



Eclipta alba extract with potential for hair growth promoting activity

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ABSTRACT

Ethnopharmacological relevance: *Eclipta alba* is traditionally known to potentiate hair growth promotion. **Aim of the study:** The study was aimed to investigate the efficacy of methanol extract of *Eclipta alba* as hair growth promoter.

Materials and methods: Pigmented C57/BL6 mice, preselected for their telogen phase of hair growth were used. In these species, the truncal epidermis lacks melanin-producing melanocytes and melanin production is strictly coupled to anagen phase of hair growth. The extract was applied topically to assess telogen to anagen transition. Immunohistochemical investigation was performed to analyze antigen specificity. Animals in anagen phase of hair growth were positive for FGF-7 and Shh and negative for BMP4, whereas the animals in telogen phase were positive only for BMP4 antigen.

Results: The methanol extract of whole plant when tested for hair growth promoting potential, exhibited dose dependent activity in C57BL6 mice. The activity was assessed by studying the melanogenesis in resected skin, follicle count in the subcutis, skin thickness and surrogate markers in vehicle control and extract treated animals.

Conclusion: These findings suggest that methanol extract of *Eclipta alba* may have potential as a hair growth promoter.

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1. Introduction

Eclipta alba has been traditionally used to check hair loss and stimulate hair growth. *Eclipta alba* Hassk. (Bhringaraja, Fam: Compositae) is a small-branched annual herb with white flower heads inhabiting tropical and subtropical regions of the world. The extracted juice if taken internally and applied to the scalp blackens the hair (Chopra et al., 1955; Kritikar and Basu, 1975). *Eclipta alba* has been reported in various polyherbal formulation (Baishiyou, 1993; Cheol, 2004; Lee, 1995, 2001, 2004; Shin, 2006; Xiulan, 1997) for hair growth promotion. The reported hair growth promoting activities of *Eclipta alba* in traditional and published literature prompted us to explore this plant for potential hair growth promoters.

The study of hair follicles, a complete regenerating system characterized by phases of growth (anagen), regression (catagen), resting (telogen), shedding and then growth again, is the underlying theme in hair biology. Numerous studies on morphological changes in the follicle show that the hair bulb extends into the deep subcutis during the anagen III–VII and catagen I–II.

Various species of animals such as mice (Chase, 1954; Hattori and Ogawa, 1983), rats (Johnson and Ebling, 1964), sheep (Hynd et al., 1986), monkeys (Uno, 1991) have been used, and the mouse model is most widely reported for hair growth promotion studies due to availability of large data base and specific mutants such as nude, hairless, rhino, and severe combined immunodeficient mice (Sundberg and King, 1996). The periodic intervals of rodent hair cycles, particularly the duration of the anagen phase are much more consistent and less susceptible to iatrogenic influences (Mori and Uno, 1990). The disadvantages associated with the mouse model include a high follicle density and the fact that the rodent hair cycle progresses in a wave pattern that sweep posterior and dorsally (Ahmed et al., 1998), unlike the mosaic pattern seen in humans (Sundberg and King, 1996). Pigmented C57/BL6 mice are the most commonly used strain as their truncal pigmentation is entirely dependent on their follicular melanocytes. The truncal epidermis in this species lacks melanin-producing melanocytes and melanin production is strictly coupled to anagen phase of hair growth. The strict coupling of follicular melanogenesis and hair follicle cycling thus leads to characteristic changes in skin pigmentation during anagen development (Slominski and Paus, 1993; Slominski et al., 1991, 1994). The C57/BL6 model has been widely reported for evaluation of Cyclosporin A (Paus et al., 1989), Oligopeptide (Hirai and Takebe, 2002), Capsaicin (Paus et al., 1994), Pyrrolidine derivative (Steiner and Hamilton, 2002) and compound Tellurium (AS101) which is an immunomodulator (Sredni et al., 2004) for hair growth

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promoting activity. *In vitro* models like the hair follicle organ cultures have been limited in their use by the inherent difficulties in preparation, variability and viability (Prouty et al., 1996).

Among a variety of hormones, growth factors, and development-related molecules identified as being involved in normal hair follicle growth. The hedgehog (Hh) family of intercellular signaling proteins is intricately linked to the development and patterning of almost every major vertebrate organ system (Paladini et al., 2005). In the skin, Sonic hedgehog (Shh) expression appears to play a key role as an initiator of hair follicle growth during both fetal hair follicle development and the postnatal hair cycle (Bitgood and McMahon, 1995). Shh serves as a key regulator of follicular growth and cycling as it is able to induce the transition from the resting (telogen) to the growth stage (anagen) of the hair follicle cycle (Sato et al., 1999; Stenn and Paus, 2001). Conversely, antibodies that block the activity of Shh are able to prevent hair growth in adult mice (Wang et al., 2000).

Epithelial stem cells located in the bulge region of a hair follicle (HF) have the potential to give rise to hair follicle stem/progenitor cells that migrate down to regenerate HFs. Bone morphogenetic protein (BMP) signaling has been shown to regulate the HF cycle by inhibiting anagen induction (Zhang et al., 2006). In resting hair follicle BMP4 predominates over noggin in the epithelium and mesenchyme. The hair growth inducer Noggin increases Shh expression in hair follicle where as bone morphogenetic protein 4 (BMP4) down regulates Shh (Botchkarev et al., 2001). Among the FGF genes, only FGF7 is expressed in the hair follicle. FGF7 RNA is localized to the dermal papilla during anagen, but expression is downregulated by the late-anagen VI stage (Rosenquist and Martin, 1996), but it is down regulated during catagen and telogen (Kawano et al., 2005).

