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

R. M. Thorat, V. M. Jadhav*, V. J. Kadam.**

Department of Quality Assurance, Bharati Vidyapeeth's College of Pharmacy, Sector 08, CBD Belapur, Navi-Mumbai – 400 614, India

Corres.author :drvmjadhav_bvcop@rediffmail.com, rupali.78@rediffmail.com
Telephone No. : (022) 27882901, 09766533037.

Abstract: Alopecia is a dermatological disorder with psychosocial implications on patients with hair loss. Eclipta alba Hassk [Asteraceae], Hibiscus rosa sinensis Linn [Malvaceae], Nardostachys Jatamansi [Valerianaceae] is a well known Ayurvedic herb with purported claims of hair growth promotion. Hair formulation of Eclipta alba Hassk [Asteraceae] 10% w/v, Hibiscus rosa sinensis Linn [Malvaceae] 10 % w/v, Nardostachys jatamansi [Valerianceae] 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 [82] which were higher as compared to control [52]. The result of treatment with oil were better than the positive control minoxidil 2 % treatment. It holds the promise of potent herbal alternative for minoxidil .
Key-words : Herbal hair formulation, Asteraceae, Malvaceae, Valerianaceae.

Introduction

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[1].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[2-4].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[5]. Synthetic drug, minoxidil is a potent vasodilator was scientifically proved for the treatment of alopecia [6-7] .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 which includes Eclipta alba Hassk
[10 % w/v], Hibiscus rosa sinensis Linn [10 % w/v], Nardostachys jatamansi [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 Hassk [Asteraceae], Hibiscus rosa sinensis [Malvaceae], Nardostachys jatamansi [Valerianaceae] is such herb with traditional claims of hair growth promotion[8] .Eclipta 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, wound healing[9] .The herb Hibiscus rosa sinensis Linn [Malvaceae] is a glabrous shrub widely cultivated in the tropics as an ornamental plant and has several forms with varying clour of flowers. The leaves and flowers are observed to be promoters of hair growth and aid in healing of ulcers. Flowers have been found to be effective in the treatment of arterial hypertension and have significant antifertility effect[10]. Dried rhizome of Nardostachys jatamansi DC [Valerianaceae] is a small herbaceous Himalayan genus, common in Garhwal, Kumaun and Sikkim Himalaya between 3000m and 5000m. It is used for hair growth , convulsions, sedative, treatment of hypertension, dysuria, cystitis and uterine inflammation[11] .

Materials and Methods

Collection and authentication of plant

The leaves of Eclipta alba, flowers of Hibiscus rosa sinensis, rhizomes of Nardostachys jatamansi were purchased from local market and authenticated by Agharkar Research Institute, Pune. The various parts of plant drugs are crushed in mixed 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, Hibiscus rosa sinensis, Nardostachys jatamansi were selected and mixed in 3 different concentration for maximum activity [Table 1]. The formula of base contain coconut oil.

Table 1 : Selection of concentration of oil for hair growth activity

Amount of drugs / 100 ml of Oil g.				
Formulations	**Eclipta alba %**	**Hibiscus rosa sinensis %**	**Nardostachys jatamansi %**	**Eclipta albaJuice ml**
DF1	5	10	10	400
DF2	10	5	10	400
DF3	10	10	5	400

Procedure

Mixed all 3 powders properly then add little quantity juice and make a paste. Heat the 100 ml 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. While doing this 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.

Primary Skin irritation test

Three 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 24 hrs prior to the test. The hair from the back of each rat of 1 cm2 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. 1 ml quantity of formulations DF1, DF2, DF3 were applied over the respective test sites of one side of the spine. The test sites were observed for erythema and edema for 48 hrs after application[12] .

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 enviromental 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 (IAEC/PR/2008/03).

The rats were divided into 5 groups of 6 rats each 6 cm2 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 1 ml of [2 % Minoxidil ethanolic solution] was applied over the shaved area, once a day. The animals of remaining groups were given application of 1 ml of formulation DF1, DF2 and DF3respectively, once a day. This treatment was continued for 30 days.

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.

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 10 days, 20 days and 30 days 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 10 µm 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 [13-15] .

Results and Discussion:

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

Table 2 : Evaluation of General Characteristics.

Sr.No.	Parameters	DF1	DF2	DF3
1.	Colour	Green	Green	Greenish black
2.	Odour	-	-	Characteristic

Table 3 : Evaluation of physical parameters.

Sr.No.	Parameters	DF1	DF2	DF3
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

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 / or 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 18 d in DF3 and 22 d in DF2. Thus on comparison DF3 and Minoxidil it has been observed that DF3 hair oil formulation application shows better growth that the patch with minoxidil. Mean hair length was 4.6 mm in DF3 and 3.6 mm in DF2.

The quantitative study revealed that formulation DF3 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 DF3 it was 82 and DF2 65. The result shows that formulation DF3 have contributed in most significant hair growth activity and also showed maximum extraction of active principles responsible for hair growth.

Conclusion

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.

Table 4 : Qualitative observation of hair growth

Sr No.	Group	No of Rats	Time taken to initiate the growth (in days)	Time taken for complete growth (in days)
1.	Control	6	8	25
2.	Standard (2% Minoxidil)	6	7	21
3.	DF1	6	10	24
4.	DF2	6	8	22
5.	DF3	6	6	18

Table 5 : Mean hair length mm

Sr.No.	Group	Mean hair length mm
1.	Control	2
2.	Standard(2%Minoxidil)	3.5
3.	DF1	3
4.	DF2	3.6
5.	DF3	4.6

Table 6 : The Rate of Hair Growth

Sr. 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.	DF1	58	2	40	59
4.	DF2	65	4	31	68
5.	DF3	82	1	17	78

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