RESEARCH ARTICLE

Formulation and *In-vivo* Assessment of Topical Polyherbal Hair Serum to Promote Hair Growth

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ABSTRACT

Male pattern baldness, or androgenic alopecia, is a term used to describe hair loss that results from the underlying sensitivity of hair follicles to androgenic shrinkage. Alopecia is caused by environmental factors, hormone imbalances, and/or inherited factors, according to research studies, however, many etiologies are still unclear. The purpose of treating androgenic alopecia is to slow down the thinning of hair and promote hair growth. A small number of natural treatments, along from allopathic medicines, have demonstrated effectiveness in the decades-long combat over alopecia. The main object of the present study was to develop and *in-vivo* evaluation of hair growth serum. Crude herbs extract of *Hibiscus rosa-sinesis, Glycyrrhizza glabra, Eclipta alba, Withania somnifera* and *Bacopa monnieria* were specifically weighed to make polyherbal serum and animal models are rats with induced androgen test to access for hair growth promoting activity. To assess the serum's potential against alopecia, it was applied topically. Testosterone had been administered subcutaneously for 20 days to cause alopecia in albino rats. Serums made of herbal extracts were administered concurrently to prevent androgen from having the ability to cause baldness. A positive control was provided with finasteride. At the conclusion hair growth activity was assessed visually, follicular density was measured using an ocular micrometer, and the anagen/telogen ratio was measured using an animal skin sample.

Keywords: Alopecia, Hair growth, Testosterone, Finasteride, Hair serum.

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INTRODUCTION

The most prevalent type in loss of hair in males, androgenic alopecia, consider as hair loss from the scalp in which there is a decrease in hair production that may ultimately result in balding. It is assumed that the continuous loss of pigmented, visible hair on the terminal on the scalp as a result of androgens in the blood.[1] The activity or concentration of enzymes, such as 5-alpha-reductase, which produces dihydrotestosterone (DHT), a powerful form of testosterone, in the scalp and hair follicles, or the sensitivity of hair follicle receptors to DHT, have been related to androgenetic alopecia.[2] 5-alpha-reductase-blocking drugs for androgenetic alopecia have been developed; however, their effectiveness has varied and they occasionally have major side effects. From ancient time we saw that most products that originated from plants, animals, and minerals used in the basic treatment of human diseases and based on the availability of traditional medicines 80% of people in developing countries still depend on natural products.[3]

Topical polyherbal serum was examined for hair growth-promoting activity. Androgenic alopecia was instigated in albino species rats by injecting testosterone for 20 days from the subcutaneous route. Here we evaluated how loss of hair was inhibited by using of polyherbal extract serum and parameters like hair follicle density, a ratio of anagen/telogen (A/T) and animal skin were observed visually. In this treatment topically finasteride is used as a standard solution.[4]

MATERIALS AND METHODS

Plant Material

Plant material of *Hibiscus rosa-sinesis* (flower), *Glycyrrhizza glabra* (roots), *Eclipta alba* (plant), *Withania somnifera* (root) and *Bacopa monnieria* (leaf) was used in this study and the specimen was examined in Botany department, Janata PG college, A.P.S University, Rewa M.P. (Herbarium Specimen No. J/Bot./2020-083BMH, J/Bot./2020-084EAWP, J/Bot./2020-085GGR, J/Bot./2020-086WSR, J/Bot./2020-088HRSSPLB).

Preparation of Test Sample

Several herbal serum formulations were created and showed that the result of this formulation was satisfactory and reliable for hair growth. Ingredients incorporated in serum are shown

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in Table 1. There were two phases to the formulation: an oil phase and an aqueous phase. At the beginning, the oil phase ingredients were placed in a beaker and homogenized thoroughly for 15 minutes at 1000 rpm. Next, tween 80, an emulsifying agent, was added. Subsequently, the oil phase was continuously stirred while the aqueous phase was gradually introduced. The extract of all herbs was added to the formulation and homogenized for 15 minutes at the same rpm to create a serum formulation. Next, both phases were adequately homogenized for more than 20 minutes at the same rpm. The prepared serum was then stored in a container with the pH adjusted to between 6 to 7.[5,6]

Chemicals

Finasteride was used as the reference standard for activity promoting hair growth. Normal saline has served as a control.

Animals

Male wistar albino rats weighing between 150 and 200 grams were chosen for the investigation. Every animal was housed in a conventional polypropylene cage with adequate ventilation, a twelve-hour light-dark cycle, and a room temperature of 27 ± 2ºC and 60–70% relative humidity. They were given water and a regular rat pellet to eat.[7] The protocols were all carried out in compliance with the guidelines set forth by the Committee on Institutional Ethics, which was established under the Ministry of Animal Welfare Division, Government of India, New Delhi, with the aim of supervising and controlling animal experiments (CPCSEA) (IAEC/SCOPE/21-22/01).