The present study enabled us to assess the potential of methanol extract of *Eclipta alba* for hair growth promotion activity. 62 day old C57/BL6 mice, which were in telogen phase of hair growth were selected. Methanol extract was formulated in 96.5% of propylene glycol and 3.5% of DMSO. The extract at a dose level of 3.2 mg/kg, 1.6 mg/kg or the vehicle was applied topically on dorsal back for 10 days using syringe plunger by applying 40 strokes. Skin specimens were taken from the dorsal back after euthanization.

From center of the treated skin 8 mm punch biopsies were excised and embedded in paraffin blocks to obtain both longitudinal and transverse sections. Follicles were counted manually in dermis and subcutis layer by a blinded observer at a fixed size. Skin thickness from epidermis to panniculus carnosus was measured using UTHSCSA image tool 300. Surrogate markers were analyzed by indirect immunohistochemistry for FGF7 and Shh antigens, which are expressed in anagen phase and BMP4 antigen, which is expressed in telogen phase of hair growth.

2. Materials and methods

2.1. Plant material

Dried whole plant of *Eclipta alba* was procured from Ayurvedic store of Dabur Research Foundation and the agrotechnologists of Research Foundation authenticated the sample. A voucher specimen has been preserved with the Ayurvedic Division of Dabur Research Foundation, India.

2.2. Extraction and fractionation

A quantity of 1000 g of the dried pulverized powder of *Eclipta alba* was extracted with 95% methanol using soxhlet. The methanol extract was filtered and concentrated under reduced pressure to provide a crude extract (100 g) Extract obtained in previous step was suspended in demineralized water and heated on water bath

at 60 °C to remove wax like matter. After filtration, water phase was partitioned with chloroform followed by ethyl acetate. Further HPTLC fingerprinting was performed for both methanol fraction using Linomet V spotter and scanned on TLC scanner-II of CAMAG with Cats 3.18. Silica gel 60 F₂₅₄ TLC plates (Merck) were used for HPTLC fingerprinting. The solvent system used was Chloroform: Ethanol: Water (7:2:0.4) and scanning was performed at 254 nm.

2.3. Phytochemical analysis

Phytochemical analysis assessed the presence of coumestans (Wagner et al., 1986; Yahara et al., 1997), triterpenoid glycosides (Singh and Bhargava, 1992), thiophene derivatives (Yahara et al., 1997), triterpenoid saponins (Zhao et al., 2001), flavanoids (Shieh and Tsai, 1985) and wedelolactone (Samiulla et al., 2003).

2.4. Animals

Healthy C57/BL6 mice were purchased from NIN Hyderabad (India) and fed with standard rat chow and water *ad libitum*. Animals were housed in polypropylene cages maintained under standard conditions of 12-h light/dark cycle, 23 ± 2 °C and 35–60% humidity. All the mice were kept in quarantine for one week prior to experimentation. All experiments were carried according to the guidelines laid by Institutional Animal Ethics Committee (IAEC) of Dabur Research Foundation, India.

2.5. Chemicals

Propylene glycol was obtained from Spectrochem Pvt. Ltd., India. Sodium Chloride injection used as control was obtained from Parth Parenteral Pvt. Ltd. India. Minoxidil was purchased from Dr. Reddy's Lab, Hyderabad, India. Methanol, ethanol, ethyl acetate and formaldehyde were obtained from Merck Germany. FGF-7 (sc-1365, Rabbit polyclonal antibody) and Shh (sc-9024, Goat polyclonal antibody) and developing Goat ABC staining system (sc-2023) were procured from Santa Cruz Biotechnology Inc. BMP-4 (5674-100, Goat polyclonal antibody) was obtained from BioVision USA. Wedelolactone used as one of the reference standards was procured from Merck, Germany.

2.6. Validation of studies with Minoxidil

The mice in all the groups were morphologically preselected for their Telogen phase (62 days) of hair growth cycle. Thirty-two animals in 4 randomized groups ($n=8$) were used for the study. The animals in group 1 served as sham control. Animals in group 2 received an equal volume of vehicle (propylene glycol 50%, alcohol 30% and water 20%) of Minoxidil. Group 3 and group 4 received 1% Minoxidil and 2% Minoxidil respectively. The drug/vehicle was applied topically on the dorsal back for 10 consecutive days using syringe plunger by applying 40 strokes. The animals were kept in isolation for half hour and then housed back to the respective cages. Animals were euthanized on 13th day of the study.

2.7. Experimental studies with methanol extract

Forty animals in 4 randomized groups ($n=10$) were used for the study. The mice in all the groups were morphologically preselected for their Telogen phase (62 days) of hair growth cycle. The animals in group 1 served as sham control. Animals in group 2 received an equal volume of vehicle (propylene glycol 96.5% and DMSO 3.5%) of the methanol extract of *Eclipta alba*. Animals in Group 3 and group 4 received 1.6 mg/15 cm² and 3.2 mg/15 cm² of methanol extract of *Eclipta alba* respectively. The extract or the vehicle was applied topically on dorsal back for 10 days using syringe plunger by applying 40

strokes. The animals were kept in isolation for half hour and were housed back to the respective cages. Skin specimens were taken from the dorsal back after euthanization.

2.8. Histopathological processing and histomorphometry

Dorsal skin measuring 15 cm² was excised between fore and hind leg and stretched out on flat glass surface and was maintained in 4% formalin. 8 mm punch biopsies were excised from center of the skin was bisected and embedded in paraffin blocks to obtain both longitudinal and transverse sections. 5 μM sections were cut and stained with haematoxylin and eosin. Digital photomicrographs were taken from representative areas at a fixed magnification of 100×. All the images were cropped in a fixed area of 700 pixels width. Follicles were counted manually in dermis and subcutis layer by a blinded observer at a fixed size. Skin thickness from epidermis to panniculus carnosus was measured (Johnson and Ebling, 1964) using UTHSCSA image tool 300.

