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Evaluating Hair Growth Activity of Herbal Hair Oil

Pushpendra Kumar Jain[1]*, Debajyoti Das[2], Puneet Jain[3]

[1]Faculty of Pharmacy, Naraina Vidyapeeth Group of Institutions, Panki-Kanpur, Uttar Pradesh, India.
[2]School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Bhubaneshwar, Odisha, India.
[3]Maharana Pratap Education Center, Kalyanpur, Kanpur, Uttar Pradesh, India.

Abstract: Herbs & herbal drugs are clinically proved good for hair growth. Hair loss problem is of great concern to both males & females & the main problems associated with hair loss are hair fading, dandruff & falling of hair. Various synthetic medicines are available for hair loss which does not treat permanently & also shows severe side effects. The main objective of this work is to develop such an herbal hair oil formulation which can resolve the problem related to hair fall & other hair diseases. Herbal drugs like *Emblica officinalis, Bacopa monniera* and *Cyperus rotundus* were selected for the formulations of poly herbal hair oil. The hair oil was prepared individually and in a varying concentration of all three herbs and a mixture of all the three herbs in fixed proportion using coconut oil as base. The formulated oil in varying concentration was evaluated physical, chemical and hair growth properties of formulated oil by applying it topically on shaved skin of albino rats. Primary skin irritation test, hair length test were performed and the hair growth was compared with standard Minoxidil 2% ethanolic solution using healthy albino rats. It was observed that hair oil formulation (HF3) showed the best result among the other formulation evaluated by showing an enlargement of follicular size and prolongation of the anagen phase.
Keywords: Hair loss, Herbal drugs**,** *Emblica officinalis, Bacopa monniera, Cyperus rotundus,* herbal hair oil, hair length.

Introduction

Hair loss is a distressing condition for an increasing number of men and women. Therefore it is of great importance, to develop new therapies for the treatment of hair loss. It is a dermatologic disorder, and the surge for discovering natural products with hair growth promoting potential is continuous[1, 2]. Hair loss, or alopecia, is a common patient complaint and a source of significant psychological and physical distress[3]. Androgens are considered to be one of the most important causes for alopecia apart from a variety of other factors[4]. Natural products in the form of herbal formulations are available in the market and are used as hair tonic, hair growth promoter, hair conditioner, hair-cleansing agent, antidandruff agents, as well as for the treatment of alopecia, dandruff and lice infection[5]. A number of herbal products have been acclaimed with hair growth–promoting activity[6]. The traditional system of medicine in India acclaims a number of herbal drugs for hair growth promotion. In our study, we have found that the Ethanolic extracts of *Emblica officinalis*, *Bacopa monniera* and *Cyperus rotundus* are useful in treating "Indralupta" (i.e., loss of hair)[7,8]. The present study was, therefore, undertaken to develop a formulation containing Ethanolic extracts of these drugs in the form of herbal hair oils in varying ratios & concentrations and evaluating the formulated oils for their hair growth initiating and hair growth promoting activity.

Emblica officinalis (Euphorbiaceae)

Fig. 1: *Emblica officinalis* fruits

Emblica officinalis is rich in vitamin C, tannins and minerals such as phosphorus, iron and calcium which provides nutrition to hair and also causes darkening of hair[11,14] in Fig.1 Part Used: Fruit.

Chemical constituents:

Alkaloids (Phyllantidine, Phyllantine), Vitamin C, Gallotannis (5%), Carbohydrates (14%), Pectin, Minerals, Phenolic acid, Gallic acid, Ellagic acid, Phyllemblic acid, Emblicol, Amino acid (Alanine, Aspartic acid, Glutamic acid, Lysine, Proline)[9]. Gupta et al investigated increase in hair growth activity of *Emblica officinalis*.

Fig. 2: *Bacopa monnieri* herb

Bacopa monnieri (Scrophulariaceae)

Bacopa monnieri is small herb with purple flowers. It grows in wet and sandy areas and near the streams in tropical regions. It is a creeping herb with numerous branches and small fleshy, oblong leaves. Flowers and fruits appear in summer. The stem and leaves of the plants are used[11,14] in Fig.2 Part used: Whole Plant.

Chemical constituents:

Alkaloids (Brahmin, Herpestine), Saponins (Monnierin, Hersaponin, Bacoside A, A3, B), betulic acid, D-mannitol Steroids (Stigmasterol, Sitosterol)[10].

Fig.3: Cyperus rotundus herb

***Cyperus rotundus* (Cyperaceae)**

Cyperus rotundus is a major weed of cultivated crops and gardens, but only a minor weed elsewhere[11,14]. It is encouraged by frequent cultivation and grows best in moist fertile soils in Fig.3 Part Used: Rhizome.

