Pharmacological Screening of Polyherbal plant extracts for Alopecia Management

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Abstract

Hair loss and alopecia is a common dermatologic disorder. Now-a-days variety of herbal Cosmetics are available in the market and are used as hair tonic, hair growth promoter, hair conditioner, hair-cleansing agent and antidandruff agents etc. these herbal products claim to have with hair growth promoting activity Herbal medicines are in great demand in both developed and the developing countries in primary healthcare because of their great efficacy and little or no side effects. These traditional systems of medicine together with homoeopathy and folklore medicine continue to play a significant role largely in the health care system of the population. The present paper deals with the evaluation of hair growth activity on polyherbal extract containing of ethanolic extract of *Trigonella foenum-graecum* L, *Eclipta alba* (L.), *Hibiscus rosa sinensis Linn*. and. *Allium cepa L* and it was concluded from the present work that combination polyherbal extract E1 have good result in management of alopecia.

Keywords: Hair fall , hydroalcohalic gel, *Hibiscus rosa sinensis Linn, Eclipta alba* (L.).

Introduction

The use of medicinal plants as raw materials in the production of new drugs is ever increasing because of their potentials in combating the problem of drug resistance in micro-organisms. Demand for medicinal plants is increasing in both developing and developed countries. Research on medicinal plants is one of the leading areas of research globally[1-2]. However, there is a need to pay closer attention to the issue of bioactivity-safety evaluation of medicinal plants.

Trigonella foenum-graecum L. (Fenugreek) commonly known as methi (in Hindi) has been used as a culinary spice, a flavoring agent and as a medicinal plant from ancient time. The seeds of fenugreek Fenugreek seeds are the most important and useful part of fenugreek plant. These seeds are golden-yellow in colour, small in size, hard and have four-faced stone like structure. The main chemical components of Trigonella foenum-graceum are fibers, flavonoids, polysaccharides, saponins, fixed oils and some identified alkaloids.

Eclipta alba (L.) commonly known as bhringraj as well as false daisy, is a species of plant in the family Asteraceae. It is a weed which grows in tropical and subtropical regions all over the world. Eclipta alba has been traditionally used in folk remedy, both Ayurveda and Siddha. Eclipta alba (L.) contains wide range of active

principles which includes coumestans, alkaloids, flavonoids, glycosides, triterpenoids. The leaves contain stigmasterol, β terthienylmethanol, wedelolactone, demethylwedelolactone and demethylwedelolactone-7-glucoside. The roots give hentriacontanol and heptacosanol. The roots contain polyacetylene substituted thiophene. The aerial part contains phytosterol, β-amyrin in the n-hexane extract and luteolin-7-glucoside, β- glucoside of phytosterol, a glucoside of a triterpenic acid and wedelolactone. The plant is known to have some important pharmacological activities such as antimicrobial, antinociceptive, analgesic, antiinflammatory, antiviral, hepatoprotective, immunomodulatory activity. Eclipta alba is used in hair oil preparations since it promotes hair growth and maintains hair black.

Hibiscus rosa sinensis Linn. (Family Malvaceae) is a plant which is widely distributed throughout the world. Its leaves, barks, roots and flowers have been used in the Indian traditional system as medicine to treat various diseases. In Ayurvedic medicine, hibiscus petal is was used to stimulate thicker hair growth and to prevent premature graying, hair loss and scalp disorders[1-7]

Material & Methods

Collection of plant material

The seeds of *Trigonella foenum greacum* and *Allium cepa L* were obtained from local market while *Hibiscus rosa sinensis, Linn* and *Eclipta alba (L.)* were collected from natural habitat and authenticated by Dr. S. N. Dwivedi, Prof. & Head, Department of Botany, Janata PG College, APS, University, Rewa, M.P.

Preparation of plant powder

The seeds of *Trigonella foenum greacum* were pulverized, sieved through 40 meshes to obtain a coarse powder. While flower of *Hibiscus rosa sinensis Linn*, leave of *Eclipta alba (L.)* were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for further use.

Preparation of extracts

About 250gm of dried powder of *Trigonella foenum greacum* seed, 250 gm flower of *Hibiscus rosa sinensis Linn* and 250 gm leave of *Eclipta alba (L.)* were subjected to soxhlation separately . It was first defatted with petroleum ether then exhaustively extracted with ethanol solvent in a Soxhlet apparatus for 36 hours. The temperature was maintained at 40-50 degree centigrade. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract.

