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Eclipta alba extract with potential for hair growth promoting activity

Kakali Datta[a], Anu T. Singh[a,*], Ashok Mukherjee[a], Beena Bhat[a,b,1], B. Ramesh[b], Anand C. Burman[a]

[a] *Molecular Oncology Lab., Dabur Research Foundation, Sahibabad, Ghaziabad 201010, India*
[b] *Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut, India*

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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

* Corresponding author. Tel.: +91 0120 4378505; fax: +91 0120 4376901.
E-mail addresses: singhat@dabur.com, kakuli2001us@yahoo.com (A.T. Singh).
[1] The scientist was associated with Dabur Research Foundation when the work was undertaken.

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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 F_{254} 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), triterpinoid glycosides (Singh and Bhargava, 1992), thiophene derivatives (Yahara et al., 1997), triterpinoid 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 cm^2 and 3.2 mg/15 cm^2 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

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