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DEVELOPMENT AND EVALUATION OF POLYHERBAL FORMULATIONS FOR HAIR GROWTH-PROMOTING ACTIVITY

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ABSTRACT

Hair loss (technically known as alopecia) is a loss of hair from the head or body. Baldness can refer to general hair loss or male pattern baldness specifically. Some types of baldness can be caused by *Alopecia areata*, an autoimmune disorder. *Eclipta alba* (Asteraceae), *Bacopa monnieri* (Plantaginaceae), *Trigonella foenugraecum* (Fabaceae) are well known Ayurvedic herbs with purported claims of hair growth promotion. Hair formulation of *Eclipta alba* 10% w/v, *Bacopa monnieri* 10 % w/v, *Trigonella foenugraecum* 5 % w/v concentration in the form of herbal oil were studied and showed excellent hair growth activity with standard (2% minoxidil ethanolic solution) in wister albino rats. Hair growth initiation time was significantly reduced to half on treatment with the oil, as compared to control animals. The time required for complete hair growth was also significantly reduced. Quantitative analysis of hair growth after treatment with oil exhibited greater number of hair follicles in anagenic phase i.e. 82 which were higher as compared to control (52). The results of treatment with oil were better than the positive control minoxidil 2% treatment. It holds the promise of potent herbal alternative for minoxidil.

KEYWORDS: Herbal Hair Formulation, Asteraceae, Plantaginaceae, Fabaceae

INTRODUCTION

Hair grows everywhere on the human skin except on the palms of our hands and the soles of our feet, but many hairs are so fine they are virtually invisible. Hair is made up of a protein called keratin that is produced in hair follicles in the outer layer of skin. As follicles produce new hair cells, old cells are being pushed out through the surface of the skin at the rate of about six inches a year. The hair you can see is actually a string of dead keratin cells (Cash, 2001). The average adult head has about 100,000 to 150,000 hairs and loses up to 100 of them a day; finding a few stray hairs on your hairbrush is not necessarily cause for alarm. Hair is one of the vital parts of the body derived from ectoderm of skin, is protective appendages on the body and considered accessory structure of the integument along with sebaceous glands; sweat glands and nails (Kapoor, 1990). They are known as epidermal derivatives as they originate from the epidermis during embryological development. Hair is an important of the overall appeal of the human body (Rathi et al, 2008).

Alopecia is dermatological disorder that has been recognized for more than 2000 years is a common problem in cosmetics as well as Primary Health Care Practice. It is common throughout the world and has been estimated to affect between 0.2% and 2% of the world population (Messenge, 2000).

There are many types of hair loss, also called alopecia: *Involutional alopecia* is a natural condition in which the hair gradually thins with age. More hair follicles go into the resting phase, and the remaining hairs become shorter and less

in number. *Androgenic alopecia* is a genetic condition that can affect both men and women. Men with this condition, called male pattern baldness, can begin suffering hair loss as early as their teens or early 20s. It's characterized by a receding hairline and gradual disappearance of hair from the crown and frontal scalp. Women with this condition, called female pattern baldness, don't experience noticeable thinning until their 40s or later. Women experience a general thinning over the entire scalp, with the most extensive hair loss at the crown (Stough et al, 2005).

Alopecia areata often starts suddenly and causes patchy hair loss in children and young adults. This condition may result in complete baldness (*alopecia totalis*). But in about 90% of people with the condition, the hair returns within a few years. *Alopecia universalis* causes all body hair to fall out, including the eyebrows, eyelashes, and pubic hair. *Trichotillomania*, seen most frequently in children, is a psychological disorder in which a person pulls out one's own hair. *Telogen effluvium* is temporary hair thinning over the scalp that occurs because of changes in the growth cycle of hair. A large number of hairs enter the resting phase at the same time, causing hair shedding and subsequent thinning (Bhalearo and Salanki, 2002).

Synthetic drug, minoxidil is a potent vasodilator was scientifically proved for the treatment of alopecia (Parker et al, 1982; Uno et al, 1987). Though the use of drugs for its side effect is not advisable, the drug of plant origin is necessary to replace the synthetic one. Hence the present study was aimed to evaluate the hair growth activity of herbal formulation (HF) which includes *Eclipta alba* (10 %w/v), *Bacopa monnieri* (10% w/v), *Trigonella foenugraecum* (5%w/v) concentration in oil. Bhavaprakash, an Ayurvedic treatise mentions the use of drug for the treatment of “Indralupta” i.e. drug used in the treatment of hair loss. *Eclipta alba* (Asteraceae), *Bacopa monnieri* (Plantaginaceae), *Trigonella foenugraecum* (Fabaceae) is such herb with traditional claims of hair growth promotion (Bone, 1996). *Eclipta alba* commonly known as bhringraj, is a species of plant in the family Asteraceae.

