

Concomitant Effect Of Present Herbal Formulation With Marketed Allopathic Formulation For The Evaluation Of Hair Growth

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ABSTRACT

Herbal formulations for all time have engrossed great attention because of their good action and relatively minor or nil side effects with synthetic drugs. The purpose of present study involves preparation of herbal extract using *Embllica officinalis* with minoxidil 2% solution, its assessment for increase in hair growth activity. Herbal extract was tested for their hair growth activity with synthetic marketed formulation. Based on this outcome mixture of crude drugs fruits of *Embllica officinalis* and minoxidil 2 % solution was prepared in varying concentration in the form of herbal extract by different concentration and were veteran for hair enlargement activity. The herbal extract of different concentrations were describe for proximate analysis including moisture content, water soluble ash, total ash, water insoluble ash, acid insoluble ash, sulphated ash. Chemical tests to decide the presence of active element in the drugs. The Herbal extract drug used for the study and showed outstanding hair growth action with standard (2% minoxidil ethanolic solution) by an enlargement of follicular size and prolongation of the anagen and telogen phase. It holds the assurance of potent herbal extract with minoxidil give outstanding results of hair growth were seen in this study.

Keywords: Minoxidil, Herbal formulation, Extraction, *Embllica officinalis*, Hair growth

How to cite this article: Sheikh SA, Purohit P, Teja Ram, Shrimali A. Concomitant Effect Of Present Herbal Formulation With Marketed Allopathic Formulation For The Evaluation Of Hair Growth. Int J Drug Deliv Technol. 2026;16(40s): 133-136. DOI: 10.25258/ijddt.16.40s.14.

INTRODUCTION

The skin of almost entire the human body contains hairs. Only the palms of the hands, the soles of the feet and nails are hairless. Hair is composed of strong structural protein known as keratin. This is the similar variety of protein that create the nails and the outer layer of skin. Hair is one of the vital parts of the body considered to be protective appendages on the body and accessory structure of the integument along with sebaceous glands, sweat glands and nails. At the present time hair loss is common problem in men and women due to excessive exposure of chemicals in daily routine on scalp.

Alopecia or hair loss is a very common and distressing symptom. Alopecia affects approximately 50% of men over 40 year of age and may also affect just as many women. Human have over 2 million hair follicles, which collectively may have significant positive and negative influence on skin health. Synthetic drug, minoxidil is a potent vasodilator was scientifically proved for the treatment of alopecia. Hence the present study was aimed to evaluate the hair growth activity of herbal formulation which includes *Embllica Officinalis*¹.

Amla is one of the most often used herbs in Indian ayurveda. *Embllica officinalis* is a small to medium sized deciduous tree belonging to family *Embllica Officinalis* and 8-18 meters height. It is small or medium size tree

found all over India, the sea-coast districts and on top of hill slopes up to 200 meters, also cultivated in plains. It is also found in Sri Lanka, Nepal and Burma. *Embllica Officinalis* is used in Skin Cancer, Antimutagenicity, Antigenotoxicity, Antibacterial, Antihyperglycemic, Antioxidant².

METHODOLOGY

Animals

The study was carried out in rats of Wister strains of either sex weighing 150-200 gm. 2-3 months old. They were procured from animal house of the Swami Keshvanand Institute of Pharmacy, Bikaenr; and were kept individually under standard laboratory condition. Food pellets and tap water were provided and libitum. Ethical clearance for experimental studies was obtained from institutional animal Ethical Committee³.

Plant material

The using crude drug (*Embllica officinalis*) was purchased from local market and its identity was confirmed. It was dried well to make powder. Coarse powder of the dried fruit was prepared with the help of the grinder. Hydro alcoholic extract of the powdered drug was prepared by Soxhalet's apparatus.

Preparation of extract

The air-dried parts of the plants were powdered and extracted with 95% ethonal, petrolium ether (40-60) and aqueous solvent systems by hot percolation method by using Soxhlet apparatus assembly at a controlled temperature. After complete extraction, marc was pressed to assemble the micelle, assorted with the contents of RBF, filtered and rigorous to obtain the extract. The color and consistency of the extract was noted⁴.

