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# *Eclipta alba* Extract with Potential for Reversing Chemotherapy-induced Alopecia: An Experimental Study in Mice

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## ABSTRACT

Chemotherapy-induced alopecia (CIA) or scalp hair loss is a well known adverse effect of conventional anticancer drugs like alkylating agents. Presently, there are no drugs which when given alongwith anticancer chemotherapy can be used for the treatment of CIA as preventing or reversing agents.

The whole plant extract of *Eclipta alba,* Hassk (Family Compositae) commonly known as Bhringraja, when used as oil applied topically on head has been reported to promote scalp hairs. In the present study ethyl acetate fraction (EAF) of methanolic extract of *Eclipta alba* has been investigated for hair growth promoting effect in a mouse model where alopecia-like state was produced by systemic administration of a cytostatic alkaylatic agent, etoposide. Etoposide was administered in a dose of 36 mg/kg, i.p. using two strains of mice: Swiss albino (with white body hairs) and C57/BL6 (with black body hairs) to inhibit the normal hair growing activity in a shaved area of skin 4x4 cm (4 $cm^2$) on the dorsal (back) surface of the trunk in groups of each species of mice. EAF or its vehicle was applied topically in the form of cream on the shaved area of skin of different groups of mice in two concentrations: 1.6%

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and 3.2%. Initially, some pilot experiments were conducted to generate the baseline data related to morphological and histological changes in shaved area of skin at different time points. Histopathological studies were conducted on skin section for hair growth activity in both strains of mice. In each strain of mice after recording the morphological changes for hair growth activity, the animals were sacrificed under deep ether anaesthesia on day 0, 1, 7, 11, 14 and 16 for recording microscopic histological changes in the transverse and longitudional sections of skin for skin thickness and hair follicle growth activity. The animals were photographed on different days and the evaluation of morphological changes were recorded by a blind observer for hair growth activity in the shaved areas and the alopecia was graded and scored by standard prescribed methods. The main study was conducted in seven groups of six animals each for obtaining the morphological and histological data of various groups treated with two doeses of EAF and the vehicle with or without administration of etoposide or its vehicle in doses as described above.

Results of morphological hair growth showed that the mice treated with etoposide-induced 100% inhibition of hair growth (considered as alopecia) during a span of subsequent 30 days post-treatment period. Topical application of EAF cream in the administered concentrations produced a dose-dependent reversal of inhibition of hair growth produced by etoposide, whereas EAF vehicle applied topically as cream failed to show any such activity. The results demonstrated that EAF of *Eclipta alba* has a potential to reverse the inhibition of etoposide-induced hair growth in both strains of the study mice.

*Keywords:* Anticancer chemotherapy, alopecia, chemotherapy-induced alopecia (CIA), etoposide, hair growth.

## Introduction

*Eclipta alba,* Hassk (Bhringaraja, Family: Compositae) has been traditionally used to check hair loss and stimulate hair growth (1). The plant 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 and has been reported to promote hair growth when used as part of several polyherbal formulations

(1-4). The reported hair growth promoting activities of *Eclipta alba* in traditional and published literature prompted us to explore this plant extract for reversing chemotherapy-induced alopecia (CIA) in an experimental model in mice.

**Alopecia**

Alopecia stands defined as hair loss and is a common human affliction resulting from changes in hair follicles or hair cycle or a combination of both (5, 6). Human hair represents an ensemble of some $10^5$ hair follicles that continually evolve over the course of time. At any time, a follicle is either growing (anagen phase) or ceasing to grow and involuting (catagen phase) but still on the scalp (telogen phase) before shedding and entering a new cycle (7). These successive phases constitute a follicular cycle. The duration of such a cycle is variable but typically ranges from a few months to several years. Each follicle can undergo repeated cycles until it eventually dies or miniaturizes to give rise to a vellus hair; the vellus hair shaft is not pigmented and has a cross-sectional diameter much thinner than normal. Whether miniaturization occurs progressively or abruptly is still unclear. If large proportions of follicles die or miniaturize, alopecia ensues, with a severity that depends on the location of lost follicles and on the amount of total hairs that are irreversibly shed (8).

**Chemotherapy-induced Alopecia (CIA)**

CIA is one of the most common side effects of anticancer chemotherapy employing cytotoxic and cytostatic agents (9, 10). CIA could be a cause of a stigma, which, in its metaphorical term, is referred to as discrediting attribute that may be physical, behavioural or biogeographical. CIA is ranked third in the list of distressing symptoms for cancer patients after nausea and vomiting (11). The adverse effect of cancer chemotherapeutic agents can manifest as thinning of hair, partial loss of hair or complete loss of hair leading to baldness (alopecia). Hair loss usually happens 2-3 weeks after the start of treatment with chemotherapeutic agent(s) or cycles of treatment. Sometimes it starts within a few days and then tends to increase 1 to 2 months into treatment. Several factors may contribute to the severity of the hair loss. The extent of hair loss depends on the type of the drug or the combination of chemotherapeutic agents, the dose of

the drug(s), root of administration (oral, I.V., I.M. or in the skin) and sensitivity of the individual to the drug (5, 11, 12).

