PharmacologyOnLine December 30, 2012
Archives • 2012 • vol.3 • 150 - 157

EFFECT OF SOME NOVEL MEDICINAL PLANTS AND POLYHERBAL FORMULATION ON STRESS INDUCED ALOPECIA

Amjadkhan Pathan[1*], Mehrajkhan Pathan[2], Navneet Garud[3], Akanksha Garud[4]
[1]Jaslok Hospital & Research Centre, Mumbai, Maharashtra, Inda
[2]R.D.Bhakt College of Pharmacy Jalna, Maharashtra, India
[3]School of Studies in Pharmaceutical Sciences, Jiwaji University, Gwalior (M.P.), India
[4]Institute of Professional Studies-College of Pharmacy, Gwalior(M.P.), India
*akanksha.garud@gmail.com

Summary

Alopecia is a medical term for hair loss or thinning of hair can be a sign of serious diseases especially if the hair loses rapidly. Alopecia is dermatological disorder.

Hibiscus rosa sinensis, *Calotropis gigantea* and Polyherbal formulation in combination of both the plants extract. The study observations and results were compared with Minoxidil in stimulating hair growth in stress induced alopecia animal model.

Thus on comparison HRSF, CGF, HCF and Minoxidil it has been observed that HRSF as well as HCF herbal formulation application shows better growth that the patch with minoxidil.

Key words: Alopecia, Hibiscus rosa sinensis, *Calotropis gigantean*, Minoxidil

Introduction

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 is a medical term for hair loss or thinning of hair can be a sign of serious diseases especially if the hair loses rapidly. Alopecia is dermatological disorder that has been recognized for more than 2000 years[7] & is common throughout the world and has been estimated to affect between 0.2 % and 2% of the world population[18]. Apart from metabolic and hereditary causes alopecia has been observed as a major side effect of anticancer, immunosuppressant and many other drug treatments. Many people have tried conventional hair restoration methods such as laser treatments, harsh chemicals, pharmaceutical drugs or even surgery. Often these methods have limited success. The usage of synthetic drugs like minoxidil and finasteride (approved by FDA) have abbreviated due to their side effects.[16]

These crises lead to the search for natural products from plant origin possessing potential hair growth activity. The folkfore claim of medicine in various regions in the country and worldwide acclaims the hair growth promotion of medicinal plants belonging to various families, but lack of scientific literatures limited the use of these plants among community. Herbal medicines are a natural alternative for hair restoration, gray hair reversal and/or overcoming the health disorders that often result in thinning of hair. Many plants such as *Cuscuta reflexa* Roxb, *Prunus dulcis* seeds[16] and herbal formulations of *Hibiscus rosa-sinensis* Linn.[1,18] are evaluated in *in-vivo* & *in-vitro* conditions and found effective in traditional system of medicine for hair growth promotion[8]. Natural products are unequivocally advocated in the cosmetic and hair care industry and about 1000 different plant extracts have been examined with respect to hair growth activity; proanthocynidine from grape seeds (*Vitis Vinifera*) and beta-sitosterol in saw palmetto (*Serenoa serrulata*) have shown remarkable effect[17]. There are many products available prepared by combination of one or more herbal drug that find acceptability as hair tonics, hair growth promoters, hair conditioners, hair cleansing agents, antidandruff agents and for the treatment of alopecia and lice infection[15]. Vitamin deficiencies, poor nutrition, chemotherapy or hormonal problems can all cause or worsen cases of hair loss. Herbal remedies can contribute to restoring the body's balance. Recently introduced a mouse model launching experimental evidence that stress-induced hair loss is fact, and not fiction, as every so often imputed by a number of dermatologists. This mouse model provides new insights in to the pathophysiology of stress-induced hair growth inhibition and permits exploration of various strategies for therapeutic intervention[2]. When an individual experiences intense stress chemicals in the body will transmit signals to the hair follicles, which causes them to enter a resting phase. During this phase there is no new hair growth. During the next few months hair will be shed normally but new growth will not occur to take its place. This uneven pattern can cause hair to appear thinner and eventually result in hair loss. In this model exposure to sonic stress inhibits the growth of a hair shaft producing (anagen) hair follicle by premature induction of hair follicle regression (catagen) and up-regulated keratinocyte apoptosis[10].