Chemicals:

Sulphuric acid, sodium hydroxide, fehling's solution (A and B), hydrochloric acid (HCl), Mayer's reagent, Dragendorff's reagent, ferric chloride, Ammonia solution, chloroform and dichloromethane were purchased & distilled water was prepared in the department of pharmacology, Swami Keshvanand Institute of Pharmacy, Bikaner^{5,6}.

Determination of acute toxicity (LD50)

The acute toxicity of petroleum ether, mathanolic and aqueous extract of plant *Embllica officinalis* were determined in wister rat. The animal were fasted overnight prior to the experiment, fixed dose method of OECD guideline no. 420; (Annexure-2d) of CPCSEA was adopted for this purpose^{7,8}.

Toxicity studies of petroleum ether and ethanolic extracts of *Embllica officinalis* were carried out on rats, when topically applied in a concentration of up to 5% did not show any toxic side effects or erythma on skin cells. Consequently the prepared extracts were considered protected for topically administration⁹⁻¹¹.

Animal Dose

Table 1 Rat were divided in to 4 groups of 4 animals in each, the various group were treated as follows:

Group	Treatment	Dose	Route of drug administration
Group I	Control	-	-
Group II	Standard Minoxidil	2% solution (0.2ml)	Topical
Group III	Extract of Embllica Officinalis	0.2 ml	Topical
Group IV	Minoxidil+ Extract	2% solution+ 0.2ml	Topical

Testing for Animals:

Testing of animals, for the purpose of pharmacological determination and analysis of results were done from PRECISION PATH LAB, Jaipur are as follows.

- Skin Biopsy
- Haematology
- Total serum protein estimation

Collection and Authentication of Plant:

Identification of the fruit of *Embllica officinalis* was done from University of Rajasthan (Jaipur, Rajasthan).

Reference no. : RUBL211430

Plant is authenticated by Vinod kumar Sharma, Botanist, UOR, Jaipur. Material was shade dried at room temperature and powdered mechanically and passed through a sieve #40.

Experimental procedure

Screening of hair growth potential was evaluated in Wister albino rats weigh 150 –250 gm. The hairs of the dorsal part of the Wister rats (2-3cm) clipped with scissor and the hair removed after the application of hair removal cream (Anne French). For the topical application, only the hair of one area on the dorsal surface was removed. After removal of hair, animals divided into four groups, each group containing 5 animals. Group I was normal without any application. Group II apply topical application of 0.2 ml of extract of *Embllica officinalis* and group III apply topical application of 0.2 ml standard Minoxidil (2%) and group IV apply topical application of combination of minoxidil (2%) + extract of *Embllica officinalis* 0.2 ml. The hair regions were observed for hair growth initiation afterward the length of hair was measured at 1st 7th, 14th and 21st day and 26th day^{13, 14}.

RESULT AND DISCUSSION

Determination of Pharmacological analysis:

Table 2: Effect of formulation on Total serum protein Count of animals in alopecia

S . N .	Biochemic al Cont ent	Rat Bioche mical Refere nce Range s	Observed Value After Treatment				Un it
			Con trol	Mino xidil	Ext rac t	Extr act + mino xidil	
1	Total Seru m Protei n	5.6-7.6	7.3	7.4	7.3	7.5	g/d L
2	Albu min	3.8-4.8	3.1	4.2	3.9	4.6	g/d L
3	Total Billir ubin	0.2- 0.55	0.4	.4	0.4	0.5	mg /d L

Table 3: Effect of formulation on Hematology Count of animals in alopecia

CB C	Rat Hemat	Observed Value After Treatment
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S.N		ologic Reference Ranges	Control	Minoxidil	Extract	Extract + minoxidil	Unit
1	Hemoglobin	11.5-16.1	12.4	12.8	12.6	13.2	g/dL
2	Total Leucocyte Count (WBC)	6.6-12.6*10 ³	7500	7600	7500	7700	mm ³
3	RBC	6.76-9.75*10 ⁶	6.87	7.52	7.12	7.81	mm ³
4	Platelet Count	150-460*10 ³	62000	65000	63000	65000	mm ³

Table 4: The Rate of Hair Growth (Histopathology/Skin Biopsy)

S.N.	Group	Anagen
1	Control	20%
2	Extract	20%
3	Minoxidil	30%
4	Minoxidil+extract	40%

Table 5: Mean Hair Length (mm)

S.N	Group	Mean Hair Length (mm)
1	Control	2.3
2	Standard (2% minoxidil)	2.7
3	Extract	2.6
4	Minoxidil+Extract	3.4