Most drugs used in cancer chemotherapy affect the growth and metabolism of not only malignant cells but certain normal tissues as well. Tissues with rapid metabolic and mitotic rates such as the roots of scalp hair are most noticeably affected (13). Ninety percent of all the scalp hair follicles are in a phase of rapid growth and the high blood flow rate around the hair bulbs results in an optimal bioavailability of many compounds to this area (14). The majority of the cancer therapeutic drugs that cause hair changes such as alopecia are alkylating agents, vinca alkaloids, antimetabolites, anthracyclin antibiotics (doxorubicin), platinum-based drugs (cisplatin, carboplatin), taxoids (docetaxel, paclitaxel), as well as some of the new classes of drugs called targeted therapeutic agents (15). However, the last class of drugs (cetuximab, afatanib, dabrafenib, dasatinib, erlotinib, ibrutinib, imatinib, nilotinib, panitumumab, trametinib, sorafenib, vemurafenib, sonidegib, vismodegib, etc.) may cause the hair loss to thin or to become curlier and drier than earlier, rather than the frank loss of hairs or dopecia.

**Therapeutic Challenges of Developing Drugs for CIA**

Till date there is no approved drug against CIA. During the situation of anticancer chemotherapy the main focus of the oncologist is the treatment of cancer and prevention of its metastasis. Further, it is thought that during the remitting period when anticancer drugs are no more given, the CIA may recover by its own. As a result not much efforts have been made in the past to study drugs which can prevent or decrease/ delay the progress of CIA even during the use of anticancer chemotherapy. With this background, in the present study, we have explored a number of plants as a source for identification of new medicaments with hair growth promoting activities, which is much safer for the humankind in clinical settings of anticancer chemotherapy.

The present investigation deals with a part of this ongoing work where the role of *Eclipta alba* plant extract has been investigated in two strains of mice which were challenged with etoposide, an anticancer cytostatic agent for inhibiting the growth of shaved hairs giving a simulation to producing CIA.

## Materials and Methods

*Animals and Animal Care*

Two strains of healthy Swiss albino (with white hairs) and C57/BL6 (with black hairs) mice were purchased from National Institute of Nutrition (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-hour light/dark cycle and 23± 2ºC with 35-60% humidity. All mice were kept in quarantine for one week prior to experimentation. All experiments were carried out according to the guidelines laid by Institutional Animal Ethics Committee (IAEC) of Dabur Research Foundation, India.

*Chemicals*

Propylene glycol was obtained from Spectrochem Pvt. Ltd., India. Sodium Chloride injection was obtained from Parth Parenteral Pvt. Ltd., India. Etoposide was purchased from Dabur India Ltd. Methanol, ethanol, ethyl acetate, benzyl alcohol, dehydrated alcohol, polysorbate 80 and formaldehyde were obtained from Merck, Germany.

## Fractionation and Sub-fractionation of the Extract

Dried *Eclipta alba* whole plant was procured from the Ayurvedic store of Dabur Research Foundation and the Agro-technologist of Research Foundation authenticated the sample. A voucher specimen was preserved with the Ayurvedic Division of Dabur Research Foundation.

An amount of 1000 g of the shade-dried whole plant powder of *Eclipta alba* was initially extracted in 95% methanol using soxhlet. The methanolic extract was filtered and concentrated under reduced pressure to provide a 100 g crude extract. This extract was suspended in de-mineralized 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. The ethyl acetate fraction (EAF) was filtered and dried using sodium sulphate and vacumm, leaving 5.8 g of light brown powder. The final EAF of methanolic extract found to be rich in coumestans was screened for hair growth promoting activity.

**Evaluation of Etoposide-induced Alopecia in Adult Swiss Albino and C57/BL6 Mice**

Based on the principle that follicles in telogen phase can be induced to enter the anagen phase by mechanical traumatization (16), such as shaving (17), a method was developed which served as a model for studying etoposide-induced alopecia.

This *in-vivo* model for etoposide-induced alopecia and screening of EAF was used in both adult Swiss albino and C57/BL6 mice. Forty five to 60 days old male mice of both the strains were used in the study. On day zero, not more than 10% of the body hairs, i.e. from an area of 4x4 cm (4 $cm^2$) of the dorsal surface were shaved off using sterile scalpel blade and care was taken not to cause nick or abrasion to the underlying dermal layer.

For generating the baseline data, some pilot experiments were coducted in groups of both strains of mice: Swiss albino and C57/BL6 mice which were sacrificed under deep ether anesthesia on day 0, 1, 7, 11, 14 and 16 day post-shaving. Skin specimens were taken from the shaved skin of areas on the dorsum (back) of the trunk and processed for histological evaluation in both longitudinal and transverse sections. The follicles in the subcutis were counted as an indication for anagen induction. The animals were photographed on different days and the evaluation of morphological changes were documented by a blind observer for hair loss and hair growth. Induction of alopecia was graded as per method described by Hussein *et al* (18) (Table 1).

**Table 1: Scoring scale for alopecia-induced by etoposide**

| Scale | Description |
|---|---|
| 0 | No detectable alopecia |
| 1 | Mild alopecia defined as less than 50% hair loss |
| 2 | Moderately severe alopecia with more than 50% hair loss |
| 3 | Total absence of hair |

To study etoposide-induced alopecia or decrease in the hair regrowth, the etoposide and the vehicle for etoposide was administered intraperitoneally (i.p.) on 9th, 11th and 13th day of post-shaving period in different groups of both the strains of mice (Table 2).