2.9. Immunohistochemical studies

Indirect immunohistochemistry for FGF-7, BMP4 and Shh antigen involved inhibition of endogenous peroxidase using 1% hydrogen peroxide in methanol. Antigen retrieval was done in Citric acid buffer followed by addition of primary antibody and subsequently secondary antibody. The antigen-antibody reaction was demonstrated using diaminobenzadine as the substrate and the sections are then counterstained with Haematoxylin. The antigens were immunohistochemically analyzed in all the groups. Amongst various molecular mediators controlling the morphogenesis of hair, FGF7 and Shh antigens were expressed in anagen phase and BMP4 antigen was expressed in telogen phase of hair growth.

2.10. Parameters for measuring hair growth promoters

Researchers have variously assessed the parameters for evaluating hair growth promoters. We have focused on melanogenesis, follicle count, and skin thickness. Various phase specific antigens like FGF-7, Shh and BMP4 were also investigated for all the studies.

2.11. Statistical analysis

All values were expressed as mean ± S.E. ($n = 10$ in each group). Statistical analysis was performed using SAS 9.1.3. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Phytochemical analysis

Phytochemical analysis revealed that methanol extract contains coumestans, triterpenoid glycosides, thiophene derivatives, triterpenoid saponins, flavonoids and wedelolactone. The HPTLC fingerprint combined with phytochemical analysis strongly suggests that

the peaks observed correspond to coumestans, triterpenoid glycosides, thiophene derivatives, triterpenoid saponins, flavonoids and wedelolactone.

3.2. Validation of studies with Minoxidil

The model was validated with Minoxidil. The histopathological and morphometric data depicts that Minoxidil at both the concentrations 1% and 2% showed approximate efficacy of 40% over vehicle treated control groups (Table 1). The experiment was repeated four times with similar results.

3.3. Experimental studies with methanol extract

Increase in thickness and presence of the follicles in the subcutis layer were taken as evidence for transition of follicles from telogen to anagen phase of hair growth. The transition of telogen phase to Anagen phase of hair growth was observed in approximately 87.5% animals treated with 3.2 mg/15 cm² (Fig. 1a) of methanol extract of *Eclipta alba*. 50% of the animals treated with 1.6 mg/15 cm² (Fig. 1b) of methanol extract of *Eclipta alba* also showed transition from telogen phase to anagen phase of hair growth (Table 2). The animals treated with vehicle (Fig. 1c) showed no anagen induction. As no erythema, edema and scaling/drying of skin was observed at the site of application in any of the animal in the total experimental procedure. The preformulated extract at all dose levels was innocuous with zero irritation potential. In the representative longitudinal sections (Fig. 2) the anagen phase skin, which is associated with increase in follicle size, lies in deep subcutis when compared to telogen phase skin where follicle lies in the dermis only.

3.3.1. Immunohistochemical findings

Animals treated with 3.2 mg/15 cm² showing evidence of anagen induction also stained positive for FGF-7 (Fig. 3a, b), Shh (Fig. 3c, d) and negative for BMP4 (Fig. 3e, f). The vehicle treated animals in telogen phase stained positive for BMP4 antigen only. The experiment was repeated four times with similar results.

4. Discussion

There exists an unmet need for identification of novel hair growth promoters in light of the fact that there are only two drugs, topical Minoxidil and oral Finasteride approved by the Food and Drug Administration (FDA) for the treatment of alopecia.

Large randomized placebo controlled trials on humans conducted by Upjohn Company for Minoxidil, a potassium channel opener showed efficacy in 54% of the treated patients as opposed to 34% in placebo control group. There are significant adverse dermatological effects associated with minoxidil viz. pruritis, dryness, scaling, local irritation, dermatitis (De Villez, 1990).

Finasteride, an oral 5 alpha reductase inhibitor is known to increase hair growth in patients with male pattern baldness (Androgenetic alopecia). Large clinical trials conducted by Merck research laboratories (MRL clinical study report: 087-03, 089-03, 092, 1996)

Table 1
Effect of Minoxidil on follicle counts and skin thickness.

Treatment	Mean follicle count	Average skin thickness (μm)	% Anagen induction ^a	P value ^b	Mean follicle count in subcutis layer
Vehicle treatment	43 ± 8.4	177.2 ± 13.4	50	–	28.3 ± 8.8
1% Minoxidil	69 ± 3.9	160.2 ± 10	87.5	<0.05	50.8 ± 3.9
2% Minoxidil	73 ± 8.2	253.25 ± 6.6	87.5	<0.05	51 ± 7.9

±: SEM value.

^a H & E stained histopathological data depicts 87.5% anagen phase of hair growth in the groups treated with 1% and 2% Minoxidil, however 50% of the animals treated with vehicle also showed similar efficacy.

^b Statistical analysis was done using SAS version 9.1.3.

