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## **Emblica (*Phyllanthus emblica* Linn.) Fruit Extract Promotes Proliferation in Dermal Papilla Cells of Human Hair Follicle**

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### **ABSTRACT**

Emblica (*Phyllanthus emblica* Linn.) has been used to promote the growth of hair in traditional medicine, however less is known regarding its pharmacological activities in hair follicles. To investigate the effects of emblica fruit extracts in terms of hair growth stimulation, the proliferative effects of extract in HaCaT keratinocytes and Dermal Papilla (DP) cells of human hair follicles were determined by MTT assay and cell counts. The results show that emblica extract stimulated proliferation of DP cells in a concentration-dependent manner, whereas it showed minimal effect on keratinocytes, suggesting that the extract might promote hair growth by prolonging the anagen phase through the proliferative effect on DP cells.

**Key words:** Emblica, *Phyllanthus emblica* Linn., keratinocyte, dermal papilla cell, hair growth

### **INTRODUCTION**

Dermal Papilla (DP) cells, specialized mesenchymal cells, play an important part in hair cycle mediating hair growth and loss (Rogers and Hynd, 2001; Botchkarev and Kishimoto, 2003; Alonso and Fuchs, 2006). The DP cells not only provide nutrient supply but also produce a number of growth factors and cytokines which influence proliferations and functions of several follicular cells namely keratinocytes (Matsuzaki and Yoshizato, 1998; Stenn and Paus, 2001; Fujie *et al.*, 2001). Anagen is an active growth phase of hair follicle, thereby the length and strength of hair is depended on the duration of this phase. In addition, prolongation of anagen is clinically shown to maintain the hair follicle growth (Paus and Cotsarelis, 1999). Many studies have reported the positive correlation between DP cell number and follicle size during anagen (Ishino *et al.*, 1997; Elliott *et al.*, 1999). In particular, cultured DP cells are currently used for evaluation of potential hair growth stimulators in several studies (Rho *et al.*, 2005; Sung *et al.*, 2006).

*Phyllanthus emblica* Linn., commonly known as emblica or amla, is distributed over tropical and subtropical areas of China, India, Indonesia and Thailand. The emblica fruit extract represents several pharmacological activities, such as anti-oxidant, anti-tumor and anti-inflammation,

attributable to its high vitamin C and polyphenolic content (Scartezzini *et al.*, 2006; Liu *et al.*, 2008). Importantly, emblica fruits have been long used as a hair growth nourishment in traditional Tibetan and Ayurvedic medicine (Dweck and Mitchell, 2005). Recent *in vivo* studies suggested emblica as one of the herbs that acclaimed with hair growth promoting activity as it is composed in the herbal formulations that effectively enlarge size and prolong the anagen phase of hair follicles (Purwal *et al.*, 2008; Jadhav *et al.*, 2009). However, its biological effects on follicular cells are still largely unknown.

In the present study, we performed *in vitro* experiments using cultured human DP cells of healthy hair follicles and HaCaT keratinocytes to investigate the effect of emblica fruit extract.

## **MATERIALS AND METHODS**

**Plant materials:** Fresh emblica fruits were collected from Srakaew Province of Thailand, where the climate is tropical. The samples were picked at commercial harvest time (May 2007) and selected for the uniformity of shape and maturity.

**Extraction and sample preparation:** Fresh emblica fruits were cut into small pieces. The materials (1 kg) were macerated and agitated in distilled water (5 L) and then filtered. The filtrate was converted to powder form by spray drying. Yield of the extract was approximately 35%. The extract contained 308-320 mg g<sup>-1</sup> of total phenolics calculated as Gallic Acid Equivalent (GAE) using Folin-Ciocalteu's reaction. Various samples of emblica extract were prepared by dissolving the dry emblica extract powder in deionized water to the indicated concentrations (10-500 µg mL<sup>-1</sup>).