The Present study is aimed at revealing the effect of some novel medicinal plants to diminish the stress induced alopecia on the basis of chemical constituents which are involved in induction of hair growth found in the above mentioned & other reported plants and herbal formulations, so that such formulations can be prepared which is likely to be more effective and less harmful compared to synthetic products available in the market. The study will evaluate the effect of various solvent extracts of selected plant on hair growth initiation and promotion in albino rats.

Materials and Methods

Animals

Wistar strain albino rats of either sex weighing between 120-150 g werw used. The animals were housed under standard laboratory conditions,maintained on a 12h light and dark cycle and had free access to food and water. Animals were acclimatized to laboratory conditions 8 days before the test. Each animal was used only once in the experiments. All experiments werw carried out between 09:00 and 15:00 h.The institutional Animal Ethics Committee approved the protocol of this study.

Collection of plant

The leaves, of *Calotropis gigantea and Hibiscus rosa sinensis* were collected from local regions of Gwalior district. They were further identified for physical characteristics of leaf morphology in Department of Botany, Jiwaji University, Gwalior (India).

Preparation of extract

The powdered leaf material (100 g) was packed into soxhlet apparatus and extracted with organic solvent namely petroleum ether (60-80 °C). The filtrate was evaporated using rotary vacuum evaporator under reduced pressure ≤10 mmHg and extracts were stored in desiccators and used for subsequent experiments.

Phytochemical Screening of extracts

Various chemical tests were carried out to identify the phytoconstituents as described by Kokate C.K. *et al* (2001).

Preparation of Test Sample

The petroleum ether extracts were incorporated into hair cream base prepared by fusion method using o/w base. The cream base consisted of glyceryl monostearate (9%, w/w), light liquid paraffin (20%, w/w), cetyl alcohol (15%, w/w), bees wax (15%), propyl and methyl paraben (0.15%, w/w), glycerol (4.5%, w/w), and water (59%, w/w). A mixture of 5% (w/w) of all the three proportionate extracts HRSF, CGF, and HCF were incorporated in the base to obtain 5% herbal creams.

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 cm^2 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 HRSF, CGF, HCF was 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.[19]

Animal Model Study for Alopecia

All groups of animals were exposed to sonic stress for the duration of 24 hours starting on day 14 post-depilation (p.d.), when all back skin hair follicles were in late anagen. The sound stress was emitted by a rodent repellant device (epilatore) at a frequency of 300 Hertz in intervals of 15 seconds. The stress device was placed into the rats cage so that the rats could not escape sound perception[2]. All animals were depilated with the help of wax applied over the 2.5 cm^2 area at the dorsal portion of rats the animals were left for 14 days for anagen induction in resting stage. After produced stress in animals all the thirty animals were divided into five groups of six animals in each group.

The groups of animals selected for the pharmacological evaluation were as follows:

Group I: Animals were given topical application of the cream base and served as control.

Group II: Animals were treated with 1ml of 2% minoxidil and served as positive control.

Group III: Animals were given topical application of *Hibiscus rosa-sinensis extract formulation. (HRSF)*

Group IV: Animals were given topical application of *Calotropis gigantea* extract formulation. *(CGF)*

Group V: Animals were given topical application of *Hibiscus rosa-sinensis and Calotropis gigantea* extract formulation in combination i.e, Polyherbal formulation. (HCF)

The treatment was followed for next 7 days, once daily and observations were for all the animals and thus the study was done for 24 days. Finally on 25th day of the study blood samples from animals were collected for biochemical parameters. Skin was collected from depilate area for histopathological studies and hair for physical parameters.

Physical parameters

i) Hair length

Hair was plucked randomly from the depilated area with the help of electric clipper and measured the hair length with the help verniercaliper and calculated the mean of hair length.

ii) Hair density

A hole of 1cm^2 was made on card board. Then the card board set on the desired depilated area (where hair fall patches observed) on the back of rat after 25 days of depilation. The hair was trimmed of desired depilated area and the hair was cut with the seizure. The hair was count manually.

Biochemical and Hematological Parameters

Total protein estimation

Total serum protein in blood estimated by Modified Biuret method. Biuret is a compound formed by heating urea to 180 degree concentration. When biuret treated with diluted copper sulfate in medium, a purple colure is obtained[18]

Total leucocytes Count (White Blood Cells)

Blood sample was collected after the treatment from retro orbital plexus of rats on the 25th day of treatment. The distal one-half centimeter of the tail was clipped and a capillary pipette containing anticoagulants (EDTA for cell counting) was used to collect 20 µl samples from the bleeding surface. The withdrawn sample was used for cell counting and immediately after collection; the cut surface of the tail was cauterized with styptic powder. The collected blood was transferred to sterile test tube containing anticoagulant at a ratio of 1: 10. The collected blood was used for hematological parameters within two hours of collection. The hematological parameters were determined with the help of pathology laboratories.