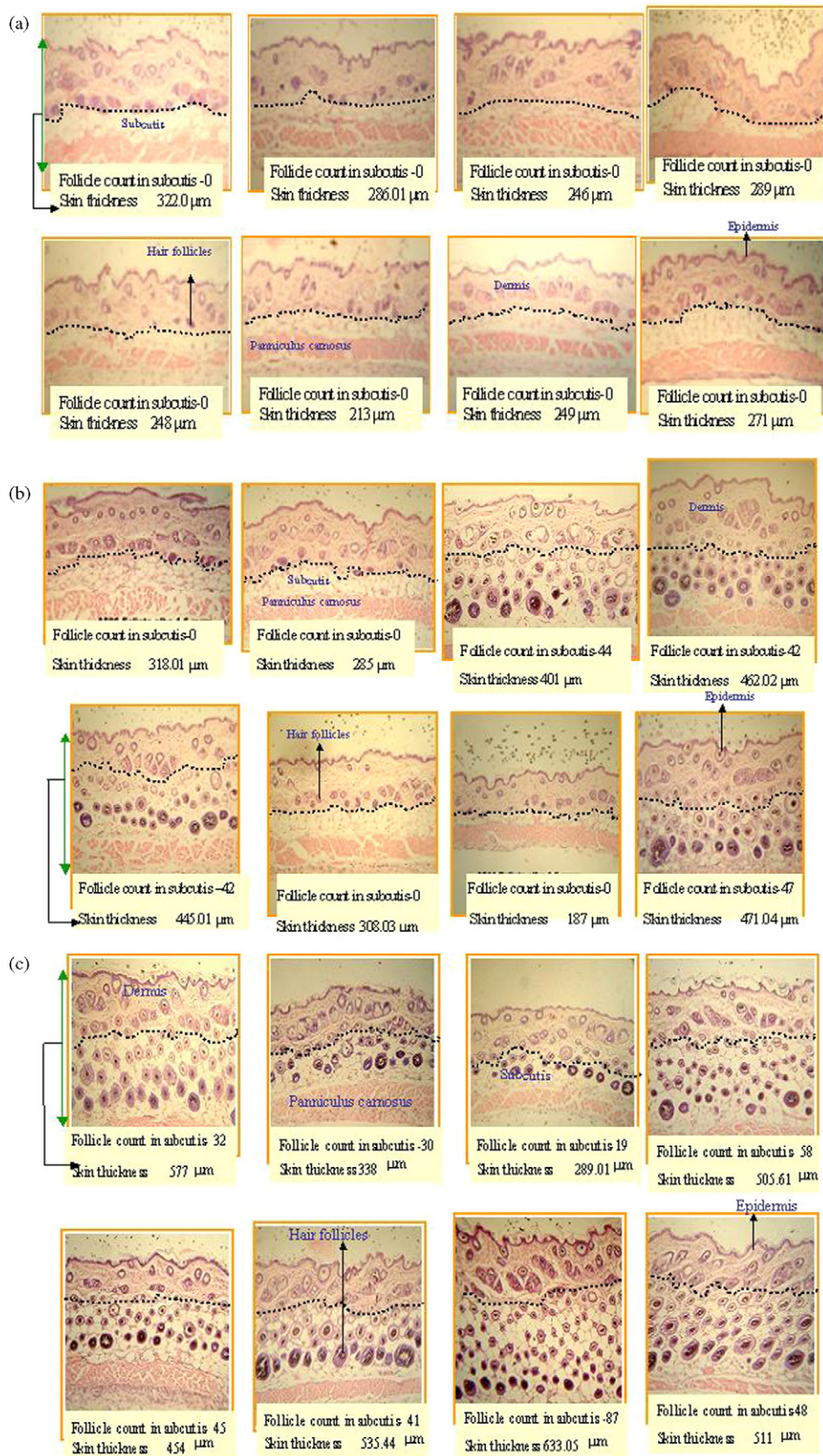


Fig. 1. (a) Effect of vehicle of methanol extract of *Eclipta alba* on morphologically selected telogen skin of C57/BL6 mice for hair growth promoting activity. Dotted line indicates junction of dermis and subcutis. (b) Effect of methanol extract of *Eclipta alba* at a concentration of 1.6 mg/15 cm² on morphologically selected telogen skin of C57/BL6 mice for hair growth promoting activity. Dotted line indicates junction of dermis and subcutis. (c) Effect of methanol extract of *Eclipta alba* at concentration of 3.2 mg/15 cm² on morphologically selected telogen skin of C57/BL6 mice for hair growth promoting activity. Dotted line indicates junction of dermis and subcutis.

Table 2
Effect of methanol extract of *Eclipta alba* plant extract on follicle count and skin thickness.

Treatment	Mean follicle count	Average skin thickness (μm)	% Anagen induction ^a	P value ^b	Mean follicle count in subcutis layer
Vehicle treatment	19.2 \pm 3	265.5 \pm 12	0	–	0.0
1.6 mg/15 cm ² of methanol extract	39 \pm 8.4	417.4 \pm 35.9	50	<0.0001	21 \pm 8.3
3.2 mg/15 cm ² of methanol extract	66 \pm 7.3	480.4 \pm 41.2	87.5	<0.0001	45 \pm 7.4

\pm : SEM value.

^a H & E stained histopathological data depicts 50% and 87.5% anagen phase of hair growth in the groups treated with 1.6 mg/15 cm² and 3.2 mg/15 cm².

^b Statistical analysis was done using SAS version 9.1.3.

