hair Growth Regulation by an Aromatic Plant Extract

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Abstract Recently, people are experiencing more stress and some have problems regarding hair growth as a part of the aging process. Resolving this hair growth problem is important for the aging society in the future. This is because not only bodily health but also mental health is important for a healthy life. Thus, we attempted to find plants having hair growth regulation activity.

The Republic of Tunisia is located in North Africa; its northern boundary faces the Mediterranean Sea while its southern side leads to the Sahara Desert. The distance during the Mediterranean Sea and the Sahara Desert is only few kilometers. This environment can be considered that dry inclination is very high. In such environment, plants can accumulate antistress factors in their system. Thus, we collected many plant extracts from Tunisia for bioprospecting purposes. We show that a Tunisian aromatic plant extract has a high activity for promotion of hair growth cycle, or induction of anagen phase from telogen phase.

Keywords Hair growth cycle • dermal papilla • MTT assay • vasodilatation

1 Introduction

The hair growth cycle has three main stages, anagen, catagen and telogen. Anagen is the growth phase of the hair cycle. In this phase, hair follicles grow deep in the dermis, and hairs are made from extracellular matrix cells as melanins are introduced into hair shafts. Anagen hair follicles are long and big, while catagen is the regression

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phase of the hair cycle. In this phase, hair follicles regress to the shallow portion of the dermis from the epidermis. Telogen is the resting phase of the hair cycle. In this phase, hair follicles do not have any activity, and hair shafts fall out. On the other hand, the falling out of hair shafts is called exogen. These three stages are cycling and hair molt occurs continuously. Normally in humans, anagen continues for 3–4 years in male hair follicles and 4–6 years in female hair follicles [1, 2].

In the hair growth cycle, the most important element of hair follicles and skin is the dermal papilla. Dermal papilla cells are found at the bottom of each hair follicle. Although most hair follicle cell lines are differentiated from the ectoderm, the dermal papilla is differentiated from the mesoderm. The most important role of the dermal papilla is the regulation of hair growth. Signals to regulate hair growth from the dermal papilla stimulate hair follicle cells (such as outer root sheath cells), and then hair growth or hair regression is promoted [3].

The most useful and rapid method for evaluating hair growth promotion is by determining the dermal papilla growth rate as influenced by various samples [4]. After that, more detailed studies should be performed such as in vivo assay [3–11].

We collected many aromatic plants from Tunisia, which is located in North Africa. The northern side of Tunisia faces the Mediterranean Sea while the south leads to the Sahara. From the Mediterranean Sea to the Sahara, the distance is only a few hundred kilometers. This denotes that the dryness gradient is high, and animals and plants living in this region harbor many physiologically active compounds in their body. For the maintenance of skin homeostasis, some physiologically active compounds are useful. Thus, we tried to screen Tunisian aromatic plant extracts for compounds having hair growth regulation activity.

Therefore we also introduce various methods for hair growth research, such as how to search for samples that effectively promote hair growth, and how to investigate the effect of samples in vivo [12].

2 Materials and Methods

2.1 Cells and Cell Culture

Human follicular dermal papilla cells (HFDPCs) were purchased from TOYOBO (Tokyo, Japan) as primary cells and grown in HFDPC growth medium (TOYOBO).

2.2 Sample Extract

Tunisian plants were collected by a Tunisian researcher and extracted in 70% EtOH. Ten grams of dried plant was immersed in 100 ml of 70% EtOH for 1 to 2 weeks. The extract was then filtered to remove plant parts and to sterilize the extract.