Statistical Analysis of data

Data will be reported as mean ± SEM. Statistical analysis of data will be carried out by one way ANOVA comparing all test groups vs control followed by Dunnet's test using Instat v 2.1 software run on Windows Xp residing in Pentium IV processor.

Results

see Table 1.

see Table 2.

see Table 3.

see Table 4.

see Table 5.

see Table 6.

see Table 7.

Discussion

The study observations and results were compared with Minoxidil in stimulating hair growth in stress induced alopecia animal model. This study was carried on albino rats model since it is often

difficult to conduct a statistical analysis of the efficacy of hair stimulants in humans.

Calotropis gigantean is commonly known as Madar and wild growing tropical plant. Human kind first utilized material found in environment on an empirical basis to cure various ailments. Previously it has been reported to contain glycosides, beta-sitosterol, madrine, saponins, alkaloids, tannins, trisacharoides and flavonols. The plant has been used for various disease conditions, including leprosy, ulcers tumors and piles. Various pharmacological activity reported like analgesic activity, anti-infertilty, anti-inflmmatory activity, hepato protective activity, antimyocardial infarction activity and antidiarrhoeal activity.

Hibiscus rosa sinensis was selected for the study of prevention of hair fall and hair growth activity of the plant extract. Since the hair growth activity of the selected plant has been reported but the combination therapy with the newly selected plant, the scientific data in stress induced alopecia animal model of which has not been reported yet. *Hibiscus rosa sinensis* is a shrub widely cultivated in the tropics. It is grown as an ornamental plant in gardens throughout India and often planted as a hedge or fence plant. Previous studies have showed that *H. rosa sinensis* possesses many biological activities, such as antipyretic, analgesic and anti-inflammatory activities. It has also been reported that the plant's flower possesses antispermatogenic, androgenic, antitumour and anticonvulsant properties in addition, the leaves and flowers have been found to be aid in the healing of ulcers. The available literature also showed that, 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 is potential and better results were obtained in animal model study.

According to traditional texts[11] it is well accepted that the leaves and flowers of *Hibiscus rosa-sinensis* have hair growth promoting and anti-greying properties. Moreover, in India the herbal products in the market intended for hair growth include the extract of various parts of *Hibiscus rosa-sinensis*. Hence, the present study is focused on the scientific investigation of the hair growth potential of the herb *Hibiscus rosa sinensis* and its therapeutic efficacy in combination with *C.gigantea*.

The hair length determination for HRSF, CGF and HCF were 4.91±0.261, 3.38±0.183 and 5.87±0.007 respectively. The hair density determination for HRSF, CGF and HCF were 1937±37.84, 1611±83.39 and 2179±51.63 respectively. The qualitative study revealed that the time taken for complete hair growth was 18 days in HRSF, 21 days in HCF and 23 days in CGF. Thus on comparison HRSF, CGF, HCF and Minoxidil it has been observed that HRSF as well as HCF herbal formulation application shows better growth that the patch with minoxidil. The results of biochemical parameters study also support the study for the best formulation outcome of the research. Effect of different prepared formulations on Total serum protein of albino rats in stress induced alopecia animal model study and Effect of different formulation on W.B.C. Count of animals in stress induced alopecia model study.

The result shows that formulation HRSF and HCF have contributed in most significant hair growth activity and also showed maximum extraction of active principles responsible for hair growth.

If stress becomes persistent and low-level, however, all parts of the body's stress apparatus (the brain, heart, lungs, vessels, and muscles) become chronically over- or under-activated. This may produce physical or psychologic damage over time. Acute stress can also be harmful in certain situations.

A number of studies have shown that subjects under chronic stress have low white blood cell counts and are vulnerable to colds. And once any person catches a cold or flu, stress can exacerbate symptoms. People who harbor herpes or HIV viruses may be more susceptible to viral activation following exposure to stress. Even more serious, some research has found that HIV-infected men with high stress levels progress more rapidly to AIDS when compared to those with lower stress

levels.

The hair growth activity of individual herb and their combination was evaluated in stress induced alopecia animal model. The hair growth activity of HRSF was found to be almost equivalent to minoxidil group. Among the various groups the *Hibiscus rosa-sinensis* leaves petroleum ether extract showed significant results, while the growth with *Calotropis gigantean* in some animals was not significant in comparison to control group. The effect in group of polyherbal formulation was significant and the potential hair growth activity was found in almost all the animals.