**Table 2: The treatment groups in etoposide-induced alopecia model**

| Group No. | Days of treatment | Treatment | Dose/Day | |
|---|---|---|---|---|
| | | | Etoposide | Vehicle |
| I | $9^{th}$ to $14^{th}$ | Untreated | Nil | Nil |
| II | $9^{th}$, $11^{th}$, $13^{th}$ | Vehicle of Etoposide injection | Nil | Equivalent dose with respect to highest dose |
| III | $9^{th}$, $11^{th}$, $13^{th}$ | Etoposide | 36 mg/ kg (i.p.) | Nil |

**Evaluation of EAF on Etoposide-induced Alopecia**

The main study was conducted in seven groups of six animals each. Treatment was given to six different groups of both strains of animals as shown in Table 3. The EAF effect was evaluated in two concentration doses: 1.6 mg/4 $cm^2$ and 3.2 mg /4 $cm^2$ in both strains of mice groups. The composition of vehicle for etoposide was benzyl alcohol (30 mg), dehydrated alcohol (30.5%v/v) and polysorbate 80, whereas the composition of vehicle for EAF was propylene glycol (93%), glycerol (3.5%) and DMSO (3.5%). Etoposide (36 mg/kg, i.p.) or its equivalent amount of vehicle was administered in the indicated dose by i.p. route, whereas EAF was applied topically in the form of cream to observe the hair growth promoting activity and effect in the shaved area on the dorsal surface of the animal's trunk. At the end of the study, the group of mice were photographed and observations were documented by a blind observer as per scale of hair loss or scale of hair growth. Induction of alopecia was graded using Hussein *et al* method (18) (Table 1), while the scale of hair growth was scored following the method of Steiner and Hamilton (19) (Table 4). Before submitting the skin from the experimental area of 4 $cm^2$ for making sections for histopathological microscopy, the regrown hairs were shaved off and weighted on a precision one-pan balance for the quantitation of hair regrowth which was expressed as hair weight in miligram (mg) for comparison of the regrown hair data among different groups of mice.

### Statistical Analysis

All the study parameters were expressed as Mean±SEM in their respective units and the obtained data were analyzed using SAS version 9.1.3. The level of statistical significance was considered at 'p' value $< 0.05$.

**Table 3: Treatment schedules in different groups of animals for screening of EAF in different group of mice**

| Group | Days of treatment | Treatment | Dose | | | |
|---|---|---|---|---|---|---|
| | | | Etoposide (mg/kg; i.p.) | EAF mg/ 4 $cm^2$ (topical) | Vehicle for etoposide (i.p.) | Vehicle for EAF (topical) |
| I | $9^{th}$ to $15^{th}$ | No treatment | Nil | Nil | Nil | Nil |
| II | $9^{th}$, $11^{th}$, $13^{th}$ | Vehicle for etoposide | Nil | Nil | Equivalent volume | Nil |
| III | $9^{th}$ to $15^{th}$ | Vehicle for EAF | Nil | Nil | Nil | Equivalent dose with respect to highest dose |
| IV | $9^{th}$, $11^{th}$, $13^{th}$ | Etoposide | 36 | Nil | Nil | Nil |
| V | $9^{th}$, $11^{th}$, $13^{th}$ | Etoposide and EAF | 36 (Administered after one hour of EAF) | 1.6 | Nil | Nil |
| | $10^{th}$, $12^{th}$, $14^{th}$, $15^{th}$ | EAF | Nil | 1.6 | Nil | Nil |
| VI | $9^{th}$, $11^{th}$, $13^{th}$ | Etoposide and EAF | 36 (Administered after one hour of EAF) | 3.2 | Nil | Nil |
| | $10^{th}$, $12^{th}$, $14^{th}$, $15^{th}$ | EAF | Nil | 3.2 | Nil | Nil |
| VII | $9^{th}$, $11^{th}$ $13^{th}$ | Etoposide and EAF vehicle | 36 (Administered after one hour of EAF (vehicle) | Nil | Nil | Equivalent dose with respect to highest dose |
| | $10^{th}$, $12^{th}$, $14^{th}$, $15^{th}$ | Nil | Nil | Nil | Nil | Equivalent dose with respect to highest dose |

**Table 4: Scoring scale for hair growth as per method used by Stiener and Hamilton (19)**

| Scale | Description |
|---|---|
| 0 | No growth |
| 1 | Beginning of growth in small tufts |
| 2 | Hair growth covering over < 25% of shaved area |
| 3 | Hair growth covering over > 25% but less than 50% of the shaved area |
| 4 | Hair growth covering over > 50% but less than 75% of the shaved area |
| 5 | Complete hair growth of shaved area |

## Results

*Baseline Data*

The histopathological evaluation was conducted on hematoxylin and eosin stained transverse sections of the cut skin specimens obtained from the respective experimental groups of animals. The changes in the skin thickness and the number of follicles in the subcutis on day 0 and on 1st, 7th, 11th, 14th and 16th day of post-shaving period were counted as an indication for anagen induction. The histopathological changes clearly indicated that there was a significant increase in the skin thickness and follicle counts in the subcutis. The consolidated results have been tabulated for Swiss albino mice and C57/BL6 mice in Table 5, 6a and 6b.

Histopathological data from the transverse sections show that the animals at 13 or 14 days of post-shaving period were in active proliferative phase, i.e. anagen phase of hair growth, characterized by an increase in skin thickness. The increase in thickness of subcutis placed just above panniculus carnosus and an increase in follicle count in subcutis layer was observed in both strains of mice. Additionally in C57/BL6 mice, there was an increase in the melanogenesis as evident in resected skin and punch biopsies. Animals at day 0-, 1-, 7-, 11- and 16-day post- depilation were at quiescent phase (telogen phase). However, 14-day post-depilated mice having hair growth at peak anagen phase of hair cycle were preselected for the evaluation of the effect of EAF for reversing or preventing etoposide-induced inhibition of hair regrowth. It was evident from the longitudinal section that during anagen phase of hair growth, hair bulb penetrates into subcutis layer (Fig. 1). The study was repeated thrice with similar results.