This plant has cylindrical, grayish roots. The solitary flower heads are 6–8 mm in diameter, with white florets. The achenes are compressed and narrowly winged. *E. alba* is small much branched annual herb with white flower heads found in moist situation throughout India ascending up to 600 feet, grows just after the first showers of rainy season. It is used for hair growth promoter, improving the luster of the hair, treatment of variety of human ailments, particularly liver disorders, and wound healing. The herb has been known for its curative properties and has been utilized as antimytotoxic, analgesic, antibacterial, antihepatotoxic, antihaemorrhagic, antihyperglycemic, antioxidant, immunomodulatory properties and it is considered as a good rejuvenator too (Roy et al, 2008).

Bacopa monniera, a member of the Plantaginaceae family, is a small, creeping herb with numerous branches, small oblong leaves, and light purple flowers. In India and the tropics it grows naturally in wet soil, shallow water, and marshes. The herb can be found at elevations from sea level to altitudes of 4,400 feet, and is easily cultivated if adequate water is available. Flowers and fruit appear in summer and the entire plant is used medicinally. The leaves of this plant are succulent, oblong and 4–6 millimeters thick. Leaves are oblanceolate and are arranged oppositely on the stem.

The flowers are small and white, with four or five petals. Its ability to grow in water makes it a popular aquarium plant. *B. monniera* is an important medicinal herb used in Ayurveda, where it is also known as "Brahmi," after Brahma, the creator God of the Hindu pantheon. Bacopa has traditionally been employed as a neurological tonic and cognitive enhancer, and it is currently being studied for its possible neuroprotective properties (Mahato et al, 2000; Chakravarty et al, 2003).

Fenugreek, *Trigonella foenum-graecum* is an herbaceous annual plant in the family Fabaceae grown for its leaves and seeds which are used as a herb or spice. The fenugreek plant may have a single stem or may be branched at the stem base. The plant has an erect growth habit and a strong, sweet aroma. The leaves of the plant are small and trifoliate with oval leaflets which are green to purple in color. The plant produces solitary pale white or purplish flowers and a straight or occasionally curved yellow pod which houses the seeds. Between 10 and 20 seeds are produced per pod and they are small, smooth and brown, each divided into two lobes. Fenugreek can reach a height of 60 cm (23.6 in) and as an annual, survives only one growing season. The origin of fenugreek is unknown but it is indigenous to the western Mediterranean (Khare, 2003).

MATERIALS AND METHODS

Collection and Authentication of Plant

The leaves of *Eclipta alba*, aerial parts of *Bacopa monniera*, seeds of *Trigonella foenum-graecum* were purchased from local market and authenticated by Dr P.K. Tiwari, Prof. and Head, Dept. of Botany, Dr. H.S. Gour, Cental University, Sagar, M.P., dried in shade, crushed and passed through the sieve number 80. The various powder drugs were subjected to pharmacognostic studies for confirmation.

Preparation of Combined Drug Herbal Hair Formulation of Different Concentration

After confirming the literature survey, *Eclipta alba*, *Bacopa monniera*, *Trigonella foenum-graecum* were selected and mixed in 3 different concentration for maximum activity (Table 1) and the formula of base contains coconut oil.

Procedure

Mixed all three drug powders properly then add little quantity juice and make a paste. Heat the 100ml of coconut oil. Then add above paste to it and mark the level. Then add remaining juice in to it. Continue to heat it on medium flame till water part is evaporated, till the level is slightly above the mark followed by continuously stirring arrangement. Confirmatory test of oil as per Ayurvedic text, Agnipariksha, Vatipariksha were performed.

Chemical Evaluation

The prepared formulations were evaluated using standard methods of general characterization, physical and chemical evaluation including Specific gravity, pH, Refractive index, Acid value, Saponification value, Iodine value (Adhirajan et al, 2001).

Primary Skin Irritation Test

Four healthy female wistar albino rats, weighed 200-250gm were selected for study. Each rat was caged individually food and water given during the test period 24hrs prior to the test. The hair from the back of each rat of $1cm^2$ was shaved on the side of the spine to expose sufficiently large test areas, which could accommodate three test sites were cleaned with surgical spirit. 1ml quantity of formulations HF1, HF2, HF3 were applied over the respective test sites of one side of the spine. The test sites were observed for erythema and edema for 48hrs after application (Uno et al, 1991).