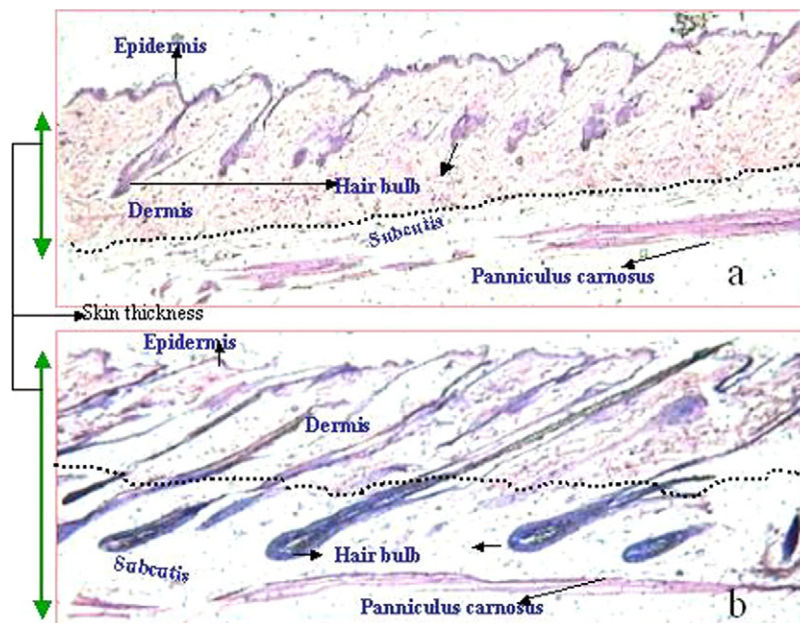


Fig. 2. Representative photomicrograph of C57/BL6 mice treated with vehicle (a) and methanol extract of *Eclipta alba* at concentration of 3.2 mg/15 cm². Dotted line indicates junction of dermis and subcutis. (a) Longitudinal sections of animals in telogen phase of hair growth (Vehicle treated). The hair bulb is shrunken and is present in the dermis above the subcutis layer. (b) Longitudinal sections of animals in anagen phase of hair growth (3.2 mg/15 cm² of methanol extract of *Eclipta alba*). The hair bulb is rigid and is present deep in the subcutis layer.

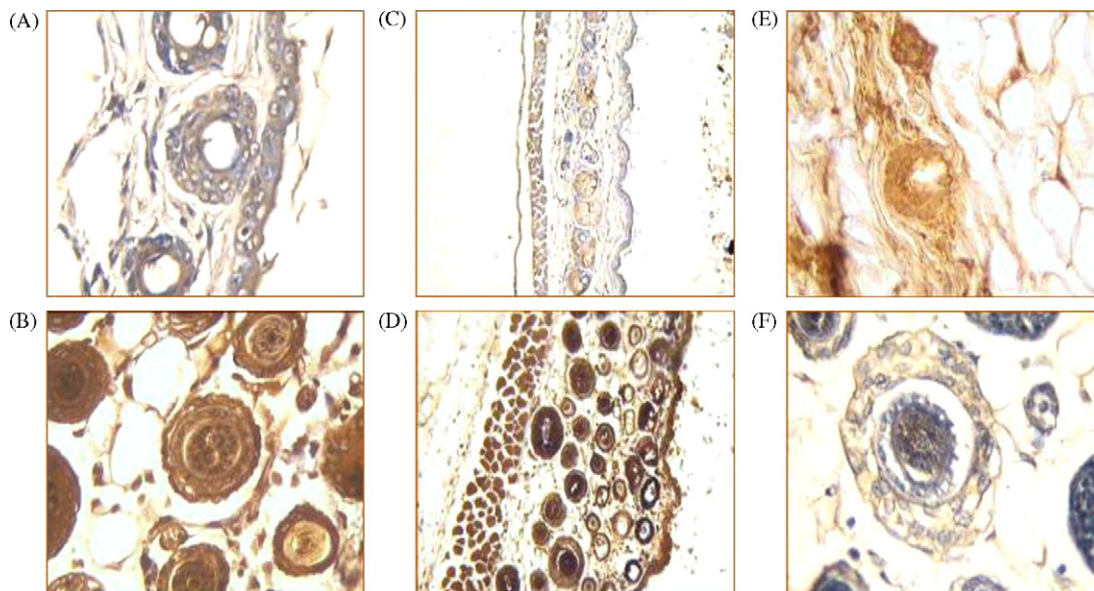


Fig. 3. Efficacy of methanol extract of *Eclipta alba* at 0.4 mg/4 cm² dose for hair growth promoting activity in C57/BL6 mice. Immunohistochemical investigation for antigen specificity: surrogate marker study for methanol extract of *Eclipta alba*. A: Vehicle treated group: Negative for FGF-7 (400 \times), B: Treated with 3.2 mg/15 cm² Methanol extract of *Eclipta alba*: Positive for FGF 7 (400 \times), C-Vehicle treated: Negative for Shh (100 \times), D: Treated with 3.2 mg/15 cm² Methanol extract of *Eclipta alba*: Positive for Shh (100 \times), E-Vehicle treated: Positive for BMP4 (400 \times), F: Treated with 3.2 mg/15 cm² Methanol extract of *Eclipta alba*: Negative for BMP4(400 \times).

report that 48% of hair regrowth is observed in finasteride recipients in one year. Patients receiving finasteride shows that it is generally well tolerated, but few patients withdrew the treatment due to drug related sexual disorders. Finasteride is not indicated for use in women (McClellan and Markham, 1999).

Few other drugs either in preclinical development or undergoing Clinical Trials are Dutasteride (Olsen et al., 2006), Spironolactone, small and large peptides like Lys-Pro-Val tripeptide (Mahe, 1998), glycyl-L-histidyl-L-lysine copper (II) and their derivatives (Pickart, 1993). Bexarotene, Capsaicin and hydroxychloroquine, Alefacept and Roxithomycin are in various phases of clinical trials (www.clinicaltrial.gov, 2007).