**Table 5: Histopathological evaluation of follicle count and skin thickness in Swiss albino mice on day 0 and other post-shaving days**

| S.No | Days post depilation | Mean follicle count * | Mean follicle count in subcutis layer * | Average skin thickness (μm) * |
|---|---|---|---|---|
| 1 | 0 | 11.5 ± 0.5 | 0 | 244.00 ± 15.27 |
| 2 | 1 | 14.0 ± 1.0 | 0 | 289.02 ± 28.15 |
| 3 | 7 | 30.5 ± 3.5 | 5.5 ± 5.5 | 296.11 ± 24.03 |
| 4 | 11 | 87.0 ± 5.0 | 70.0 ± 4.0 | 491.51 ± 38.6 |
| 5 | 14 | 113.0 ± 0.0 | 95.5 ± 1.5 | 638.69 ± 32.5 |
| 6 | 16 | 64.5 ± 0.50 | 41.5 ± 1.5 | 391.31 ± 9.14 |

* Mean ± SEM.

**Table 6a: Histopathological evaluation of follicle count and skin thickness in C57/BL6 mice on day 0 and relative post-shaving days**

| S.No | Days post depilation | Mean follicle count * | Mean follicle count in subcutis layer * | Average skin thickness (μm) * |
|---|---|---|---|---|
| 1 | 0 | 20.5 ± 7.5 | 0 | 388.9 ± 59.30 |
| 2 | 1 | 28.0 ± 6.0 | 0 | 380.0 ± 57.99 |
| 3 | 7 | 24.5 ± 5.50 | 0 | 383.0 ± 45.15 |
| 4 | 11 | 45.5 ± 4.5 | 17.5 ± 2.25 | 580.07 ± 8.00 |
| 5 | 14 | 86.0 ± 4.0 | 52.0 ± 4.5 | 666.51 ± 12.40 |
| 6 | 16 | 37.0 ± 1.0 | 7.0 ± 0.35 | 457.53 ± 43.60 |

* Mean ± SEM.

**Etoposide-induced Alopecia**

The results of both strains of mice: Swiss albino and C57/BL6 mice have been shown in Fig. 1, 2 and 3, respectively. Observations of alopecia/ hair growth revealed that the treatment of Group IV animals with etoposide-induced 100% visible alopecia. Onset of alopecia was seen in all the animals in Group IV on day 18 of the experiment and complete alopecia was documented in this group till 30 days post-treatment, whereas normal hair growth was seen in Group I, II and Group III animals during the period of 18 - 21 days post-treatment (Table 6b).

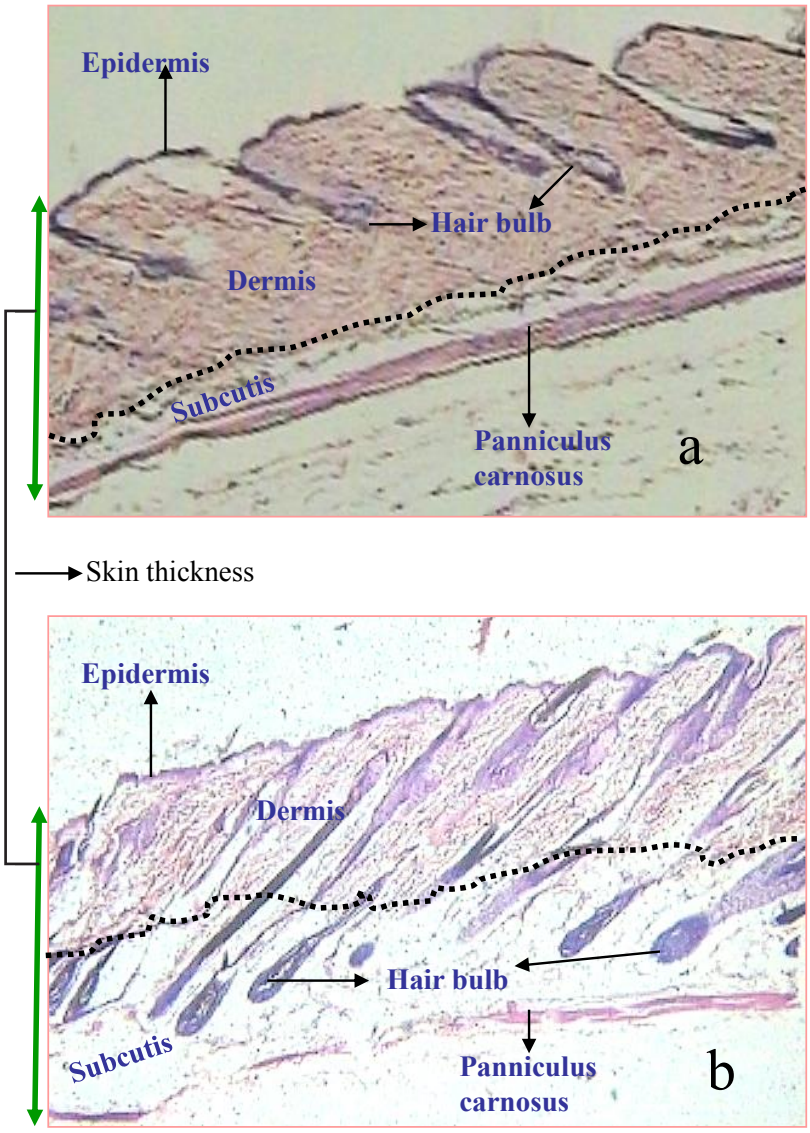



**Fig. 1: Hair growth cycle in C57/BL6 mice-post birth.**

a) Longitudinal sections of skin of animals in telogen phase of hair growth (0,7, 11 and 16 day old). The hair bulb is shrunken and is present in the dermis above the subcutis layer.

b) Longitudinal sections of skin of animals in anagen phase of hair growth (14 day old). The hair bulb is rigid and is present deep in the subcutis layer.

