WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 17, 477-484. Research Article ISSN 2277– 7105

EVALUATION OF HAIR GROWTH ACTIVITY OF *HIBISCUS ROSA-SINENSIS* AND *CALOTROPIS GIGANTEA* LEAVES ON STRESS INDUCED ALOPECIA

[1]*Dr. Namrata Singh, [2]Dr. Mukul Tailang and [3]Dr. Anupam K. Pathak

[1]M.Pharm Ph.D. Asso. Professor, Shreeyash Inst. of Pharmaceutical Education and Research, Aurangabad (M.S.) India.

[2]M.Pharm Ph.D. Professor, School of Studies in Pharmaceutical Sciences, Jiwaji University, Gwalior (M.P.) India.

[3]M.Pharm Ph.D. Professor and Dean, Department of Pharmacy, Barkatullah University, Bhopal (M.P.) India.

Article Received on
24 October 2017,

Revised on 14 Nov. 2017,
Accepted on 04 Dec. 2017

DOI: 10.20959/wjpr201717-9905

***Corresponding Author**
Dr. Namrata Singh
M.Pharm Ph.D. Asso. Professor, Shreeyash Inst. of Pharmaceutical Education and Research, Aurangabad (M.S.) India.

ABSTRACT

In the present hair growth study, the various parameters like hair length, hair density, total serum protein, testosterone, total leucocytes count and histology were performed in stress induced alopecia albino rat model. It can be affirmed that 5% ointment of hydrolcholic leaves extract of *Hibiscus rosa-sinensis*, *Calotropis gigantea* and Polyherbal formulation of *H. rosa-sinensis* + *C. gigantea* showed reflective results. From the observations it was concluded that *H. rosa-sinensis* show significant hair growth as compare to *C. gigantea* and Polyherbal formulation of *H.rosa-sinensis* + *C. gigantea*. 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. Against the stress not any herbal preparation were formulated, but these results indicate that *Hibiscus rosa-sinensis*, *Calotropis gigantea* and their polyherbal formulation promoting hair growth activity. Further study will be carrying out to find out chemical active constituents of these plants which promoted hair growth.

KEYWORDS: *Hibiscus rosa-sinensis*, *Calotropis gigantea*, sonic stress, Cyclophosphamide, Alopecia, Histopathology.

INTRODUCTION

Hair loss during extreme periods of stress, it is more likely that the hair loss will first be noticed after the stressful period has pasted. This is due to the effects of stress on the hair growth pattern. 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.[1] Substantial efforts have been expended and consequently, multiple crude drugs like the fruits of *Embelica officinalis*, leaves of *Bacopa monnieri*, leaves of *Murraya koeniigi*, seeds of *Trigonella foenugraecum*[2], *Glycyrrhiza glabra*[3], green tea[4], *Ginkgo biloba* leaf[5], *Lawsonia alba*[6], *Rosmarinus officinalis*[7], grape seeds[8], *Cuscuta reflexa roxb*[9], have been developed, to manage the hair loss, but no effective treatments for stress induced alopecia are available at present. This work was done with an attempt to collect some experimental evidences, use of herbs, *Hibiscus rosa-sinensis* and *Calotropis gigantea* and their polyherbal formulation that having the potential of preventing hair loss in such cases of stress-induced alopecia.

2. MATERIALS AND METHODS

2.1. Plant material

Leaves of *Hibiscus rosa-sinensis* Linn. and *Calotropis gigantea* Linn. were collected in the month of September-October from the campus of Barkatullah University Bhopal. Plants were identified and authenticated in the Department of Pharmacy, Barkatullah University, Bhopal (M.P.). Voucher specimen no. BUPH- 4024/A for *Calotropis gigantea* Linn. and voucher specimen no. BUPH- 4024/B for *Hibiscus rosa-sinensis* Linn. was deposited.