The formulation did not exhibit any signs of erythema and edema after topical application of prepared oil. In control group animals, initiation of hair growth in denuded area was observed in second week. Hair growth initiation was recorded in the first week in animals of minoxidil treated standard group as well as test group. Similarly the time taken for the complete hair growth on shaved area was also affected by minoxidil treatment as well as treatment with formulated herbal hair formulation when compared with control group. Complete hair growth was observed on 19th day with minoxidil treatment, on 20th day with herbal hair formulation treatment. Significant increase in hair count and hair length was observed in formulated herbal hair formulation which was comparable to standard and control groups.

It was also observed that in herbal formulation treated group the texture of hair was coarse, rough and hard as compared to the hair of minoxidil treated group which were short and silky. However the exact mechanism of hair growth stimulation is still not known and not available in literature.

Conclusion

The hair growth studies finally prove that formulation HRSF have excellent hair growth promoting activity by an enlargement of follicular size and a prolongation of the anagen phase. The hair growth activity was also observed in CGF but less in comparison to HRSF while the hair growth activity in animals treated with formulations in combination of both the herbal extracts was found to be significant when all the groups were compared statistically. When compared to the standard, it holds the promise of potent herbal alternative for minoxidil. The hair growth studies showed that formulation HCF has excellent potential to be developed as herbal alternative to minoxidil. 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.

References

1. Adhirajan N. Ravi Kumar T. Shanmugasundaram N. In vivo and in vitro evaluation of hair growth potential of Hibiscus rosa-sinensis Linn. J Ethnocol 2003; 88:235–239
2. Arck PC, Handjiski B, Hagen E, et al. Indications for a "brain-hair follicle axis (BHA)": inhibition of keratinocyte proliferation and up-regulation of keratinocyte apoptosis in telogen hair follicles by stress and substance J Eur Mol Bio Org 2001;5:2536–2538.
3. Budd D, Himmelberger D, Rhods T,et al. The effects of hair loss in European men. Eur J Dermat 2000;10: 122.
4. InaokaY. Shakuya A.Fukazawa H. et al.Studies on active substances in herbs used for hair treatment. I. Effects of herb extracts on hair growth and isolation of an active substances from Poluporus umbellatus F. Chemical & Pharmaceutical Bulletin 1994;42:530–533.
5. Jain Ritu, Jain Neetesh, Singh Namrata et al. Development and evaluation of polyherbal ointment for hair growth activity. Int J Pharma and Pharma Sciences 2011;Vol 3: Suppl 2
6. Kabyashi N. SuzukiR. Suzuki T.1993: Effects of leaves of Ginkgo biloba on hair regrowth in C3H strain mice 1993; Yakugaku Zasshi :113: p.718–724.
7. Kwon OS, Han JH, Yoo HG,et al .Human hair growth enhancement in vitro by green tea epigallocatechin-3-gallate (EGCG), Phytomedicine 2007;14 :551–555
8. Mahalaxmi Mohan, Amol Shinde, Bhagyashree Khade Effect of Anthocyanidin Fraction of Hibiscus Rosa-Sinensis on Blood Pressure in Deoxycorticosterone cetate (DOCA)-Salt-Hypertensive Rats Pharmacologyonline 2011; 3: 1097-1111.
9. Milena E., Peters J , Handjiski B Nurogenic Inflammation in Stress Induced Termination of Murine Hair Growth is Promoted by Nerve Growth Factor, American Journal of Pathology .July 2004; Vol.165, No.1, p.259-271.
10. Nadkarni A K, India material medica, Popular prakashan pvt. Ltd., Bombay, 1954;p.631.
11. Peter EMJ, Arck PC, Paus R, Hair Growth Inhibition by psycho emotional stress: a mouse model for neural mechanisms in hair growth control Exp Dermat 2006; Vol.15:p.1-13.
12. Roy RK, Mayank Thakur , Dixit VK, Effect of Citrullus colocynthis. on Hair Growth in Albino Rats, Pharmaceutical Biology 2007; Vol. 45, No. 10 , Pages 739-744.
13. Saraf S, Pathak AK, Dixit VK Hair growth promoting activity