**Chemical reagents:** Vitamin C (L-ascorbic acid) was obtained from Ajax Finechem (Auckland, New Zealand). Ketoconazole was obtained from Crosschem International Company, Derb and Co. (Lugano, Switzerland). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sigma Chemical, Inc. (St. Louis, MO, USA).

**Cells and cell culture:** Human follicle dermal papilla cells (DP cells) were obtained from PromoCell (Heidelberg, Germany). Cells were cultured in DP cell growth medium (PromoCell) containing 100 units mL<sup>-1</sup> of penicillin and 100 µg mL<sup>-1</sup> of streptomycin (Gibco, Gaithersburg, MA, USA) in a 5% CO<sub>2</sub> environment at 37°C.

Human immortalized keratinocyte HaCaT cells were obtained from Cell Lines Service (Heidelberg, Germany) and maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, Gaithersburg, MA, USA) supplemented with 2 mmol L<sup>-1</sup> L-glutamine, 10% fetal bovine serum, 100 units mL<sup>-1</sup> of penicillin and 100 µg mL<sup>-1</sup> of streptomycin in a 5% CO<sub>2</sub> at 37°C.

**Cell proliferation:** Cell proliferation was determined by MTT colorimetric assay (Mosmann, 1983) and cell counts. For MTT assay, cells at a density of 5×10<sup>3</sup> cells per well (3 replicates per treatment) were seeded in a 96-well plate and incubated with emblica fruit extracts (0-500 µg mL<sup>-1</sup>) for 24 h. After that cells were incubated with 500 µg mL<sup>-1</sup> of MTT for 4 h at 37°C. The supernatant was then removed and dimethylsulfoxide (DMSO) was added to dissolve the formazan product. The intensity was spectrophotometrically measured at 550 nm using an ELISA reader (Anthros, Durham, NC, USA). All analyses were performed in three independent replicate cultures. The intensity readings were normalized to a non-treated control and represented in the terms of relative cell proliferation.

For cell counts, cells at a density of  $2 \times 10^4$  cells well<sup>-1</sup> were plated in 24-well plates. Cells were cultured in a control medium or emblica extract-containing medium. At each time point (0, 24 and 48 h), the number of DP cells was counted using hemocytometer under light microscope.

**Statistical analysis:** Statistical analysis is performed using the Student's t-test. A p-value of less than 0.05 would be considered as statistically significant.

## RESULTS

**Effects of emblica fruit extract on cell proliferation:** The cell growth promoting effect of emblica fruit extract was investigated in immortalized keratinocytes (HaCaT) and primary human hair DP cells using MTT assay. Cells were incubated with various concentrations ( $10$ - $500 \mu\text{g mL}^{-1}$ ) of emblica extract and cell proliferation was determined after 24 h. The results indicated that emblica extract caused a dose-dependent increase in DP cell proliferation ( $1.11 \pm 0.18$ -fold,  $1.19 \pm 0.19$ -fold,  $1.88 \pm 0.05$ -fold,  $2.31 \pm 0.11$ -fold and  $2.67 \pm 0.39$ -fold at  $10$ ,  $50$ ,  $100$ ,  $250$  and  $500 \mu\text{g mL}^{-1}$  of extract, respectively), whereas, it had minimal proliferative effect on HaCaT keratinocytes (Fig. 1a). At various concentrations ranging from  $100$ - $500 \mu\text{g mL}^{-1}$ , the extract significantly increased DP cell growth with the maximal effect by approximately 3-fold compared to the non-treated control. Notably, at the treatment dose of  $500 \mu\text{g mL}^{-1}$ , the proliferative effect of emblica extract was only slightly increased compared to that of  $250 \mu\text{g mL}^{-1}$ .

To confirm the proliferative effect of emblica extract on DP cells, DP cell numbers were counted after cultivation in emblica extract ( $100 \mu\text{g mL}^{-1}$ )-containing medium at various times. Consistent with the MTT assay, the number of DP cells markedly increased in the emblica-treated group over a non-treated control (Fig. 1b).