Approximately 200g of powdered crude drugs were separately extracted with hydro-alcoholic solvent (*H. rosa-sinensis* Linn. 50:50, *C. gigantea* Linn. 70:30) by double maceration process. Phytochemical tests reveals the presence of carbohydrate, alkaloid, flavonoid, steroids, protein, tannin, amino acids in *H. rosa-sinensis* whereas the hydro-alcoholic extract *C. gigantea* tested positive for carbohydrate, alkaloid, flavonoid, steroids, protein, tannin, amino-acids and tannins.[10,11]

2.2. Formulation

Formulation were prepared by fusion method, test one containing *H.rosa-sinensis* leaf extract (5% w/w), test two containing *C.gigantea* leaf extract (5% w/w) and test third containing polyherbal (*H.rosa-sinensis C.gigantea* ratio 1:1) total 5% w/w in hydrophilic USP base.[12]

2.3. Animals

Albino rats, weighing 120-150, carried out as per the guidelines of Institutional Animal Ethical Committee (IAEC), Bhopal, MP, India and used for hair growth studies, based on the OECD Guideline No. 423 of CPCSEA. The rats were placed in cages and kept in standard environmental conditions, fed with standard and diet *ad libitum* and allowed free access to drinking water.

3. HAIR GROWTH ACTIVITY (STRESS-INDUCED ALOPECIA ALBINO RAT MODEL)

All albino rats were depilated with the help of wax (2.5cm^2 area) and rest for 14 days for anagen induction. After 14 days of depilation, stress was produced for three days i.e. 72 hr. with the help of rodent repilatore (300 htz) with every 15 second interval. After produced stress all albino rats were divided into 4 group and administered different drug extract through in group 1, received ointment base, group 2 received 5%w/w ointment (hydroalcoholic extract of *H. rosa-sinensis*), group 3 received 5%w/w Ointment (hydroalcoholic extract of *C.gigantea*) and group 4 received total 5%w/w Ointment (hydroalcoholic extract of Polyherbal) for next 7 days, once daily. On 25th day of depilation blood were collected from retro-orbital plexus for biochemical parameter, skin was collected (from depilate area) for histological parameter, and hair for physical parameter.[1,13]

3.1. Hair length determination

Hair was plucked randomly from the depilated area from each group on 25th day of the treatment with the help of clipper and measured the hair length of each rat with the help vernier caliper and the mean of hair length was calculated.[14]

3.2. Hair density

A hole of 1 c.m.^{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 45 days of depilation. The hair was trimed of desired depilated area and the hair was cut with the seizure. The hair was count manually.[15]

3.3. Total Protein estimation

The total protein content of tissue homogenate measured by Lowery's method(121). 0.5 ml of tissue homogenate was mixed with 0.5 ml of 10% TCA and centrifused for 10 min. The precipitate obtained was dissolved in 1.0 ml of 0.1 N NaOH. From this 0.1 ml used for the protein estimation.[16]

3.4. Total leucocytes Count (White Blood Cells)

The blood was taken directly from the tail of rat. 20µl of blood was diluted in 0.4 ml of the diluting fluid in a test tube. The cover slip was put on the counting chamber and then a small quantity of the diluted blood was put between the cover slip and ruled the platform of the counting chamber. The solution was allowed to settle for two minutes and then the counting was done under the microscope. The WBC count was made in large (1 mm) corner squares of neubauer chamber. The numbers of cells in the 4 corner groups of 16 squares are counted. The dilution was done 1 to 20 then the total number of cells were counted in millions per mm^3 of blood.[17,18]

3.5. Histology

Skin biopsies were taken from the depilated area and fixed in 10% formalin buffer. Tissues were embedded in paraffin wax and sectioned into uniform thickness of 10 µm and were stained with haematoxylin and eosin. From the sections, the number of hair follicles per millimeter of the skin and the percentage ratio of different cyclic phases, like anagen and telogen, of hair follicles were determined using the microscope fitted with an ocular micrometer.[14,19]

4. RESULTS

Phytochemical tests reveals the presence of carbohydrate, alkaloid, flavonoid, steroids, protein, tannin, amino acids in *H. rosa-sinensis*[20] whereas the hydro-alcoholic extract *C. Gigantea*[21] tested positive for carbohydrate, alkaloid, flavonoid, steroids, protein, tannin, amino-acids and tannins. In stress-induced alopecia albino rat model hair growth was observed from depilated area, the physical parameter like hair length and density of group II having best result as compare to control and all groups, shown in table 1. Biochemical parameter like Total Protein estimation and testosterone, in comparison to control, group II having best result. shown in table 2 and in histological parameter the percentage ratio of different cyclic phases, like anagen and telogen, of hair follicles of group II having best result as compare to control and all groups, shown in table 3.