For *Allium cepa L*. extraction first 100gms of freshly bulbs of allium cepa were cutted in small pieces. These pieces take into the mixer apparatus crush the content of allium cepa then collect it. This extract is passed through the muslin cloth to take the pure extract in filtrate. This extract is used for the formulation. [8-9]

Pharmacological Screening of Plant Extract[10-15]

Three different combinations of plants extracts: E1, E2 and E3 selected for in-vivo hair growth study. Both qualitative hair growth and quantitative hair growth activity performed. Qualitative hair growth analysis was

undertaken by visual observation of two parameters: hair growth initiation time and hair growth completion time. While hair length, hair weight and histological study performed in quantitative hair growth analysis.

Plant Extract	**E_1**	**E_2**	**E_3**
Seed extract of *Trigonella foenum greacum* (% w/w)	2.5	5	2.5
Flower extract of *Hibiscus rosa sinensis Linn*(% w/w)	2.5	2.5	5
Leave extract of *Eclipta alba (L.)* (% w/w)	5	2.5	2.5
Bulb extract of Allium cepa L. (% w/w)	0.5	0.5	0.5

Animals:

Healthy Wistar rats (200-250g) were used for the study. Rats were housed in small cages in environmentally controlled (25 ± 20C, 12h light and dark cycle, with free access to food and water *ad libitum*). Rats were fed with the standard laboratory chow diet during the period of study.

Treatment for hair growth activity in-vivo study:

In which research study to determine the Polyherbal extract to improve hair growth on the wistar albino rat. Thirty six wistar rats are taken in that study. Those 30 rats are divided in to the five groups each group contain six rats. In which first group served as control group where there no drug treatment. Second group as a standard where 2 % minoxidil was applied over the shaved area. Third group to fifth group was topically applied with different combination of extracts (E1,E2, E3) and served as test group .Hair from 3 cm^2 area at the dorsal portion of all the rat were shaved using electrical shavers and applied with marketed hair remover to completely remove hair. All the polyherbal extracts and minoxidil gel formulation were applied to the denuded area of the respective groups two times in a day and control group received no treatment. This treatment was continued for 30 days during which qualitative and quantitative parameter of hair growth was observed and recorded. *In-vivo* skin irritation study was conducted for 7 days and observed for any sensitivity and the reaction if any was graded as under -

A – No reaction, B – Slight patchy erythema, C –Slight but confluent or moderate but patchy erythema, D – Moderate erythema, E – Severe erythema with or without edema.

Qualitative Studies on Hair Growth study

Qualitative hair growth analysis was undertaken by visual observation of two parameters: hair growth initiation time (i.e. minimum time to initiate hair growth on denuded skin region) and hair growth completion time (i.e. minimum time taken to complete cover the denuded skin region with new hair).

Quantitative hair growth study

Hair length determination: Hairs were plucked randomly using sterile forceps from the shaved area of selected rats, from each group on 15th, 20th and 30th day of the treatment. The average length of 25 hairs was randomly selected and measured in millimeter and the results were expressed in mean ± SEM.

Histological studies: On the 10th, 20th and 30th day of treatment one rat from each group was authenticated and skin biopsies were taken from the shaved area and fixed in 10% formalin buffer. Sections of tissue were embedded in paraffin wax and sectioned in to uniform thickness of 10µm. The sectioned tissues were stained with haematoxylin and eosin. From the stained tissue the number of hair follicles per millimeter of the skin and the percentage ratio of different cyclic phases were examined using microscope fitted with an ocular micrometer facility. [10-15]

Results and Conclusion

Qualitative Evaluation of In Vivo Hair Growth.

Throughout the 30 days study period, all the groups of animals were observed closely to determine the hair growth initiation and completion time. This was achieved using a magnifying lens that enabled observation of minute changes in the hair growth pattern. The point at which a tiny prickle of hair growth was observed and it was noted as the initiation time. Combination of extract E3 treated animals showed significant reduction in hair growth initiation and completion time as compared to control and minoxidil treated animals (Table 1).