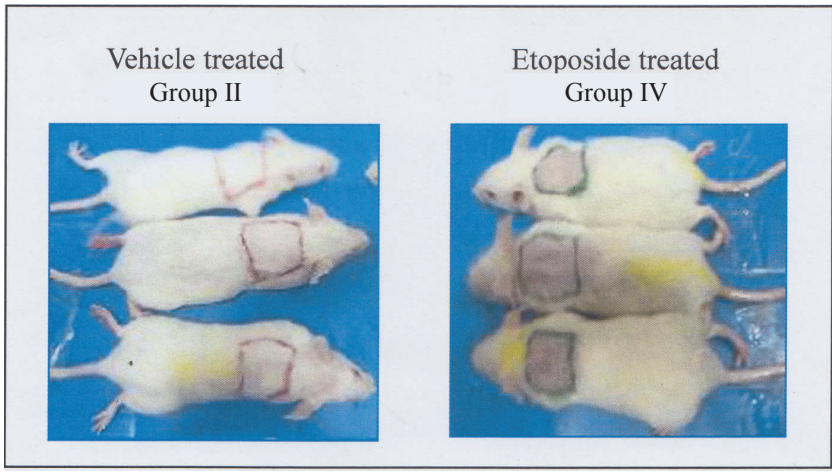



**Fig. 2 : Induction of etoposide-induced alopecia in adult Swiss albino mice**

On 21st day the scale of growth in etoposide treated group is zero as compared to vehicle treated group where scale of hair growth is 5.

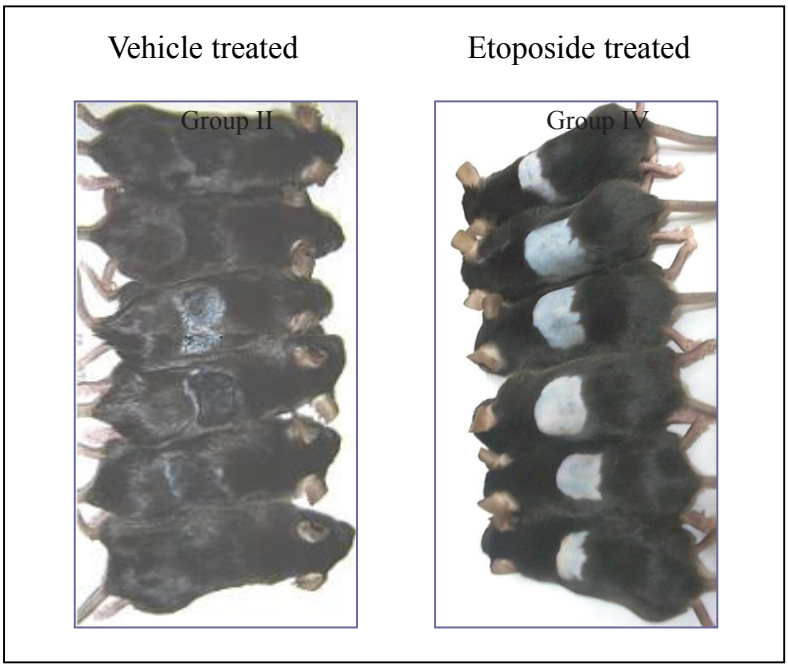



**Fig. 3 : Induction of etoposide-induced alopecia in adult C57/BL6 mice.**

On 21st day the scale of growth in etoposide treated group is zero as compared to vehicle treated group where scale of hair growth is 5.

**Protective Effect of EAF**

The hair growth was evaluated every day by the blind observer and documented for day 18, 19, 20, 21 and 30 after treatment. After the treatment with EAF, the quantitation of alopecia/ hair regrowth was expressed as percentage of shaved area covered by new hair growth, scored by blinded observer on a scale of 0 as no hair growth to 5 as complete hair growth. The data on hair growth after the treatment with EAF are presented in Table 6a and b.

**Table 6b: Analysis of Scale of hair growth in adult Swiss albino and C57/BL6 mice after treatment with EAF**

| Days | Group I | Group II | Group III | Group IV* | Group V* | Group VI* | Group VII* |
|---|---|---|---|---|---|---|---|
| ***Alopecia %/ Hair growth on indicated days*** | | | | | | | |
| 18th Day | Normal hair growth | Normal hair growth | Normal hair growth | 0-10 alopecia | 0-10 alopecia | 0-10 alopecia | 0-10 alopecia |
| 19th Day | Normal hair growth | Normal hair growth | Normal hair growth | 10-40 alopecia | 10-40 alopecia | 10-40 alopecia | 10-40 alopecia |
| 20th Day | Normal hair growth | Normal hair growth | Normal hair growth | 40-80 alopecia | 40-80 alopecia | 40-80 alopecia | 40-80 alopecia |
| 21st Day | Normal hair growth | Normal hair growth | Normal hair growth | 100 alopecia | 100 alopecia | 100 alopecia | 100 alopecia |
| ***Hair growth score on indicated days*** | | | | | | | |
| 30th Day | 5 | 5 | 5 | 0 | 2 | 5 | 0 |

* Data shown is % alopecia or hair regrowth on different days (18th to 21st).