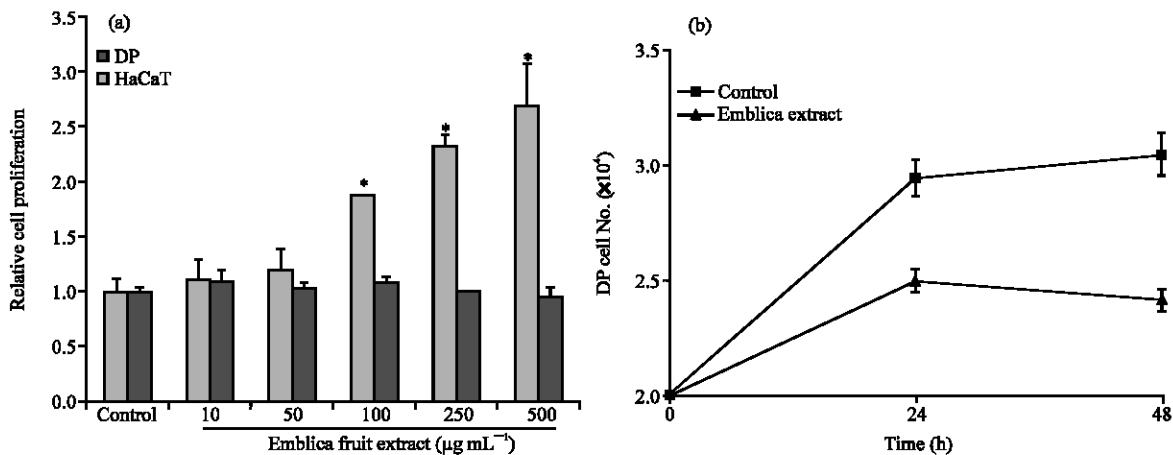


Fig. 1: Emblica fruit extract increases DP cell proliferation. (a) HaCaT and DP cells were treated with various concentrations of emblica extract ( $10$ - $500 \mu\text{g mL}^{-1}$ ) for 24 h and cell proliferation was determined by MTT assay and (b) DP cells were cultured in emblica extract ( $100 \mu\text{g mL}^{-1}$ )-containing medium. Cell numbers were counted using hemocytometer at 24 and 48 h. Values are mean of triplicate samples  $\pm$  SD. \* $p < 0.05$  versus non-treated control

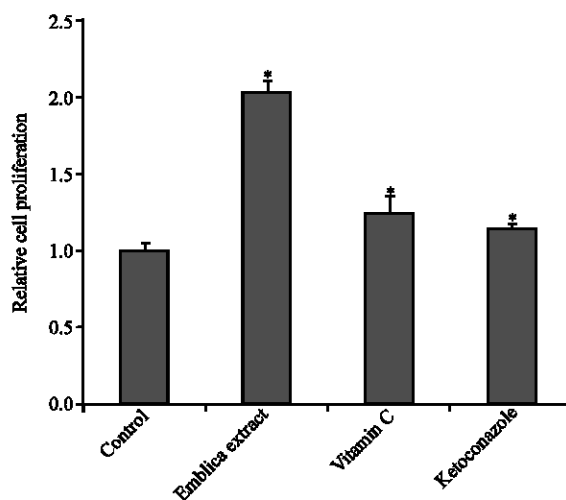


Fig. 2: Proliferative effects of emblica extract, vitamin C and ketoconazole on DP cells. Cells were treated with emblica extract ( $100 \mu\text{g mL}^{-1}$ ), vitamin C ( $50 \mu\text{g mL}^{-1}$ ) or ketoconazole ( $50 \mu\text{g mL}^{-1}$ ) for 24 h and cell proliferation was determined by MTT assay. Values are mean of triplicate samples  $\pm$ SD. \* $p < 0.05$  versus non-treated control