Table 2. Effect of different formulation on Hair Length and Hair density of albino rats in stress induced alopecia model.

Groups	Drug	Formulation	Hair Length mm (mean±s.d.)	Hair Density Per cm^2 (mean±s.d.)
Group 1	Vehicle	Ointment Base	4.32±0.12	1954±21.9
Group 2	Hydroalcoholic Extract of *H.rosa-sinensis*	Ointment (5% w/w)	5.97±0.13*	2058±19.23*
Group 3	Hydroalcoholic Extract of *C.gigantea*	Ointment (5% w/w)	5.460±09	1972±19.23
Group 4	Polyherbal hydroalcoholic Extract of (H.r.s+C.g.)	Ointment (5% w/w)	5.820±0.06*	2020±22.36*

Group-1: Control, Group-2: *H.rosa-sinensis*, Group-3: *C.gigantea*, Group-4: Polyherbal

*Significance (P value < 0.01) as campared of control group to to all 3 test group.

Table 2. Effect of different formulation on Total serum protein and Total leucocytes Count of albino rats in stress induced alopecia model.

Groups	Drug	Formulation	Total serum protein (g/dl) (mean±s.d.)	W.B.C. Count/mm^3) (mean±s.d.)
Group 1	Vehicle	Ointment Base	4.932±0.13	17640±296.64
Group 2	Hydroalcoholic Extract of *H.rosa-sinensis*	Ointment (5% w/w)	5.35±0.10*	12230±258.84
Group 3	Hydroalcoholic Extract of *C.gigantea*	Ointment (5% w/w)	5.04±0.10	12900±223.60
Group 4	Polyherbal hydroalcoholic Extract of (H.r.s+C.g.)	Ointment (5% w/w)	5.25±0.04*	12360±288.09

Group-1: Control, Group-2: *H.rosa-sinensis*, Group-3: *C.gigantea*, Group-4: Polyherbal

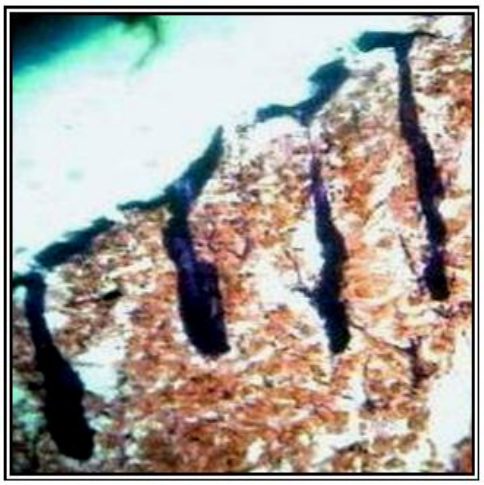
*Significance (P value < 0.01) as campared of control group to to all 3 test group.

Table 3. Effect of different formulation on Number of hair follicles in different stages of albino rats in stress induced model.

S.No	Drug	Formulation	Hair Follicle Population %		
			Anagen	Catagen	Telogen
Group-1	Vehicle	Ointment Base	50.6	2	47.4
Group-2	Hydroalcoholic Extract of *H.rosa-sinensis*	Ointment (5% w/w)	54	1.4	44.6
Group-3	Hydroalcoholic Extract of *C.gigantea*	Ointment (5% w/w)	52	1.4	46.6
Group-4	Polyherbal hydroalcoholic Extract of (H.r.s+C.g.)	Ointment (5% w/w)	53.2	1.2	45.6

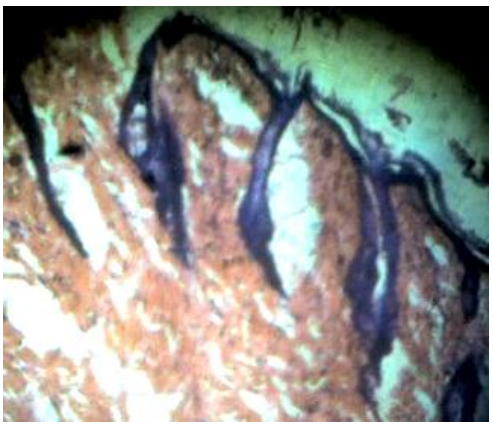
Group-1: Control, Group-2: *H.rosa-sinensis*, Group-3: *C.gigantea*, Group-4: Polyherbal.