2.3 *MTT Assay*

HFDPCs were used for the 1st screening of Tunisian samples with hair growth promotion ability. Subcultured HFDPCs were used within two passages for the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay and cell growth is evaluated. HFDPCs were trypsinized and plated at a density of 1×10^4 cells per well in 1.0 ml of HFDPC growth medium in collagen type I-coated 24-well plate (SUMILON, Tokyo, Japan). After overnight culture, the medium was changed to starvation medium (Dulbecco's Modified Eagle's Medium (DMEM) containing 0.5% charcoal-absorbed fetal bovine serum). After 96 h, the cells were stimulated with 50 μ l of Tunisian aromatic plant samples (70% EtOH extract) at various concentrations for 8 h, after which 50 μ l of 5 mg/ml MTT (Dojindo, Tokyo, Japan) was then added to the medium. After 18 h, the cells were washed with PBS, followed by the addition of 0.5 ml of PBS, and then 0.5 ml of 10% SDS was added per well. The SDS was allowed to dissolve the formazan for at least 24 h, and then the absorbance at 570 nm was determined using a plate reader [4, 7]. After the measurement, Tunisian samples that show potential for hair growth regulation were selected for further experiments.

2.4 *Cell Cycle Assay*

Cultured HFDPCs were subcultured at a density of 4×10^5 cells in collagen type I-coated 100 mm dish (SUMILON, Tokyo, Japan). After overnight culture, medium was changed to starvation medium. After 48 h, the cells were stimulated with Tunisian aromatic plant samples (1/300 dilution) for 12, 24, 48 and 96 h. The treated cells were harvested by trypsinization and washed with PBS, and then fixed in ice-cold 75% EtOH (approximately 1 ml of 75% EtOH for every 1×10^6 cells) at 4 °C for more than 12 h. The fixed cells were stained with Guava Cell Cycle Reagent (GE Healthcare Bio-Science Corp., USA) and the cell cycle kinetics was then determined using the Guava PCA according to the manufacturer's instructions.

2.5 *Hair Growth Promotion Assay In Vivo*

To evaluate hair growth activity, Tunisian aromatic plant extract should be applied to telogen skin. For this assay, 16 seven-week-old male C3H/He mice were obtained and maintained on a standard laboratory diet and water *ad libitum*. After conditioning for 1 week, all mice were anesthetized with an intraperitoneal injection of pentobarbital and dorsal hair shafts were trimmed to maintain the telogen phase. Tunisian aromatic plant extract or PBS (for negative control) was then injected subcutaneously into the test area ($n = 5$ in each group). Until results were observed, the mice were maintained normally; they were then sacrificed by CO₂ inhalation and photographed

to detect skin colorization by anagen induction. Their skin was then isolated and the reverse side was photographed to evaluate anagen induction.

3 Results and Discussion

3.1 *Effect of Tunisian Aromatic Plant Extract on Human Dermal Papilla Cell Growth*

For the 1st screening, the potential of Tunisian aromatic plants to promote the growth of HFDPCs was evaluated by the MTT assay. We evaluated seven extracts using HFDPCs, and found that only one plant has the ability to promote the growth of HFDPCs. During treatment, cell shape was not changed and cytotoxicity was not detected. This result suggested that one Tunisian aromatic plant has for the potential to promote hair growth (Fig. 1).

3.2 *Effect of Tunisian Aromatic Plant Extract on HFDPCs Cell Cycle*

After the 1st screening, we examined the effect of the Tunisian aromatic plant extract on HFDPCs cell cycle. HFDPCs were treated with the Tunisian aromatic plant extract for 12, 24, 36 and 48 h. While the 24, 36 and 48 h treatments did not show any effect, the 12 h treatment of HFDPCs seemed to stimulate the cell cycle. The G_0/G_1 phase was decreased while the G_2/M phase was increased by the 12 h treatment with the Tunisian aromatic plant extract. This denotes that the Tunisian

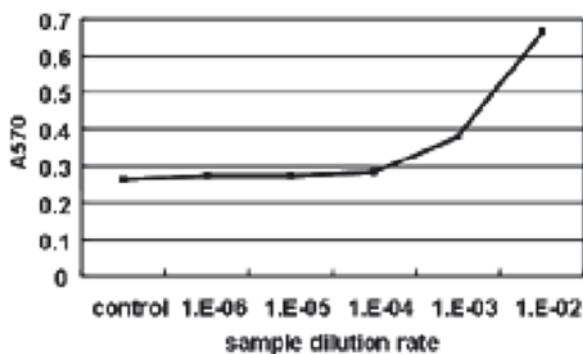


Fig. 1 Growth promotion activity of Tunisian aromatic plant on HFDPCs. Cell growth was stimulated by treatment with Tunisian aromatic plant extract in a concentration-dependent manner