#### **Proliferative effects of emblica fruit extract and hair growth stimulators on DP cells:**

Previous study has shown that vitamin C derivative can induce hair growth *in vivo* and *in vitro*. This action of vitamin C derivative was shown to be caused by its ability to induce DP cell growth, while having a minimal effect on other follicular cells (Sung *et al.*, 2006). Moreover, clinical evidence indicated that anti-fungal ketoconazole possesses an ability to promote hair growth (Pierard-Franchimont *et al.*, 1998; Ellis and Sinclair, 2008). Therefore, we compared the proliferative effect of emblica extract with vitamin C and ketoconazole.

Figure 2 shows that emblica extract, vitamin C (L-ascorbic acid) and ketoconazole exhibited significant proliferative effects on DP cells. The proliferative effect of emblica extract at its minimal effective concentration ( $100 \mu\text{g mL}^{-1}$ ) was stronger than those of vitamin C and ketoconazole at the concentration of  $50 \mu\text{g mL}^{-1}$  ( $2.03 \pm 0.21$ -fold vs.  $1.25 \pm 0.10$ -fold and  $1.15 \pm 0.09$ -fold, respectively) (Fig. 2). Notably, higher concentrations ( $>50 \mu\text{g mL}^{-1}$ ) of vitamin C and ketoconazole could not be used in the present study due to their cytotoxic effect.

#### **DISCUSSION**

Emblica extract has been used for centuries in traditional medicines to stimulate hair growth (Dweck and Mitchell, 2005). However, the mechanisms underlying its activity are largely unknown. We document for the first time that emblica fruit extract stimulated the growth of cultured DP cells. The increase in DP cell growth could be extrapolated to the hair growth stimulating potential since: (1) DP cells secrete several cytokines which are required for follicular cell growth and functions; (2) an increase of DP cell number or activity is tightly related to enlarged hair follicle and prolonged anagen phase and (3) a reduction of DP cell number caused by several stimuli contributed to hair loss (Ishino *et al.*, 1997; Elliott *et al.*, 1999; Hoffmann, 2002; Rho *et al.*, 2005; Krause and Foitzik, 2006). Thus, the results of this study provide evidence supporting emblica fruit extract as a good candidate to be developed for the treatment of hair loss. Long-term use of available synthetic drugs, minoxidil and finasteride, is limited by its side effects. In this

regard, biologically active herbal extracts such as emblica fruit extract along with the suitable hair follicle-targeted delivery system are currently in demand in the industry. Since, it is widely known that emblica extract contains high content of vitamin C and polyphenolic compounds (Scartezzini *et al.*, 2006; Liu *et al.*, 2008) and vitamin C derivative has been reported to stimulate DP cell growth *in vitro* (Sung *et al.*, 2006), vitamin C may at least in part contribute to an enhancing effect of emblica extract on DP cell proliferation. However, our comparative study on DP cell proliferation revealed that emblica extract, at the minimal effective dose, had a higher proliferative effect compared to vitamin C, suggesting that the effect of emblica extract might not be attributed to vitamin C in the form of ascorbic acid but to its derivatives contained in emblica extract and/or other components such as polyphenols. In addition, we first reported the direct enhancing effect of ketoconazole on DP cell proliferation; however, this effect of ketoconazole seemed weak when compared to the effect of emblica extract.

In summary, the present study shows the strong proliferative effect of emblica extract on DP cells, which may prolong the anagen phase of hair cycle. The pharmacologic activities particularly its effects on DP cell-derived growth factors may be further investigated.