Application of Test Formulations

Female wistar albino rats, 200-250gm, were used for hair growth studies. They were placed in cages and kept in

(23°C±10, 60% ±10 RH) standard environmental conditions, fed with standard diet and allowed free access to drinking water for two days. All animal experiments were carried out in accordance with guidelines of CPCSEA and the study was approved by the Institutional Animal Ethical committee (379/01/ab/CPCSEA). The rats were divided into 5 groups of 6 rats each 6cm^2 area of dorsal portion of all the rats shaved area to remove all hair. Group I was kept as control, where there was no drug treatment. Group II was treated as standard, where 1ml of (2% Minoxidil ethanolic solution) was applied over the shaved area, once a day. The animals of remaining groups were given application of 1ml of formulation HF1, HF2 and HF3respectively, once a day. This treatment was continued for 30 days (Adhirajan et al, 2001).

Qualitative Hair Growth Study

Qualitative hair growth analysis was undertaken by visual observation of three parameters:

Hair growth initiation time i.e. minimum time taken to initiate hair growth on denuded skin region, hair growth completion time i e. minimum time taken to completely cover the denuded skin region with new hair, mean hair length (Purwa et al, 2008).

Quantitative Hair Growth Study

The method reported by uno[11] was followed for the quantitative evaluation of treatment. Two rats from each group was euthanized after 10days, 20days and 30days of treatment: skin biopsies were taken from shaved area and specimen was preserved in 10% formalin. Tissues were embedded in paraffin wax and sectioned into uniform thickness of 10µm and stained with hematoxylin and eosin. Sections from all the groups were evaluate for the number of hair follicles per mm area of skin and percentage ratio of hair follicles in different cyclic phases, like anagen (growth phase), catagen and telogen (resting phase) was determined microscopically (Roy et al, 2006).

RESULTS AND DISCUSSIONS

The results of general characteristic, physical and chemical evaluation are summarized in Table 2 and Table 3.

Primary Skin Irritation Test

Primary skin irritation test was conducted to evaluate the irritation by the prepared formulations on intact skin of rats. All of the prepared formulations were not showed any erythema and edema; this indicates that the prepared formulations were non-irritant on skin of rats.

Hair Growth Activity

The results are shown in the table 4, 5 and 6. The qualitative study revealed that the time taken for complete hair growth was 16d in HF3 and 20d in HF2. And on comparison HF3 and Minoxidil it has been observed that HF3 hair oil formulation application shows better growth that the patch with minoxidil. Mean hair length was 4.6mm in HF3 and 3.6mm in HF2. The quantitative study revealed that formulation HF3 considerable increase in number of hair follicle in anagen phase of hair growth cycle, when compare to standard and control. In standard group percentage of population of anagen follicle 67, where as in formulation HF3 it was 82 and HF2 65. The result shows that formulation HF3 have contributed in most significant hair growth activity and also showed maximum extraction of active principles responsible for hair growth.

CONCLUSIONS

The hair growth studies finally prove that formulation DF3 have excellent hair growth promoting activity by an enlargement of follicular size and a prolongation of the anagen phase. When compared to the standard, it holds the promise of potent herbal alternative for minoxidil.

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APPENDICES

Table 1: Selection of Concentration of Oil for Hair Growth Activity

Amount of Drugs / 100ml of Oil g.				
Hair Formulation	***Eclipta alba* (%)**	***Bacopa monniera* (%)**	***Trigonella foenum-graecum* (%)**	***Eclipta alba* Juice (ml)**
HF1	5	10	10	400
HF2	10	5	10	400
HF3	10	10	5	400

Table 2: Evaluation of General Characteristics

Sl. No	Parameters	HF1	HF2	HF3
1	Colour	Green	Light Green	Greenish black
2	Odour	Characteristic	-	Characteristic

Table 3: Evaluation of Physical Parameters

Sl. No	Parameters	HF1	HF2	HF3
1	Specific gravity	0.9292	0.9383	0.9461
2	pH	9	8.3	7.5
3	Refractive index	1.504	1.472	1.434
4	Acid Value	2.38	2.16	1.9
5	Saponification value	256	257	258
6	Iodine value	8.91	9.81	10.62

Table 4: Qualitative Observation of Hair Growth

Sl. No	Group	Time Taken to Initiate the Growth (in Days)	Time Taken for Complete Growth (in days)
1	Control	8	25
2	Standard (2% Minoxidil)	7	21
3	HF1	10	24
4	HF2	8	20
5	HF3	6	16

Table 5: Mean Hair Length mm

Sl. No	Group	Mean Hair Length mm
1	Control	2
2	Standard (2% Minoxidil)	3.5
3	HF1	3
4	HF2	3.6
5	HF3	4.6

Table 6: The Rate of Hair Growth

Sl. No	Group	Anagen	Catagen	Telogen	% Hair Follicles > 0.5 mm in Length
1	Control	52	6	42	40
2	Standard (2% Minoxidil)	67	3	30	70
3	HF1	58	2	40	59
4	HF2	65	4	31	68
5	HF3	82	1	17	78