Table 1 Human dermal papilla cell cycle promotion activity of the Tunisian aromatic plant extract. HFDPCs were treated with the Tunisian aromatic plant extract (1/300 dilution) for 12 h. Dermal papilla cell cycle was stimulated and the number of cells at the G₂/M phase was increased

	G ₀ /G ₁	S	G ₂ /M
Control	72.6%	4.9%	8.2%
Tunisian aromatic plant	68.5%	4.8%	10.0%

aromatic plant extract has the ability to promote dermal papilla cell growth. This result suggested that some factors that can regulate the hair growth cycle via stimulation of dermal papilla cell growth might be included in the Tunisian aromatic plant extract (Table 1).

3.3 *Hair Growth Promotion Activity of Tunisian Aromatic Plant Extract*

From the cell growth promotion and cell cycle assays using HFDPCs, we confirmed that the Tunisian aromatic plant has the potential to promote the hair growth cycle via dermal papilla cell growth stimulation. We then tried to evaluate the hair growth regulation potential of the Tunisian aromatic plant extract on mouse dorsal skin. Male C3H/HeN mice were selected to eliminate the influence of sex hormones. Because 8-week-old mice are known to be in telogen phase at the same time, mice at this phase were used at the start of this experiment. These mice were anesthetized and their dorsal hair was trimmed to maintain the telogen-phase hair follicle on the back side of their skin even after treatment. After trimming, the Tunisian aromatic plant extract (1/300 dilution) or PBS (negative control) was injected subcutaneously into the test area (trimmed dorsal skin area). After injection, these mice were observed daily to determine whether their dorsal skin color changed to gray from white and whether hair growth was stimulated to the anagen phase from the telogen phase. Gray skin color was detected at 15 days after injection, and then hair growth on skin surface were detected at 3 weeks after injection. The graying skin color denotes that the next anagen phase was induced under the skin but hair on the skin surface was not detected yet. We checked the reverse side of the skin to determine the anagen hair follicle ratio. The reverse side of the dorsal skin of mice injected with the extract showed not only hair follicles at the next anagen stage but also at the next cycled telogen phase. This denotes that normal hair growth cycle was stimulated. Moreover, we observed vasodilation on skin near the anagen-stimulated area. If the anagen stage was stimulated directly by injection of the Tunisian aromatic plant extract, a gray colored skin should be detected 8 to 10 days after. However, in this case, gray colored skin was detected 3 weeks after injection. It suggested that the Tunisian aromatic plant extract stimulated the hair growth cycle indirectly. Because the growth and cell cycle of HFDPCs were promoted, we consider that the Tunisian

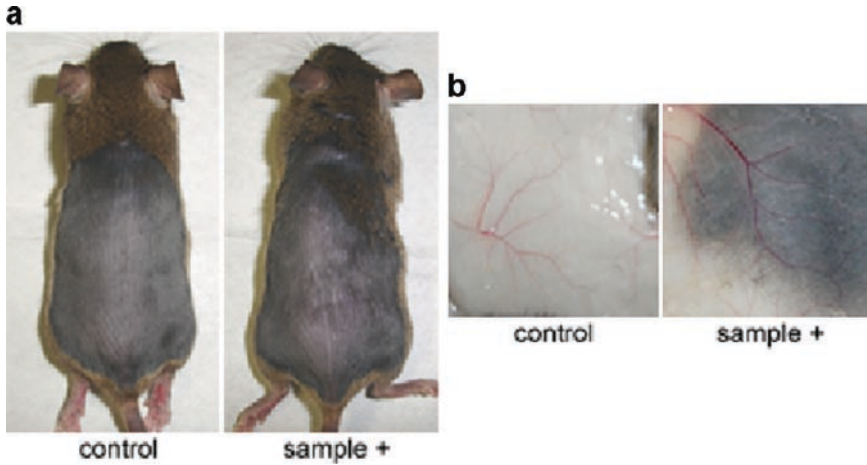


Fig. 2 Hair growth effect of the Tunisian aromatic plant extract. **(a)** Skin surface hair growth was detected on the dorsal skin of mice injected with the Tunisian aromatic plant extract. **(b)** On the reverse side of the skin, dark gray color was detected. This colored skin indicates the presence of anagen-phase hair follicles under the skin. In skin injected with sample, vasodilation was also detected. While control mouse skin showed no vasodilation, vascular thickness was increased in the sample-injected skin