Not surprisingly, the demand for drugs that alter hair growth and appearance has led to a multibillion-dollar industry. In United States hair loss sufferers spend more than 3.5 billion dollars in a year for treating hair loss. The *American Hair Loss Association* recognizes that hair loss is an extremely emotionally distressing disease that makes afflicted patients vulnerable (The Washington post, 2006).

Based on the seminal work of Sven Muller-Rover et al. (2001) we investigated the follicular growth pattern through sequential histological studies of the back skin of Indian strain of C57/BL6 mice from approximately 23 days to 62 days of age. 62 day old animals were in stable telogen phase and thus selected for screening hair growth promoters. In hair growth promoters the transition of telogen phase to anagen phase was immunohistochemically complemented by positive staining for FGF-7 and Shh protein and negative staining for BMP4 antigen.

In both mice and human, hair cycle is regulated by interplay of stimulatory and inhibitory growth factors (Stenn and Paus, 2001). Various members of fibroblast growth factor family are expressed in the skin and are involved in the dynamics of dermal function including physiological processes as wound healing and hair growth. Expression of these FGF varies throughout hair growth cycle; FGF7 and FGF10 are expressed in dermal fibroblasts and papilla cells and stimulate proliferation of keratinocytes, which is characteristic of anagen phase of hair growth (Kawano et al., 2005). In resting hair follicles, BMP4 mRNA predominates over noggin in epithelium and mesenchyme. Anagen development is accompanied by down regulation of BMP4 and increased noggin mRNA in hair follicle (Botchkarev et al., 2001). Shh expression and target gene induction occurs only in the anagen phase of hair growth. At the start of catagen when apoptosis occurs expression of Shh ceases and its expression is undetectable in the quiescent telogen hairs (Oro and Higgins, 2003). Under physiological conditions, expression of FGF 7 and Shh is restricted to anagen phase of hair growth and BMP4 to telogen phase of hair growth.

Based on the previous study of cyclical follicular growth, mice were morphologically preselected for telogen phase of hair cycle. The mice were then used further to validate the model with 1% and 2% minoxidil. The microscopic data obtained from the validation study shows that the topical administration of minoxidil affects the normal cycle by inducing the resting follicles to anagen phase of hair growth in approximately 87% of the treated animals as opposed to 50% efficacy in vehicle treated animals. Our present data may reflect the data reported for large randomized placebo controlled trials on humans conducted by the Upjohn company for Androgenetic alopecia where investigators observed 54% of the minoxidil treated patients experienced new hair growth at week 32 as compared with 34% of the placebo treated patients (De Villez, 1990).

Finally methanol extract of *Eclipta alba* was screened in C57/BL6 mice, preselected morphologically for telogen phase characterized by completely pink skin with resting hair follicles. Results showed that the rate of anagen induction was influenced by the concentration of methanol extract of *Eclipta alba*, with 50% of the animals topically treated with 3.2 mg/15 cm² extract entering anagen phase after 7 application and 87.5% of the animals after 10 applications.

The microscopic data obtained tended to support the association of increasing skin thickness, follicle count and the macroscopic development of skin pigmentation with anagen induction. The findings from the above *in vivo* assays shows that the topical administration of 3.2 mg/15 cm² methanol extract of *Eclipta alba* affects the normal cycle by inducing the resting follicles to enter anagen phase of hair growth. The follicle counts and skin thickness data is complemented by our immunohistochemical analysis for FGF-7 and Shh, which may serve as surrogate markers for anagen Phase of hair growth. This is as opposed to BMP4, a marker for telogen phase of hair growth. Similarly the ethyl acetate enriched fraction containing two coumestans viz. wedelolactone and demethyl wedelolactone was screened for hair growth promoting activities.

Hair loss or hair thinning has always been a common disorder in clinical dermatology. Parameters like hair density, hair diameter, hair growth rate and anagen/telogen ratio are assessed using phototrichograph based techniques with digital image analysis such as Trichoscan.

In our present studies, analysis of hair growth promoters included melanogenesis, follicle count, and skin thickness. Various phase specific antigens like FGF-7, Shh and BMP4 are also investigated. In C57/BL6 mice melanin synthesis of follicular melanocytes is strictly coupled to the growth stage of hair cycle (anagen), cease during follicle regression (catagen), and is absent throughout the resting stage (telogen) (Slominski et al., 1994). Owing to the strict coupling of follicular melanogenesis and hair follicle cycling in anagen development C57/BL6 mice have been used widely as a model for screening hair growth promoters (Slominski and Paus, 1993; Slominski et al., 1991, 1994). The active phase of hair follicle cycling is also accompanied by increase in size and number of follicles resulting in increase in thickness of subcutis layer between dermis and panniculus carnosus. No erythema, edema and scaling/drying of skin were observed at the site of application of the extract in any animal in the total experimental procedure and are not known from traditional use.

Traditionally the plant is reported to blacken the hairs and it has also been reported as one of the constituents of hair oils. The present study was performed in C57/BL6 mice, which has naturally growing lustrous black hair, so the property of blackening the hairs could not be evaluated.

5. Conclusion

In conclusion, methanol extract of *Eclipta alba* definitely promotes hair growth by inducing anagen in telogen (resting) phase hair follicles. Animals treated with 3.2 mg/15 cm² of methanol extract of *Eclipta alba* showed better efficacy as compared to lower doses. Identification and isolation of molecules from the extract may provide new directions for the treatment of hair loss. Further elucidation of mechanism of action for the extract is currently under investigation in our laboratory.

In conclusion, methanol extract of *Eclipta alba* definitely induces anagen transformation in telogen (resting) phase hair follicles. This activity was dose related as animals treated with 3.2 mg/15 cm² of methanol extract of *Eclipta alba* showed better efficacy as compared to lower doses. The results suggest that this fraction of *Eclipta alba* has substantial potential of acting as a hair growth promoter.