**Table 7: Effect of EAF at a concentration of 3.2 mg/4 $cm^2$ on morphologically selected telogen skin of C57/BL6 mice for hair growth promoting activity**

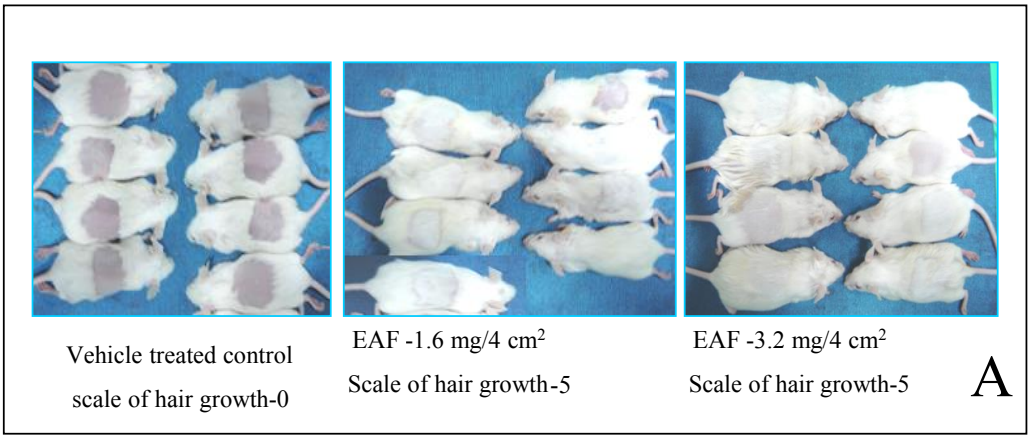
| S. No | Treatment | Mean follicle count* | Average skin thickness (µm) * | % Anagen Induction | P value | Mean follicle count in subcutis layer* |
|---|---|---|---|---|---|---|
| 1 | Vehicle for EAF | 19.12 ± 3.0 | 265.50 ± 11.92 | 0.0 | - | 0.0 |
| 2 | 1.6 mg/4 $cm^2$ of EAF | 39.00 ± 8.34 | 417.39 ± 35.87 | 50.0 | <0.0001 | 21.00 ± 8.31 |
| 3 | 3.2 mg/4 $cm^2$ of EAF | 66.00 ± 7.31 | 480.38 ± 41.22 | 87.5 | <0.0001 | 45.00 ± 7.37 |

*Data is presented as Mean ± SEM.

Increased hair growth was observed in Group VI animals of both strains (Fig. 4 and 5), which were treated with the higher dose (3.2 mg/$cm^2$) dose of EAF alone or alongwith etoposide as compared to Groups IV and VII (that received etoposide alone or etoposide plus vehicles, respectively), where scale of growth observed was 0; whereas in Group V, animals of which were exposed to the lower dose of EAF alongwith etoposide, the scale of hair growth observed was 2. A statistically significant anagen induction of 50% and 87.5% was observed in Group V and VI, respectively (Table 7). The observations obtained from animals in Group V and Group VI indicate stimulation of hair growth on treatment of topical application with EAF in two concentrations in skin but treated systemically with etoposide. All the animals in Groups I, II, III showed similar hair growth as observed in animals of Group VI (Fig. 6), where histopathological observations from transverse section of skin taken from C57/BL6 mice have been shown.

**Discussion**

CIA is a frequent and emotionally distressing side effect of cancer chemotherapy for which currently no effective preventive therapy is available (20, 21). CIA is thought to occur when anticancer drugs ablate the proliferating epithelium and block normal maturation of precursor epithelial cells to the final hair strand. The sensitivity of hair



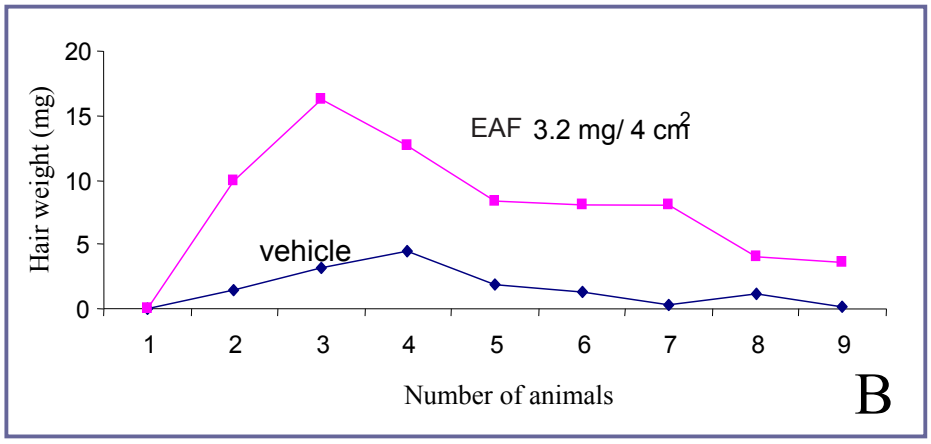




**Fig. 4 : Efficacy of EAF on etoposide-induced alopecia in adult Swiss albino mice.**

A. Pictures of animals treated with vehicle and EAF on 30th day of the treatment.

B. Comparative graph of hair weight in different treatment groups.

follicle cells to anticancer agents is related to their state of proliferation. Many anticancer agents that cause CIA in target specific phases of the cell cycle and are therefore selectively toxic to tissues undergoing cell division (22, 23). In a recent review of 38 articles, authors consistently concluded that the hair loss ranked amongst the most troublesome side effects experienced by patients undergoing anticancer chemotherapy for breast cancer (24). In a study by