Application of Test Sample

Hair clippers and electric shavers were both employed to remove the rats' dorsal hair. To completely remove all hair from a 1 sq cm area, Veet, an advanced hair remover, was used. Surgical spirit was used to clean the area that had been shaved.[8,9]

Table 1: Formulation

Ingredient	*Quantity %*
Hibiscus rosa-sinesis	10
Glycyrrhizza glabra	5
Eclipta alba	5
Withania somnifera	5
Bacopa monnieria	5
Coconut oil	3
Glycerin	5
Vitamin E	1.5
PEG 6000	1
Tween 80	2
1% Sodium citrate	q.s
Salicylic acid	2
Cetosteryl alcohol	2
Potassium sorbate	0.5
Water	q.s

Primary skin irritation test

Measured quantity of hair serum was applied over the preselected and already marked test sites of all animals. These sites were under observation for erythema and edema for 48 hours after application of test serum. [10]

In-vivo screening of serum against hormonal-induced alopecia

In male albino rat alopecia was induced by administered testosterone 20 days.

Test solution (Testosterone)

A suspension of sterile testosterone (1% w/w) was made in an aqueous carboxyl cellulose solution.

Finasteride solution

A 2% standard finasteride solution was made in an 8:1:1 vehicle containing ethanol, propylene glycol, and water.

Animals study for test treatment

Three groups of six rats each comprised the experimental animals. Except for group I, all groups' animals received subcutaneous testosterone injections. The following was how each of the groups was handled:

Group I: Normal control (without Testosterone)

Group II: Standard group (1% Testosterone solution + 2% Finasteride solution)

Group III: Test group (1% Testosterone solution + HHS formulation solution)

All groups of rats received 0.1 mL of testosterone (SC) per day. Group I, II, and III animals received topical applications of finasteride, vehicle, and polyherbal hair serum. Three groups of six mice each were used for the formulation, reference, and control of the animals. Approximately 1-mL of a vehicle and finasteride serum formulation were applied topically to the back skin of groups II–III animals once a day for 20 days.[11] Following a 21-day treatment period, randomly selected rats from each group were sacrificed. Through visual observation, the variations in hair growth within each group were noted. Each rat group's balding site was also used for skin biopsies, which were preserved in formalin that has been buffered with phosphate for paraffin sectioning.[12]

RESULTS AND DISCUSSION

After topical application of hair serum, any unwanted sign of erythema and edema did not see. The percentage ratio and quantity of hair follicles per millimeter of skin were assessed in sections from each group. The result of hair follicles during several cyclic stages, such as the anagen between the telogen (resting phase) and development phase, is ascertained under a microscope in Tables 2 and 3. Visual observation of rats in groups II and III did not exhibit any symptoms on day three, however, rats in group I displayed alopecic signs and began shedding hair from the upper dorsum on day seven. In experimental animals of group II and III alopecic condition was not visible, in this group standard Figure 1 C and serum extract Figure 1D was applied along with testosterone.[13]

Table 2: Effect of polyherbal serum on hair growth

Groups	*Animal hair length 21 days (in mm) Mean ± SD*
Normal control	11. 263 ± 2.57
Toxic group (Testosterone solution)	5.551 ± 2.10
Standard group (Finasteride solution + Testosterone)	11.235 ± 5.21
HHS+ Testosterone)	11.216 ± 0.26

Table 3: Different groups of animals for follicular density and A/T ratio

Groups	*Follicular density (no./mm) Mean ± SD*	*Anagen/telogen ratio*
Normal control	2.75 ± 2.57	1.61
Toxic group (Testosterone solution)	1.05 ± 2.10	0.86
Standard group (Finasteride solution + Testosterone)	2.76 ± 5.21	1.50
Test extract extract (TBPE 5% w/v + Testosterone)	2.22 ± 0.26	1.20

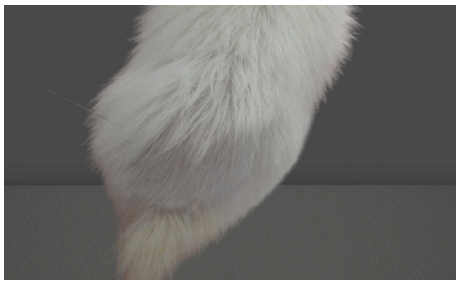

Figure 1A: Animal of control group (without testosterone)