of Tridax procumbens. Fitoterapia .1991; 62:495-498.
14. Semwal BC, Agrawal K K, Singh K et al.:Alopecia: Switch To Herbal Medicine J Pharma Res Opi 2011;1: 4 101 – 104.
15. Sinis RT ,The measurement for Hair Growth as an Index of protein Synthesis in Malnutrition. 1998 ;22 P 229.
16. Suraja R.Rejithaa G. Anbu Jeba et al. In vivo hair growth activity of Prunus dulcis seeds in rats. Biology and Medicine 2009; Vol 1 (4): 34-38.
17. Takahashi T, Kamiya T, Yokoo Y.Proanthocyanidins from grape seeds promote proliferation of mouse hair follicle cells in vitro and convert hair cycle in vivo Acta Derm Venereol 1998;78, p.428–32.
18. Thorat RM, Jadhav VM.,Kadam VJ Development and evaluation of polyherbal formulations for hair growth-promoting activity International Journal of PharmTech Research 2009; Vol.1, No.4, pp 1251-1254.
19. Uno H, Kurata S Chemical agents and peptides affect hair growth. Journal of Investigative Dermatology 1993;101:143S-147S.

S.No.	Activeconstituent	*Hibiscusrosa sinensis*	*Calotropis gigantea*
1	Alkaloids	+	+
2	Carbohydrates	+	-
3	Glycosides	+	+
4	Saponins	-	-
5	Phytosterols	+	++
6	Phenols	-	-
7	Tannins	+	+
8	Flavonoids	+	++
9	Proteins and aminoacids	++	++
10	Diterpenes	++	+

Table 1: Phytochemical analysis for the presence of active constituents present in chosen plants leaves extract

Note: Where "++" = means most prominent "+" = Presence of compound, "-" = Absence of compound.

S.No	HRSF		CGF		HCF	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
1.	-	-	-	-	-	-
2.	-	-	-	-	-	-
3.	-	-	-	-	-	-

Table 2. Showing the primary skin irritation test observations of different formulations in animals

Where (-) = signifies the absence of erythema or edema or very negligible.

S.No	Groups	Formulation	Hair Follicle Population %		
			Anagen	Catagen	Telogen
Group I	Control	Ointment Base	51	6	43
Group II	Positive control	2% Minoxidil	71	2	27
Group III	HRSF	Ointment (5% w/w)	59	3	38
Group IV	CGF	Ointment (5% w/w)	48	5	47
Group V	HCF	Ointment (5% w/w)	69	2	29

Table 3: Effect of different formulations on number of hair follicles in different stages of albino rats in stress induced alopecia animal model.

S.No.	Groups	Drug applied	Formulation	Hair length in mm (mean±s.d.)
1	Group I	Control	Cream base	2.21±0.108
2	Group II	Positive control	2% Minoxidil	6.06±0.431
3	Group III	HRSF	5% w/w	4.91±0.261
4	Group IV	CGF	5% w/w	3.38±0.183
5	Group V	HCF	5% w/w	5.87±0.007

Table 4: Effect of different extract formulation on hair length of albino rats in animal model study

S.No.	Groups	Drug applied	Formulation	Hair count per cm^2 area (mean±s.d.)
1	Group I	Control	Cream base	1071±41.69
2	Group II	Positive control	2% Minoxidil	2315± 05.78
3	Group III	HRSF	5% w/w	1937±37.84
4	Group IV	CGF	5% w/w	1611±83.39
5	Group V	HCF	5% w/w	2179±51.63

Table 5: Effect of different formulations on Hair Density of albino rats in hair growth activity

S.No.	Groups	Drug applied	Formulation	Total serum protein (g/dl) (mean±s.d.)
1	Group I	Control	Cream base	4.97±2.20
2	Group II	Positive control	2% Minoxidil	6.89±1.70
3	Group III	HRSF	5% w/w	4.80±0.38
4	Group IV	CGF	5% w/w	3.82±1.61
5	Group V	HCF	5% w/w	6.02±1.13

Table 6: Effect of different prepared formulations on Total serum protein of albino rats in stress induced alopecia animal model study.

S.No.	Groups	Drug applied	Formulation	Total WBC Count (Thousand per Cu-mm) (mean±s.d.)
1	Group I	Control	Cream base	6.12±0.45
2	Group II	Positive control	2% Minoxidil	11.37±1.26
3	Group III	HRSF	5% w/w	9.11±3.10
4	Group IV	CGF	5% w/w	7.34±6.15
5	Group V	HCF	5% w/w	9.87±2.71

Table 7: Effect of different formulation on W.B.C. Count of animals in stress induced alopecia model study.