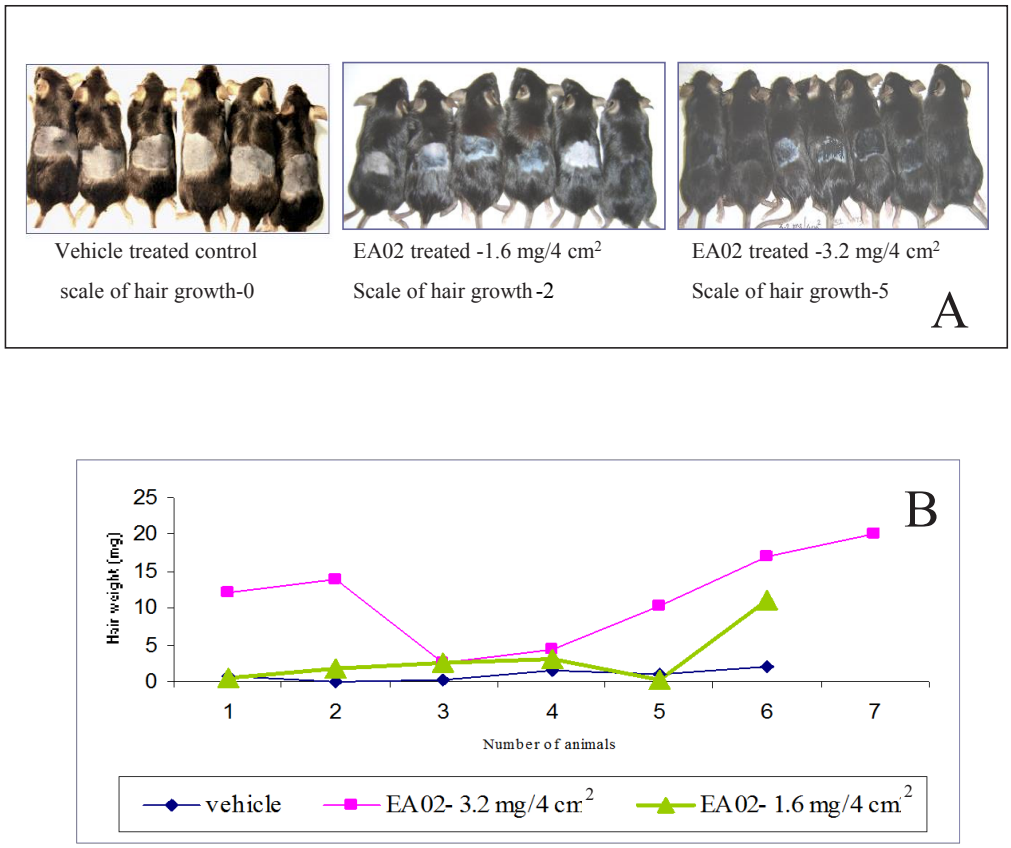



**Fig. 5 : Efficacy of EAF on etoposide-induced alopecia in adult C57/BL6 mice.**

A-Scale of hair growth observed in animals treated with vehicle and EAF at a concentration of (3.2 mg/4 $cm^2$) on 30th day of treatment.

B-Comparative graph of hair weight in different treatment groups.

Tierney *et al,* 35 of 46 patients receiving chemotherapy ranked alopecia as a more important side effect than vomiting (25). A systematic review of 10-year data on randomized controlled trials on breast cancer treatment, the incidence of alopecia was a noted adverse event (26). In 19 anti-cancer drug therapy regimens that had 95-100% of the patients suffering some degree of alopecia (grades 1-3), the common alopeciogenic agents were doxorubicin, docetaxel, epirubicin and