Chemical Constituents:

Sesquiterpenes like: α-Cyperone, β selinene, cicerone, cyperotundone, patchoulenone, sugeonol, it also contain other terpenes like: pinene (monoterpene) and other derivatives of sesquiterpenes such as cyperol, isocyperol and cyperone.[12]

Materials and Methods

Collection and authentication of plant

Dried ripe fruits of *Emblica officinalis*, entire herb of *Bacopa monniera*, rhizomes of *Cyperus rotundus* were purchased from Institute Herbal garden and also from local market. Specimen sample was authenticated from NBRI, Lucknow vide Specimen No NBRI SOP 202 dated 23.07.2010. The desired parts of plant drugs are cleaned to remove unwanted foreign material, shade dried and then crushed in with the help of mechanical device and passed through the sieve number 80. The powdered drugs were subjected for pharmacognostic screening for confirmation and characterization of phyto-constituents present in the drug sample.[13]

Procedure

After authenticating the drug and confirming from the literature survey. Herbal oil was prepared in a fix proportion containing all the three drugs (*Emblica officinalis*, *Bacoopa monari, Cyperus rotundus*). The oil of mixture of all the three herbs hair oil was formulated in varying concentration using coconut oil as base. There are various methods available for the preparation of hair oils, direct boiling method, paste method and cloth pouch method [10]. The preparation were prepared in the strength of (HF1) 5%, (HF2) 7.5% & (HF3) 10% as mentioned in Table 1, which were further evaluated for their Physical, Chemical and hair growth property.

Table 1: Selection of concentration of herbal extract % for preparing oil for hair growth activity.

Formulations	*Emblica officinalis*	*Bacopa monniera*	*Cyperus rotundus*
HF1	5%	5%	5%
HF2	7.5%	7.5%	7.5%
HF3	10%	10%	10%

Physical & Chemical Evaluation of Prepared Formulation

Formulated herbal oil were evaluated physically and chemically for the phyto constituent present as per the ayurvedic pharmacopeial standard for its general characterization which includes pH, Acid value, Refractive index, Specific gravity , Colour & Odour as mentioned in Table 2, 3 and 4.

Table: 2 Evaluation of General Characteristics of prepared formulation.

S.no	Parameter	HF-1	HF-2	HF-3
1	Colour	Greenish brown	Greenish brown	Greenish brown
2	Odour	Characteristic	Characteristic	Characteristic

Table: 3 Evaluation of Physical Parameters of prepared Formulation.

Physical Parameters	Concentrations		
	5%	7.5%	10%
pH	9.2	8.9	7.8
Acid Value	2.76	2.19	1.42
Specific Gravity	9.454	9.621	9.769
Refractive Index	1.498	1.426	1.361

Table: 4 Chemical evaluations of herbs used in formulation for the Phyto-constituents.

Name of analysis	Emblica officinalis *(Euphorbiaceae)*	Cyperus rotundus *(Cyperaceae)*	Brahmi *(Bacopa monnieri)*
Alkaloids	-	+	+
Glycosides	-	+	+
Carbohydrates	-	+	-
Saponins	-	-	+
Flavonoids	+	+	+
Proteins	-	+	-
Vitamin C	+	-	-
Tannin	+	+	+
Terpenoids	-	+	-

Primary skin irritation test

The protocol of the experiment was approved by the Institutional animal ethical committee as per the guidance of the committee for the purpose of control and supervision of experiments on animals by Animal Ethics Committee (Letter No.1122/ac/07/CPCSEA) Six healthy male albino rats, weighed 200-250g were selected for the study. Each rat was caged individually and kept at room temperature (24 ± 2^0C) on a normal day night cycle (06.00 hour to 18.00 hours) with standard diet and water. The animals were kept on fasting before the test for 24 hours. The hair from the back of each rat of $1cm^2$ was shaved as shown in Figure 4 on the side of the spine to expose sufficiently large test areas, which could accommodate three test sites were cleaned with surgical sprit 1mL quantity of formulations HF1, HF2 ,HF3, were applied over the respective test sites. The test sites were observed for erythema and edema for 72 hours after application of the prepared formulations. [15]