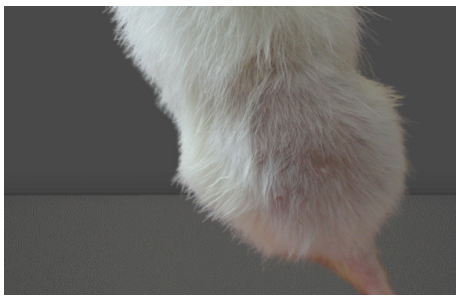

Figure 1B: Animal of toxic group testosterone

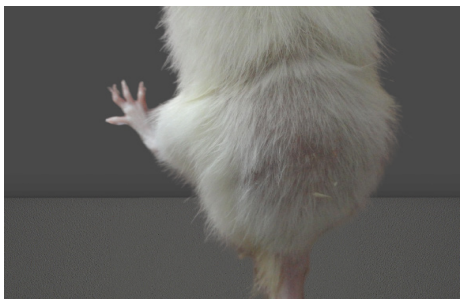

Figure 1C: Animal of standard group

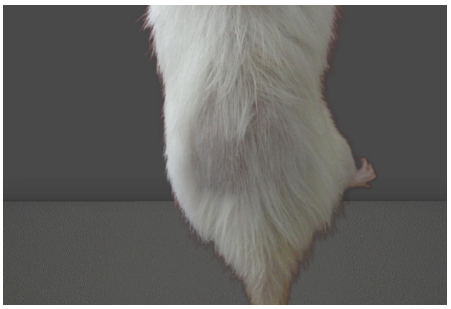

Figure 1D: Animal of test group

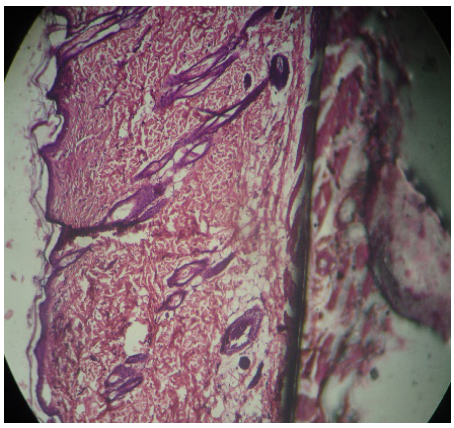

Figure 2A: Histology of skin section of control group

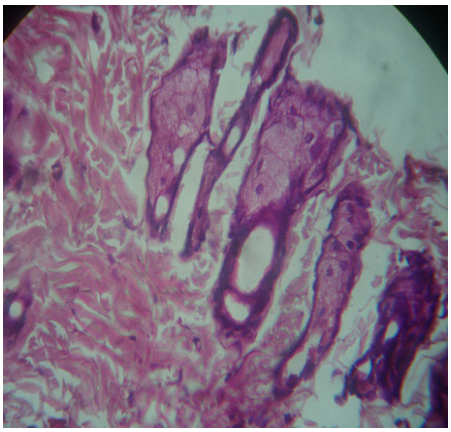

Figure 2B: Histology of skin section of standard group

Observation revealed that extracts prevented the action of testosterone and blocked testosterone-induced hair loss in Figure 1B. Rat in group I (Control) had hair loss on a regular basis, observed in Figure 1A with the posterior back of the animal affected as well. At the conclusion of the research period on day 21, group I showed signs of scattered alopecia, but hair loss in the other groups persisted from the posterior back rather than the upper dorsum. These animal groups did not exhibit the alopecic condition, indicating that the finasteride and extract suppressed testosterone-induced hair loss by blocking its activity. The group I animals' skin sections under a microscope showed that the shrinkage of hair follicles was caused by testosterone.[14] Result of histopathology of group II and III were shown in Figure 2B and 2C and many of the hair follicles in the group I rats were in the telogen phase Figure

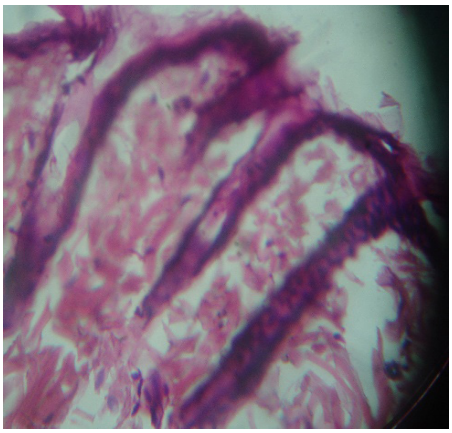

Figure 2C: Histology of skin section of test group

2A because they exhibited traits common to telogen follicles, such as being hollow and short, having necrosis, having more follicles damaged, and shrinking, which is defined as a drop-in diameter rather than a deeper follicle. Because extract prevented the effect of testosterone on hair follicles, the number of follicles in the anagen phase was significantly enhanced and the number in the telogen phase was decreased in group III mice. The process of miniaturization was also impeded by petroleum ether extract. It was also observed that the quantity of hair follicles had increased. Rats from groups II and III had follicles that exhibited anagen follicle features. [15]

CONCLUSION

At the end of the research period, saw group I exhibit scattered alopecia; however, hair loss continued from the posterior back, not the upper dorsum, in the other groups. As a result of the finasteride and extract's ability to inhibit the activity of testosterone, the alopecic condition was not present in these animal groups. Treatment with an herbal formulation had a noticeable impact on the start and completion times of hair growth in albino rats with balding skin. The treatment was superior to the conventional finasteride treatment and not only shortened the duration of hair growth beginning over control. The hair had a lustrous, silky, and smooth texture having impactful crude extract in the formulation and the promising activity was caused by the hair follicles transitioning from the telogen phase to the anagen phase and maintaining the late anagen hair follicle more.

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