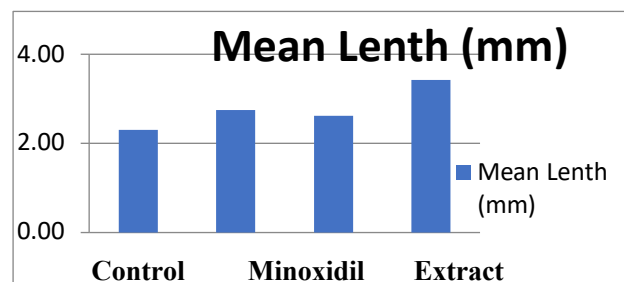


Fig 1: Mean hair length (mm)

Table 6: Qualitative Observation of Hair Growth

S.N	Group	Time Taken to Initiate the Growth (in days)	Time Taken for Complete Growth (in days)
1	Control	10	23
2	Standard (2% minoxidil)	8	20
3	Extract	9	20
4	Minoxidil+Extract	7	19

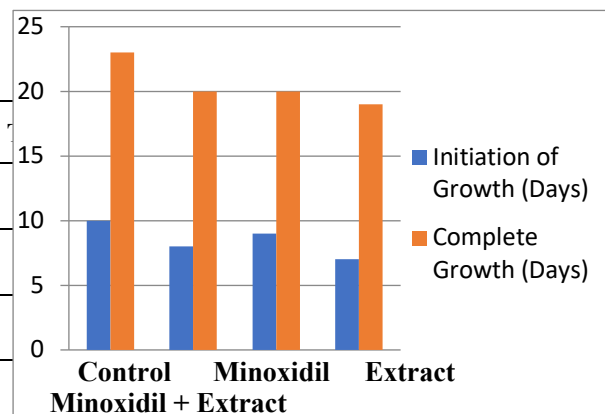


Fig 2: Qualitative Observation of Hair Growth

SUMMARY

Baldness is a common problem found in both male and female. There are very limited drug treatments available for the baldness among these Minoxidil is a popular drug and is found to be safe also. While after few year use there is decline in the regrowth of hair. There are also reported some side effects with the minoxidil. Thus the objective of the present study was to evaluate the effect of *Emblca officinalis* (Amla) on hair growth pattern in Wister rats. Primary skin irritation test was conducted to evaluate the irritation by the prepared herbal extract of *Emblca officinalis* (Amla) on skin of Wister rats. After evaluation of herbal, the prepared formulations were not showed any erythema or edema; this indicates that the prepared formulations were nonirritant on skin of Wister rats.

The results of preliminary hair growth initiation tests were encouraging. They were recorded on the basis of time taken for initiation of growth and number of hair follicles and also estimation of total leukocytes content

in blood and plasma protein estimation. This parameter is recorded for finding minimum effective concentration of drug. Amla extract showed significant growth with minoxidil solution and growth was observed in 7-8 days. Results obtained after the biopsy of skin.

The significant quantitative changes shown by herbal extract prompted the hair growth activity with minoxidil (standard drug). It was observed that the herbal Preparation showed excellent activity in combination with standard better than the single formulation of extract and standard minoxidil drug. While minoxidil be evidence for entire hair growth in 20 days and combination of minoxidil with Amla showed complete hair growth in 17 days.

The quantitative study revealed that formulation of minoxidil with Amla extract showed considerable increase in number of hair follicle in anagen phase of hair growth cycle when compared to control and standard. In standard group, percentage of population of anagen follicle was 30% while in combination formulation it was 40%. Only extract formulation were shown time of initiation of hair growth late when compared with standard with extract. It also observed that the time taken for complete hair growth the late initiation and completion of hair growth was 23 days in extract indicating late initiation and completion of hair growth. The result shows that formulation of minoxidil with extracts have contributed in most significant hair growth activity.

CONCLUSION

The hair growth studies finally prove that formulation of Minoxidil with extract of Amla have significant increase in hair growth activity when compared to the standard. It holds the promise of potent herbal combination alternative intended for minoxidil. Also recommend admirable results for hair growth in formula. The various constituents of the herbal extracts such as minerals and amino acids may be the cause for the significant hair growth activity. All these drugs not only show remarkable activity but are also devoid of potential side effects as compared to synthetic drugs.

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