The degree of anagen induction with methanol extract of *Eclipta alba* and minoxidil were comparable. On basis of the similarities observed between the minoxidil and *Eclipta alba* studies, it is expected that *Eclipta alba* will have similar hair growth promoting activity as shown by minoxidil in humans.

Identification and isolation of molecules from the extract may provide new directions for the treatment of hair loss. Further elucidation of mechanism of action for the extract is currently under investigation in our laboratory.

References

- Ahmed, W., Faiyaz, U., Haque, M., Brancolini, V., 1998. Alopecia universalis associated with a mutation in the human hairless gene. *Science* 279, 720–724.
- Baishiyou, Ri., 1993. Hair tonic. JP5201833.
- Bitgood, M.J., McMahon, A.P., 1995. Hedgehog and BMP genes are coexpressed at many diverse sites of cell–cell interaction in the mouse embryo. *Developmental Biology* 172, 126–138.
- Botchkarev, V.A., Botchkarev, N.V., Nakamura, M., Huber, O., Funa, K., Lauster, R., Paus, R., Gilchrist, B.A., 2001. Noggin is required for induction of the hair follicle growth phase in postnatal skin. *The FASEB Journal* 15, 2205–2214.
- Chase, H.B., 1954. Growth of hair. *Physiological Reviews* 34, 113–126.
- Cheol, G. Ryu, 2004. Hair-restorer and manufacturing process thereof. KR20040016331.
- Chopra, R.N., Nayar, S.L., Chopra, I.C., 1955. Glossary of Indian Medicinal plants C.S.I.R., New Delhi.
- De Villez, L. Richard, 1990. The therapeutic use of topical minoxidil. *Dermatologic Clinics* 8, 367–375.
- Hattori, M., Ogawa, H., 1983. Biochemical analysis of hair growth from the aspects of aging and enzyme activities. *Journal of Dermatology* 10, 45–54.
- Hirai Yohei, Takebe Kyoko, 2002. Method for screening a substance having promoting activity on hair growth. US 2002/0051760A1.
- Hynd, P.L., Schlink, A., Phillips, P.M., Scobie, D.R., 1986. Mitotic activity in cells of the wool follicle bulb. *The Australian Journal of Biological Sciences* 39, 329–339.
- Johnson, E., Ebling, F.J., 1964. The effect of plucking hairs during different phases of the follicular cycle. *Journal of Embryology & Experimental Morphology* 12, 465–474.
- Kawano, M., Kuramochi, K., Akiko, A.M., Suzuki, M., Oki, J., Jiang Ju, Imamura, T., 2005. Comprehensive analysis of FGF and FGFR expression in skin: FGF 18 is highly expressed in hair follicles and capable of inducing anagen from telogen stage hair follicles. *The Journal of Investigative Dermatology* 124, 877–885.
- Kritikar, K.R., Basu, B.D., 1975. *Chronica Botanica Indian Medicinal plants*: New Delhi.
- Lee in Geol, 2004. Kit for hair growth. KR20040039550.
- Lee Sang Kuk, Ha Byong-Jo, Mun Song-Jun, Kang Kil-Sung, 1995. Hair growth composition. KR950006061B.
- Lee Won Gyu, 2001. Agent for preventing hair loss and stimulating or promoting hair growth using skin of peach. KR20010044451.
- Mahe Yann, 1998. Modulating body/cranial hair growth with derivatives of the alpha-type melanocyte-stimulating hormone. US 5739111.
- McClellan, J. Karen, Markham, Anthony, 1999. Finasteride. A review of its use in male pattern hair loss. *Drugs* 57 (1), 111–126.
- Mori, Osamu, Uno, Hideo, 1990. The effect of topical minoxidil on hair follicular cycles of rats. *The Journal of Dermatology* 17, 276–281.
- MRL clinical study report, multicenter study: a double blind randomized placebo controlled, multicenter study to determined the effect of finasteride on hair loss in men with androgenetic alopecia. Merck Research Laboratories 1996: (protocol 087-03).
- MRL clinical study report, multicenter study: a double blind randomized placebo controlled, multicenter study to determined the effect of finasteride on hair loss in men with androgenetic alopecia. Merck Research Laboratories 1996: (protocol 089-03).
- MRL clinical study report, multicenter study: a double blind randomized placebo controlled, multicenter study to determined the effect of finasteride on hair loss in men with androgenetic alopecia. Merck Research Laboratories 1996: (protocol 092).
- Muller-Rover Sven, Handjiski Bori, Carina van der veen, Eichmuller Stefan, Foitzik Kerstin, McKay A. Ian, Stenn S. Kurt, Paus Ralf, 2001. A comprehensive guide for the accurate classification of Murine hair Follicle in distinct hair cycle stages. *The Journal of Investigative Dermatology* 117, 3–15.
- Olsen, E.A., Hordinsky, M., Whiting, D., Stough, D., Hobbs, S., Ellis, M.L., Wilson, T., Rittmaster, R.S., 2006. Importance of dual 5alpha-reductase inhibition in the treatment of male pattern hair loss: results of a randomized placebo-controlled study of dutasteride versus finasteride. *Journal of the American Academy of Dermatology* 55, 1014–1023.
- Oro, A.E., Higgins, K.M., 2003. Hair cycle regulation of hedgehog signal reception. *Developmental Biology* 255, 238–248.