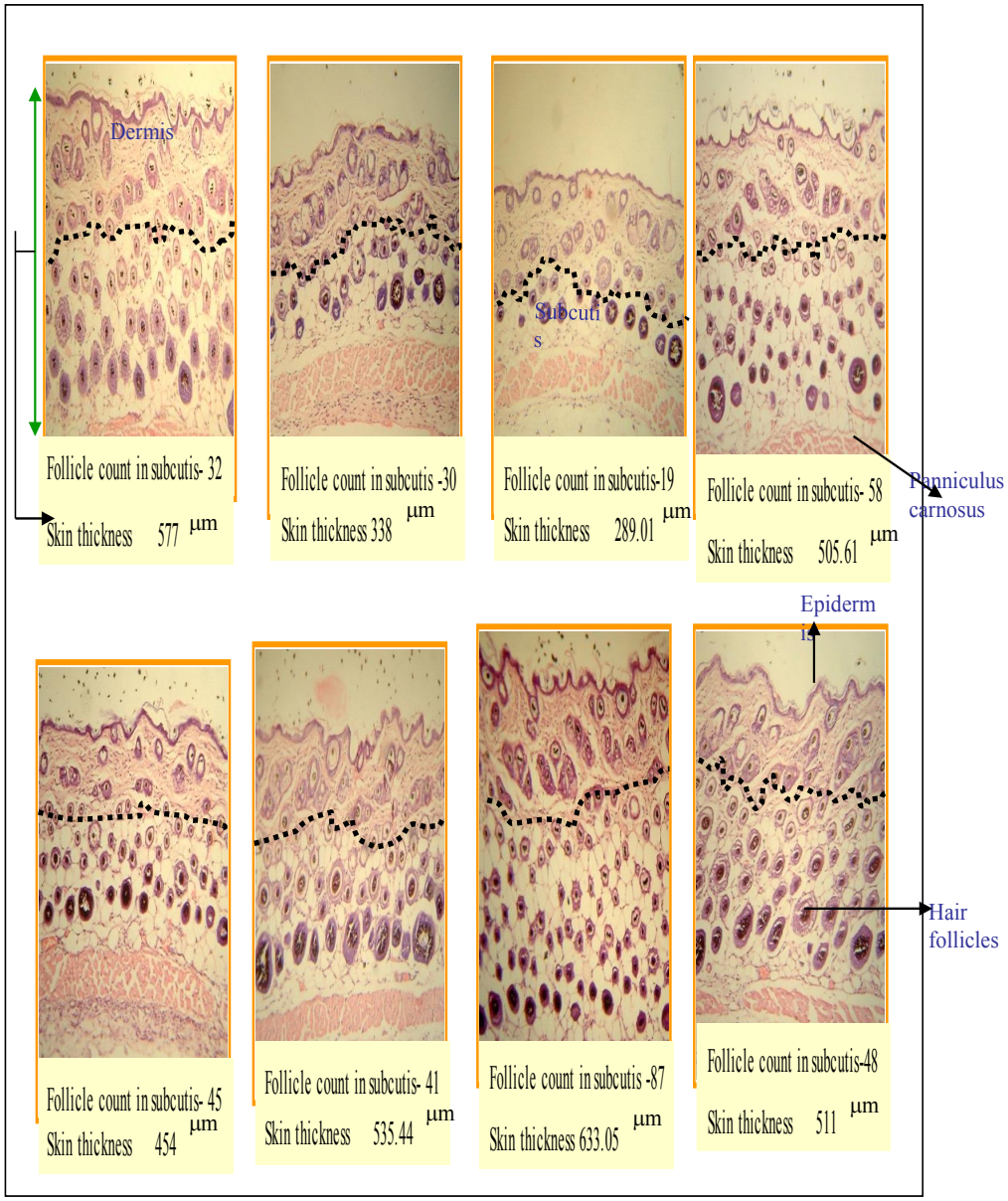



**Fig. 6 : Dotted line indicates the junction of dermis and subcutis. 87.5% animals treated with 3.2 mg/15 cm$^2$ of EAF show telogen to anagen transition.**

TS of skin showing hair growth promoting activity in C57/B26 mice treated with EAF in a concentration of 3.2%/15 cm$^2$.

platinum drugs, which are the main chemotherapeutic agents for solid cancers (27).

Hair regrowth after chemotherapy can take from 3 to 6 months to recover, and a small percentage of patients fail to experience complete recovery (27). CIA is particularly devastating because it is an outward sign of an otherwise hidden disease, leading some patients to refuse systemic anticancer chemotherapy (28). Methods currently utilized to prevent CIA are unsatisfactory, e.g. in two studies on randomized trial with local application of minoxidil in 48 patients in one study (29) and six evaluable patients in the second study (30) did not find it to be effective in preventing the CIA (29, 30). Therefore, it is important to investigate the CIA in appropriate experimental models that allow examination of pathobiology as well as the exploration of new strategies for its management. Two models which have been widely reported for the study and pharmacological manipulations of CIA are: models using both the neonate and adolescent mice in the situations of alopecia occurring both normally as well as in disease states. The latter model strikingly mimics the characteristic hair follicular pathophysiology during drug-induced alopecia.

The murine model allows the study of the effects of chemotherapy on well-defined, homogeneous and mature population of precisely the type of hair follicles that are severely damaged by chemotherapy, resulting in alopecia and disturbance in hair growth. The murine model also allows as yet unparalleled insights into the basic pattern of hair follicle response to recovery from chemotherapy, which is further based on the high degree of hair cycle synchrony displayed by the mouse strain. It has been reported that ImuVert, a biological response modifier prepared from the bacterium *Serratia marcescens,* protected the animals from alopecia-induced by cytosine arabinoside (31). In subsequent studies, similar protection from cytosine arabinoside-induced alopecia was observed with recombinant interleukin-1 β and later with epidermal growth factor and fibroblast growth factor (32). However, when used under similar conditions none of these agents offered protection from alopecia-induced by cyclophosphamide (Cytoxan). In the clinical setting, chemotherapy more often involves the use of alkylating agents. Accordingly, we continued our efforts to explore various compounds in the mouse model and ways to prevent alopecia from alkylating agents like etoposide.

The results of the present study clearly demonstrate that the murine model used for CIA resembles the clinical situation more closely than any other currently available experimental models for this clinical entity.

Development of *in vivo* model for etoposide-induced alopecia included generation of baseline as well as post-depilation data in Swiss albino and C57/BL6 mice. Animals at 14-day post-depilation that were in active anagen phase were selected for screening the EAF for reversing etoposide-induced decrease in hair growth. Etoposide was observed to terminate the anagen phase prematurely and caused severe decrease in hair regrowth.

The results of the present study have demonstrated that the topical application of EAF accelerated the anagen phase of hair growth in etoposide-induced alopecia, unlike the challenging agent which induced alopecia. Further, EAF accelerated normal pigmentation of regrowing hair shafts and retarded the occurrence of etoposide-induced alopecia and diminished the severity of hair regrowth. These results and the used model strongly encourages one to explore and develop extracts of the plants claimed in traditional system of medicine for enhancing the hair growth and find out the active principles there in as drugs for accelerating and improving the clinically and psychologically important regrowth of a normally pigmented hair coat after CIA in human subjects.

The novel strategies for the therapeutic management of CIA should more systematically take into account that, like the vast community of patients with abnormal hair loss or gain seen in clinical practice, CIA and its subsequent hair regrowth predominantly reflect defined alterations of normal patterns of hair follicle cycling, rather than of hair shaft production. This implies that real progress in the prevention and treatment of CIA can only be accomplished by understanding the molecular interactions of alopecia reducing agents.

In the end it is pertinent to mention that for evaluating the safety of EAF, acute toxicity studies were performed in Swiss albino mice in accordance with OECD guidelines and the resulting hematological, biochemical and all pathological findings were within normal limits after topical application of EAF as per doses and schedule of treatment used in the present study.

Based on the above observations, the

EAF of *Eclipta alba* has a potential for not only the hair growth stimulation but also preventing the CIA, as exemplified by the reversal of inhibition of hair growth by etoposide in both strains of mice.

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