plant extract initially stimulated dermal papilla cell growth, and then some signals were transferred to skin dermis components; vasodilation then occurred and hair growth was stimulated. The reason for the delayed stimulation of hair growth was the indirect stimulation of anagen phase from telogen phase (Fig. 2).

References

1. K. S. Stenn and R. Paus, Control of hair follicle cycling, *Physiol. Rev.*, vol. 81, 2001, pp. 449–494
2. R. Paus and G. Cotsarelis, The biology of hair follicles, *N. Engl. J. Med.*, vol. 341, 1999, pp. 491–497.
3. Y. Ota, Y. Saitoh, S. Suzuki, K. Ozawa, M. Mitsuko, and T. Imamura, Fibroblast growth factor 5 inhibits hair growth by blocking dermal papilla cell activation, *Biochem. Biophys. Res. Commun.*, vol. 290, 2002, pp. 169–176.
4. S.-S. Rho, S.-J. Park, S.-L. Hwang, M.-H. Lee, C. D. Kim, I.-H. Lee, S.-Y. Chang, and M.-J. Rang, The hair growth promoting effect of *Asiasari radix extract* and its molecular regulation, *J. Dermatol. Sci.*, vol. 38, 2005, pp. 89–97.
5. B. G. Howell, N. Solish, C. Lu, H. Watanabe, A. J. Mamelak, I. Freed, B. Wang, and D. N. Sauder, Microarray profiles of human basal cell carcinoma: insight into tumor growth and behavior, *J. Dermatol. Sci.*, vol. 39, 2005, pp. 39–51.
6. T. Midorikawa, T. Chikazawa, T. Yoshino, K. Takada, and S. Arase, Different gene expression profile observed in dermal papilla cells related to androgenic alopecia by DNA macroarray analysis, *J. Dermatol. Sci.*, vol. 36, 2004, pp. 25–32.

7. M. Kawano, A. Komi-Kuramochi, M. Asada, M. Suzuki, J. Oki, J. Jiang, and T. Imamura, Comprehensive analysis of FGF and FGFR expression in skin: FGF18 is highly expressed in hair follicles and capable of inducing anagen from telogen stage hair follicles, *J. Invest. Dermatol.*, vol. 124, 2005, pp. 877–885.
8. M. Kawano, S. Suzuki, M. Suzuki, J. Oki, and T. Imamura, Bulge- and basal layer- specific expression of fibroblast growth factor 13 (FHF-2) in mouse skin, *J. Invest. Dermatol.*, vol. 122, 2004, pp. 1084–1090.
9. K. J. McElwee, A. Huth, S. Kissling, and R. Hoffman, Macrophage-stimulating protein promotes hair growth ex vivo and induces anagen from telogen stage hair follicles in vivo, *J. Invest. Dermatol.*, vol. 123, 2004, pp. 34–40.
10. P. Komminoth, Digoxigenin as an alternative probe labeling for in situ hybridization, *Diag. Mol. Pathol.*, vol. 1, 1992, pp. 142–150.
11. C.-M. Huang, K. W. Foster, T. DeSilva, J. F. Zhang, Z. Shi, N. Yusuf, K. R. Van Kampen, C. A. Elmets, and D. C. Tang, Comparative proteomic profiling of murine skin, *J. Invest. Dermatol.*, vol. 121, 2003, pp. 51–64.
12. M. Kawano, T. Imamura, and H. Isoda, Methods for searching and evaluating effective hair growth regulation factors from Tunisian samples, *J. Arid Land Studies*, vol. 15–4, 2006, pp. 443–446.