- Paladini, R.D., Saleh, J., Qian, C., Guang-Xin Xu, Lee L. Rubin, 2005. Modulation of hair growth with small molecule agonists of the hedgehog signaling pathway. *The Journal of Investigative Dermatology* 125, 638–646.
- Paus Ralf, Stenn S. Kurt, Link E. Richard, 1989. The induction of anagen hair growth in Telogen mouse skin by Cyclosporin A administration. *Laboratory Investigation* 60(3), 365–369.
- Paus, R., Heinzlmann, T., Schultz, K.D., 1994. Hair growth induction by substance P. *Laboratory Investigation* 71, 134–140.
- Pickart R. Loren, 1993. Method for stimulating hair growth using GHL-CU complexes, US5177061.
- Prouty, S.M., Lawrence, L., Stenn, K.S., 1996. Fibroblast-dependent induction of a murine skin lesion with similarity to human common blue nevus. *American Journal of Pathology* 148, 1871–1885.
- Rosenquist, T.A., Martin, G.R., 1996. Fibroblast growth factor signalling in the hair growth cycle: expression of the fibroblast growth factor receptor and ligand genes in the murine hair follicle. *Developmental Dynamics* 205, 379–386.
- Samiulla, S., Mundkinajeddu, D., Shivanna, Y., Arun, C., Keerthi, M., Prashanth, D., Amit, A., Venkataraman, B.V., 2003. Trypsin inhibitory effect of wedelolactone and demethylwedelolactone. *Phytotherapy Research* 17, 420–421.
- Sato, N., Leopold, P.L., Crystal, R.G., 1999. Induction of the hair growth phase in postnatal mice by localized transient expression of Sonic hedgehog. *Journal of Clinical Investigation* 104, 855–864.
- Shieh, W.C., Tsai, J.L., 1985. Studies on flavonoids of the common angiosperms in Taiwan. *Guoli Zhongxing Daxue Ligong Xuebao* 22, 13–24.
- Shin Endo, 2006. Hair growing and beautifying agent. JP2006151934.
- Singh, P., Bhargava, S., 1992. A dithienylacetylene ester from *Eclipta erecta*. *Phytochemistry* 31, 2883–2884.
- Slominski, A., Paus, R., 1993. Melanogenesis is coupled to murine anagen: toward new concepts for the role of melanocytes and the regulation of melanogenesis in hair growth. *The Journal of Investigative Dermatology* 101, 905–975.
- Slominski, A., Paus, R., Costantino, R., 1991. Differential expression and activity of melanogenesis related proteins during induced hair growth in mice. *The Journal of Investigative Dermatology* 96, 172–179.
- Slominski, A., Paus, R., Plonka, P., Chakraborty, A., 1994. Melanogenesis during the anagen-catagen-telogen transformation of the murine hair cycle. *The Journal of Investigative Dermatology* 102, 862–869.
- Sredni Benjamin, Gal Rivka, Cohen J. Ian, Dazard Jean-Eudes, Givol David, Gafter Uzi, Motro Benny, Eliyahu Siona, Albeck Michael, Lander M. Harry, Kalechman Yona, 2004. Hair growth induction by the Tellurium immunomodulator AS101: association with delayed terminal differentiation of follicular keratinocytes and ras-dependent up-regulation of KGF1 expression. *The FASEB Journal* 18, 400–402.
- Steiner, P., Joseph, Hamilton, S. Gregory, 2002. Pyrrolidine derivative hair growth composition and uses. US 2002/0198250A1.
- Stenn, K.S., Paus, R., 2001. Controls of hair follicle cycling. *Physiological Reviews* 1, 449–494.
- Sundberg P. John, King, E. Lloyd, 1996. Mouse models for study of human hair loss. *Dermatologic Clinics* 14(4), 619–632.
- The Washington post, 2006. American hair loss association.
- Uno, H., 1991. Quantitative models for the study of hair growth in vivo. *Annals of the New York Academy of Sciences* 642, 107–124.
- Wagner, H., Geyer, B., Kiso, Y., Hikino, H., Rao, G.S., 1986. Coumestans as the main active principles of the liver drugs *Eclipta alba* and *Wedelia calendulacea*. *Planta Medica* 5, 370–374.
- Wang, L.C., Liu, Z.Y., Gambardella, L., Delacour, A., Shapiro, R., Yang, J., Sizing, I., Rayhorn, P., Garber, E.A., Benjamin, C.D., Williams, K.P., Taylor, F.R., Barrandon, Y., Ling, L., Burkly, L.C., 2000. Conditional disruption of hedgehog signaling pathway defines its critical role in hair development and regeneration. *J. Invest. Dermatol.* 114, 901–908.
- www.clinicaltrial.gov, 2007. NCT00055991, NCT00176969, NCT00311883, NCT00167102, NCT00176982.
- Xiulan Wang, 1997. Hair growth, beauty-care and health care medicinal liquor and method for preparing same. CN1154250.
- Yahara, S., Ding, N., Nohara, T., Matsuda, K., Ageta, H., 1997. Taraxastane glycosides from *Eclipta alba*. *Phytochemistry* 44, 131–135.
- Zhang, J., Xi C. HE, WEI-Gang Tong, Teri Johnson, Leanne M. Wiedemann, Yuji Mishina, Jian Q. Feng, Linheng Li, 2006. Bone morphogenetic protein signaling inhibits hair follicle anagen induction by restricting epithelial stem/progenitor cell activation and expansion. *Stem Cells* 24, 2826–2839.
- Zhao, Y., Tang, H., Jiang, Y., Wang, Z., Yi, Y., Lei, Q., 2001. Triterpenoid saponins from *Eclipta prostrata* L. *Yaoxue Xuebao* 36, 660–663.