In control group animals, initiation of hair growth in denuded area was observed in 10 days. Hair growth initiation was noted in the first week (6 days) in mice of minoxidil treated standard group. The extract combination E1 exhibited hair growth initiation on 7th day whereas with E2 and E3 both extract combination recorded on 9 day respectively. Similarly the time taken for complete hair growth on shaved area was promoted with minoxidil treatment as well as gel formulation. Complete hair growth with minoxidil and control group as observed in 20 and 29 days respectively and in extract E1,E2 and E3 combination was 22,25 and 24th days respectively. (Table 1).

Qualitative observation of hair growth

Table No 1 Effect of Extracts on Hair Growth Initiation and Completion Time

Group	Treatment	Time taken to initiate the growth (in days)	Time taken for complete growth (in days)
Group I	Control	10	29
Group II	2% Minoxidil	6	20
Group III	E1 Extract combination	7	22
Group IV	E2 Extract combination	9	25
Group V	E3 Extract combination	9	24

Quantitative hair growth Study

Hair length Determination

The length of the hair began to increase until the end of the treatment course. The E1 combination of extract produced a greater effect on the length of hair when compared to other group animals hair being 1.39 mm at the end of the course (30^{th} day), compare to 1.29 mm in the E2 ,1.29 mm in the E3 and 1.38 mm in minoxidil and 1.29 mm in control groups which received simple gel treatment. E1 combination of extract treated groups produced a greater effect on the length of hair when compared to other groups. This may be due to the premature switching of follicles from the telogen to anagen phase of hair growth cycle. Average hair length of each group at 10^{th} day, 20^{th} day and 30^{th} day has been given in Table.2.

Table No 2 Effect of Extracts different combination on Hair length

Group of	Formulation	Hair Growth in mm Mean+ S.D		
		10 days	20 days	30 days
Group I	Control	0.0	0.312	1.21
Group II	2% Minoxidil	0.6	1.16	1.38
Group III	E1 Extract combination	0.6	1.18	1.39
Group IV	E2 Extract combination	0.62	0.92	1.29
Group V	E3 Extract combination	0.69	1.01	1.37

Histological studies

E1,E2 and E3 different combination of extract on the development of hair follicles

The hair follicle count, skin thickness and color appearance were observed. E2 and E3 combination of extract on topical application on rats shows increase in the appearance of colour was only from day 20 to day 30. Whereas E1 combination of extract positive changes observed from day 10. E1 combination showed significantly considerable results compared to control. Combination E1 exhibited significant increase in hair regrowth. Increase in the thickness and presence of hair follicles in the subcutis layer were taken as an evidence for transition of follicles from telogen to anagen phase of hair growth.

A considerable difference in cyclic phases of hair growth was observed in groups treated with minoxidil, E1 ,E2 and E3 combination extracts. An increase in the number and size of hair follicles has been designated as an indicator for the transition of hair growth from the telogen to anagen phase.

To examine the progression of hair follicles in the hair cycle, hematoxylin – eosin staining was performed, since an increase in size and number of hair follicles can be observed in the deep subcutis (Datta *et al.,* 2009). The photomicrographs obtained indicated that control (simple) treated animals had less percentage of anagenic hair follicles (53.3 %) while the E1 (70.8 %), E2 (58.8%) ,E3 (66.4%) and minoxidil (71.4 %) treated animals showed maximum percentage of anagenic hair follicles and higher follicle density. (Table.3)

Table No 3 Effect of Extracts different combination on per cent of hair follicles

Group of 20 days	Formulation	Anagen	Telogen	T/A ratio
Group I	Control	53.3	45.4	0.85
Group II	2% Minoxidil	71.4	28.2	0.39
Group III	E1 Extract combination	70.8	25.5	0.36
Group IV	E2 Extract combination	58.8	39.3	0.67
Group V	E3 Extract combination	66.4	30.2	0.45

Skin Irritation Study

The skin irritation test was conducted for a period of seven days and the results are tabulated in Table 4. The results indicated that the control preparation, test extract combination E1 and marketed products did not cause any skin reaction. It can be assured that plants extracts did not cause any skin irritation and can be used in the gel formulation.

Table 4: Skin irritation study

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	A	A	A	A	A	A
E1 extract combination	A	A	A	A	A	A	A
Voveran emulgel	A	A	A	A	A	A	A

A – No reaction, B – Slight patchy erythema, C –Slight but confluent or moderate but patchy erythema, D – Moderate erythema, E – Severe erythema with or without edema.

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