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#### **REFERENCES**

- Alonso, L. and E. Fuchs, 2006. The hair cycle. *J Cell Sci.*, 119: 391-393.
- Botchkarev, V.A. and J. Kishimoto, 2003. Molecular control of epithelial-mesenchymal interactions during hair follicle cycling. *J. Investigative Dermatol. Symp. Proc.*, 8: 46-55.
- Dweck, A.C. and D. Mitchell, 2005. A review of amla (*Emblica officinalis*). *Personal Care Magazine*, 5: 39-45.
- Elliott, K., T.J. Stephenson and A.G. Messenger, 1999. Differences in hair follicle dermal papilla volume are due to extracellular matrix volume and cell number: Implications for the control of hair follicle size and androgen responses. *J. Investigative Dermatol.*, 113: 873-877.
- Ellis, J.A. and R.D. Sinclair, 2008. Male pattern baldness: Current treatments, future prospects. *Drug Discovery Today*, 13: 791-797.
- Fujie, T., S. Katoh, H. Oura, Y. Urano and S. Arase, 2001. The chemotactic effect of dermal papilla cell-derived factor on outer root sheath cells. *J. Dermatol. Sci.*, 25: 206-212.
- Hoffmann, R., 2002. Male androgenetic alopecia. *Clin. Exp. Dermatol.*, 27: 373-382.
- Ishino, A., M. Uzuka, Y. Tsuji, J. Nakanishi, N. Hanzawa and S. Imamura, 1997. Progressive decrease in hair diameter in Japanese with male pattern baldness. *J. Dermatol.*, 24: 758-764.
- Jadhav V.M., R.M. Thorat, V.J. Kadam and S.B. Gholve, 2009. Kesharaja: Hair vitalizing herbs. *Int. J. Pharm. Tech. Res.*, 1: 454-467.
- Krause, K. and K. Foitzik, 2006. Biology of the hair follicle: The basics. *Seminar Cutaneous Med. Surgery*, 25: 2-10.
- Liu, X., C. Cui, M. Zhao, J. Wang, W. Luo, B. Yang and Y. Jiang, 2008. Identification of phenolics in the fruit of emblica (*Phyllanthus emblica* L.) and their antioxidant activities. *Food Chem.*, 109: 909-915.

- Matsuzaki, T. and K. Yoshizato, 1998. Role of hair papilla cells on induction and regeneration, processes of hair follicles. *Wound Repair Regeneration*, 6: 524-530.
- Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival application to proliferation and cytotoxicity assays. *J. Immunol. Method*, 65: 55-63.
- Paus, R. and G. Cotsarelis, 1999. The biology of hair follicles. *N. Engl. J. Med.*, 341: 491-497.
- Pierard-Franchimont, C., P. De Doncker, G. Cauwenberg and G.E. Pierard, 1998. Ketoconazole shampoo: Effect of long-term use in androgenic alopecia. *Dermatology*, 196: 474-477.
- Purwal, L., S. Prakash, B.N. Gupta and M.S. Pande, 2008. Development and evaluation of herbal formulations for hair growth. *E-J. Chem.*, 5: 34-38.
- Rho, S.S., S.J. Park, S.L. Hwang, M.H. Lee and C.D. Kim *et al.*, 2005. The hair growth promoting effect of *Asiasari radix* extract and its molecular regulation. *J. Dermatol. Sci.*, 38: 89-97.
- Rogers, G.E. and P.I. Hynd, 2001. Animal models and culture methods in the study of hair growth. *Clin. Dermatol.*, 19: 105-119.
- Scartezzini, P., F. Antogni, M.A. Raggi, F. Poli and C. Sabbioni, 2006. Vitamin C content and antioxidant activity of the fruit and of the ayurvedic preparation of *Emblica officinalis* Gaertn. *J. Ethnopharmacol.*, 104: 113-118.
- Stenn, K.S. and R. Paus, 2001. Control of hair follicle cycling. *Physiol. Rev.*, 81: 449-494.
- Sung, Y.K., S.Y. Hwang, S.Y. Cha, S.R. Kim and S.Y. Park *et al.*, 2006. The hair growth promoting effect of ascorbic acid 2-phosphate, a long-acting vitamin C derivative. *J. Dermatol. Sci.*, 41: 150-152.