Study on the effects of *Phyllanthus emblica* extract on hair follicle growth

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Introduction:

It is important to improve the scalp barrier function, when the scalp and hair become fragile and sensitive which could finally influence the health of hair. The hair follicles are embedded in the scalp. The dermal papilla cells are a group of dermal mesenchymal cells at the base of the hair follicle. Throughout the hair growth phase, the DPCs act as a signaling center for the epithelial–mesenchymal cross-talk that regulates the balance between matrix cell proliferation and hair production. DPCs as in vitro screening model to investigate hair growth, which may be helpful for evaluating the efficacy of hair care products[1].

Phyllanthus emblica is a plant for people's health and wellbeing which has been widely popularized. It has multiple bioactivities, such as treatment of dermatitis, eczema and other skin diseases[2]. As a candidate with great potential for use as an active ingredient in skin care products, *P. emblica* extract attenuated the inflammatory response to UVB irradiation by inhibiting AP-1, NF-κB[3]. The potent 5α-reductase inhibitor indicated *P. emblica* extract may be useful for hair loss prevention and treatment[4]. The objective of this research was to investigate the hair follicle growth effects of *P. emblica* extract.

Materials & Methods:

- **Preparation of *P. emblica* extract**

Dried *Phyllanthus emblica* fruit were extracted three times with deionized water at room temperature for 30 minutes. The combined solution were filtered and the filtrate was freeze-dried to afford a gummy extract.

- **Study on dermal papilla cells**

1) Cell culture

Dermal Papilla Cells (DPCs), purchased from Promocell, were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 100μL/mL of penicillin, 100 μg/mL of streptomycin,1μg/mL of amphotericin B and 10% fetal bovine serum (FBS, Gibco). The cells were incubated in a 5% CO_2 atmosphere at 37 °C.

2) Cell viability and ATP content assay

DPCs were cultured with or without sample in 96-well plates and exposed to different concentrations of *P. emblica* extract. The viability of non-treated and treated cells was measured and compared by Cell Counting Kit-8(CCK-8) assays, by which the cytotoxicity of *P. emblica* extract could also be evaluated. The bioenergetics effect was evaluated by measuring cellular ATP content using CellTiter-Glo® Reagent. The assay procedures followed the protocol provided by the manufacturer.

3) DPCs spheroid culture for related gene expression

DPCs were trypsinized and grown in 3D dermal spheroid structures. Hanging drop method for spheroid formation[5]. Hanging drops consisted of 10 000 cells in 20μL of medium with or without sample. Cultures were maintained for 72h after which time spheres were collected for RT-PCR.

- **Study on in vitro human hair follicles**

In vitro human hair follicles isolated from human scalp tissue with hair follicles, were cultured in William'E medium[6]. In vitro human hair follicles were divided into the non-treated and treated group contained of 10 hair with different concentrations of *P. emblica* extract. The human hair follicles were incubated in a 5% CO_2 atmosphere at 37°C for 7 days. The growth rate was examined to acquire its effects on hair follicle growth.

hair follicle growth rate(%) = (hair follicle length after / hair follicle length before – 1) ×100%

- **Date statistics**

For all data, the statistical significance was assessed by the t-test. In the statistical results, "*" referred to the P <0.05 compared with the control group, "#" referred to the P <0.01.

Results & Discussion:

1.The effect of *P. emblica* extract on DPCs cell viability and the ATP content

The results suggested that suitable concentration of *P. emblica* extract is supposed to facilitate hair follicle growth through promoting cell proliferation. The ATP level in DPCs was significantly increased at the concentration from 0.005% to 0.01%(P<0.01), which indicate *P. emblica* extract may enhance DPCs cellular energy.

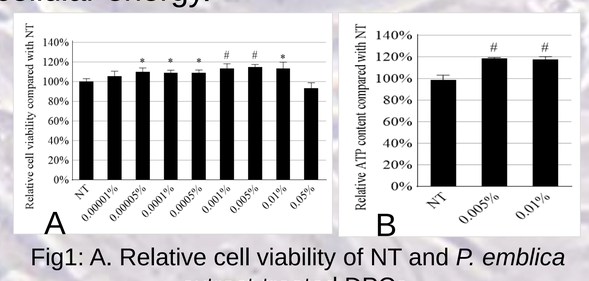

Fig1: A. Relative cell viability of NT and *P. emblica* extract-treated DPCs.
B. Relative ATP content of NT and *P. emblica* extract-treated DPCs.

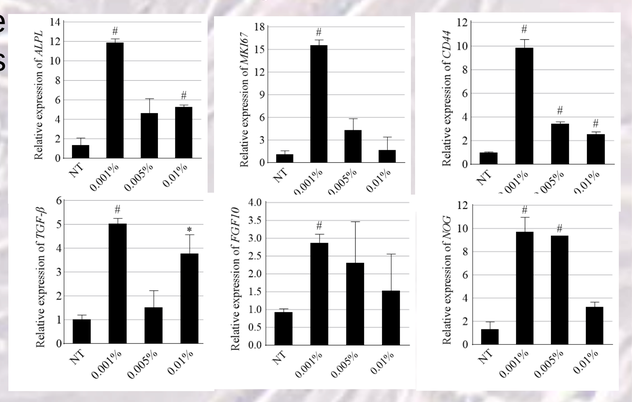

Fig 2: Relative gene expression of NT and P. emblica extract-treated DPCs spheroid

2.The effects of *P. emblica* extract on DPCs spheroids related gene expression

The related gene relative expression level was significantly increased at the concentration from 0.001% to 0.01%. As the characteristic gene of DPCs, ALPL gene of *P. emblica* extract-treated DPCs spheroids relative expression was significantly higher than NT, indicating that the concentration *P. emblica* extract was better to maintain the cellular properties. MKI67 and CD44 genes related to cell proliferation and migration and TGF-β, FGF10, NOG genes related to hair induction both resulted in significant improvement. Their direct effects on *P. emblica* extract-treated DPCs spheroids indicated 0.01% of *P. emblica* extract could maintain the DPCs cellular properties, promote the proliferation and enhance its ability of hair induction.

3.The effects of P. emblica extract on ex vivo human hair follicles growth

The results showed that 0.005% and 0.05% *P. emblica* extract could significantly promote the growth of *ex vivo* human hair follicles.

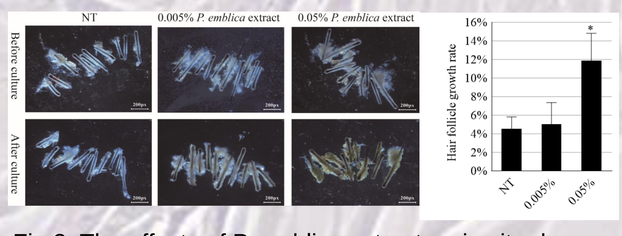

Fig 3: The effects of P. emblica extract on in vitro human hair follicles growth

Conclusions:

The DPCs viability, cellular energy and the expression of genes related to cell growth and proliferation and hair follicle induction were significantly different in the *P. emblica* extract treatment group compared with the control group. *Ex vivo* human hair follicle culture also indicated that *P. emblica* extract had a tendency to promote the growth of hair follicles. In conclusion, the appropriate concentration of the *P. emblica* extract can promote the hair follicle growth by promoting the proliferation of DPCs, increasing the ATP content and improving the follicle-induced ability. We believe that *P. emblica* extract may have the potential to prevent alopecia and protect hair. This discovery may lead to the development of new alternative medicines for loss prevention and treatment.

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