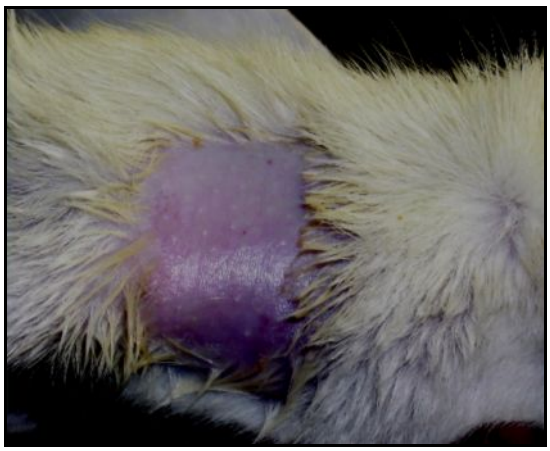

Fig. 4: Skin irritation test

Application of test formulation

Mouse model for the study of hair growth initiation was followed. The albino Wister rats were divided into three groups (five animals in each group) as group A, B, C respectively $2cm^2$ area of surface of each rat was shaved or denuded to remove all the hairs in Fig.4. Each albino Wister rat was caged individually and kept at room temperature (24 ± 2^0C) on a normal day night cycle (06.00 hour to 18.00 hours) with standard diet and water under the supervision of animal house Supervisor. In each group there were 05 animals and Animal No. 1 was treated with Coconut oil (base) and kept as controlled, Animal No.2 was with Minoxidil 2% solution and kept as Standard or Reference, Animal No.3, 4 & 5 served as Test Animals. Group A was treated with HF1, Group B was treated with HF2, Group C was treated with HF3, and with 0.5 mL of prepared formulations per day respectively .The hair growth initiation pattern was observed and recorded. The treatment was continued for 30 days and the hair growth activity was recorded at following stages described in Table 5. Hair growth activity was recorded at 00 days, 10 days, 20 days and 30 days.

Hair Growth study

Hair growth initiation time was observed and recorded i.e. the minimum time required to initiation of hair growth on shaved or denuded skin of albino wister rats, hair growth completion time was recorded i.e. minimum time required for the complete hair growth to cover the shaved or denuded skin completely. Mean hair length was calculated by plucking 25 hair from the shaved area / region were herbal hair oil formulation was applied after 10 days, 20 days and 30 days and measured using a vernier caliper or scale carefully .

Results & Discussion

The result and the observation which were recorded during the present work for general appearance of formulated herbal hair oil, physical and chemical evaluation of prepared formulations are shown in Table 2, 3 and 4.

Primary skin irritation test

Test was performed in order to evaluate the irritation effect of the prepared formulation on the denude intact skin of rats. The test sites were observed for erythema and edema for 72 hours after application of the prepared formulations[15]. It was observed that no erythema or edema was developed on the skin of test animals (albino rats) hence prepared formulation were non irritant to rat skin.

Hair growth activity

The results of preliminary hair growth initiation tests were quite satisfactory. They were recorded on the basis of time taken for initiation of growth and number of hair follicles. This parameter is recorded for finding minimum effective concentration of drug. The control group was treated with Coconut oil (base) and kept as controlled; Group 2 was treated with Minoxidil 2% solution and kept as Standard or Reference, Group

3, 4 & 5 served as Test Animals. The results for hair growth activity are shown in table 5. The hair growth studies finally prove that formulation HF2 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. HF-2 showed growth in 6-7 days at a concentration of 7.5% in Fig. 4, 5, 6, & 7 shows the results obtained after the biological screening. Thus the optimum growth was observed in HF-2 which shows that the herbal hair oil formulated is having promising hair growth activity.

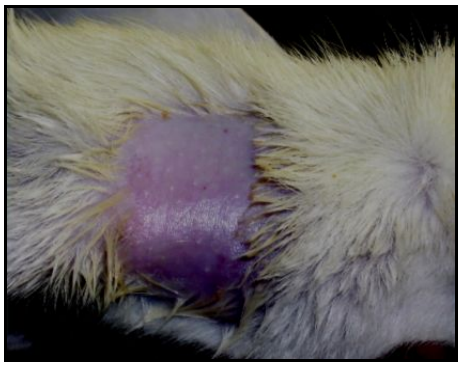

Fig. 4: At 0 day

Fig. 5: Hair growth initiation at 6th day with HF2.

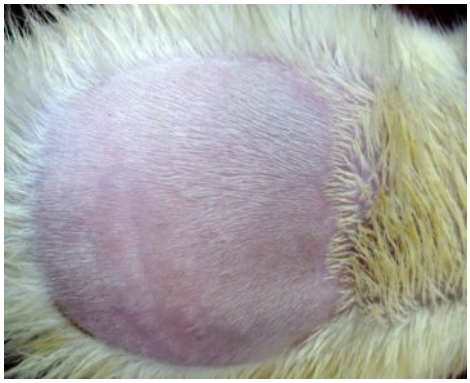

Fig. 6: Hair growth at 10th day with HF2

Fig. 7: Hair growth at 20th day with HF2

Conclusion

Among the various formulation HF2 showed good and satisfactory result for hair initiation and hair growth activity, it also shows the remarkable improvement in the length of hair and its diameter as compared to control , standard and other test formulations HF1 & HF3 . Hence it can be concluded that HF2 shows excellent hair growth promoting activity.

Table: 5 Qualitative analysis of Hair growth for the prepared Formulation.

Formulation	Number of Rats	Time required for Initiation of Hair Growth (in days)	Time required for Complete hair Growth (in days)
Control (Base)	05	07	23
Standard (2% Minoxidil Solution)	05	05	18
HF1	05	08	25
HF2	05	06	20
HF3	05	08	24

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