Histology Photographs (Stress-induced alopecia model)

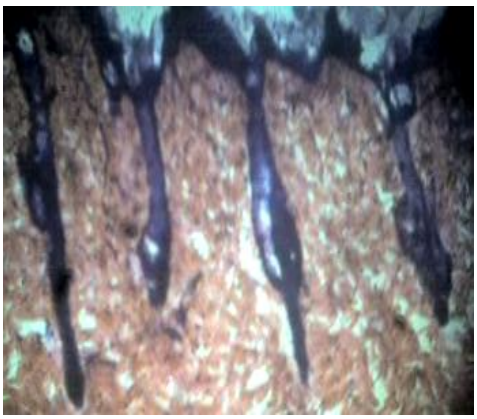


Photograph 1. Control.

Photograph 2. *H.rosa-sinensis.*



Photograph 3. *C.gigantea.*



Photograph 4. Polyherbal.

5. DISCUSSION AND CONCLUSION

In the present hair growth study, the various parameters like hair length, hair density, total serum protein, testosterone and histology, were performed in stress induced alopecia albino rat model. To conclude it can be affirmed that hydrolcholic extract of *H. rosa-sinensis, C. gigantea* and Polyherbal formulation of *H. rosa-sinensis* (Hr) + *C. gigantea* (Cg) showed reflective results.

The hydrolcholic extract of *H. rosa-sinensis* (Hr) show significant hair growth as compare to single drug extract of *C. gigantea* (Cg) and Polyherbal formulation of *H.rosa-sinensis* (Hr) + *C. gigantea* (Cg). Further study will be carrying out to find out chemical active constituents of these plants which promoted hair growth. Presence of active constituents like flavonoids, tannins, may be responsible for hair growth activity.

6. ACKNOWLEDGEMENT

The work was supported by Department of Pharmacy Barkatullah University Bhopal, India. I would like to thanks Dr. Anupam Kumar Pathak, Head of the Department, Barkatullah University Bhopal, India.

7. REFERENCE

1. 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, 2004; 165(1): 259-271.
2. Pande M, Paliwal A, Joshi A and Pathak AK, Herbal hair formulation based on Seasame oil using cloth pouch production technique, I. J. Applied life Science, 2007; 3(2): 23-25.
3. Shirode D, Tailang M, Singhai AK, and Singh A, Hair growth activity of Hibiscus rosa-sinensis and Glycyrrhiza glabra, Biosciences, Biotechnology Research Asia, 2005; 3(2): 423-424.
4. Kwon OS, Han JH, Yoo HG, Chung JH, Cho, KH, Eun HC and Kim KH, Human hair growth anhancement in vitro by green tea epigallocatechin-3-gallate (EGCG), Phytomedicine, 2007; 14: 551-555.
5. Kabyashi N, Suzuki R, Suzuki T, Effect of leaves of Ginkgo biloba on hair regrowth in C3H strain mice. Yakugaku Zasshi, 1993; 113: 718–724.
6. Dweck AC, African plants, Cosmet. Toiletories, 1997; 112: 41-51.
7. Aruoma OI, Spencer JP, Rossi R, Food Chem. Toxicol, 1996; 34: 449-456.
8. 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: 428–32.
9. Roy RK, Thakur M, Dixit VK, Effect of Cuscuta reflexa Roxb. on hair growth in albino rats, Indian Drugs, 2006; 43(12): 951–6.
10. Mukherjee, P.K., Quality control of herbal drug, An approach to evaluation of Botanicals, 1st edition, Business Horizons, Pharmaceutical publishers, New Delhi, 2003; 187: 282.
11. Harborne JB, Phytochemical methods, Chapman and hall, New York, 1984; 6.
12. Allen Loyd V, Popovich Nicholas G, Ansel Howard C, Pharmaceutical Dosage Form and Drug Delivery Systems, Edition 8th, 2005; B. I. Publications pvt. Ltd., 278.
13. Peter EMJ, Arck PC, Paus R, Hair Growth Inhibition by psychoemotional stress: a mouse model for neural mechanisms in hair growth control, Experimental Dermatology, 2006; 15: 1-13.

14. Adhirajan N, Ravi TK, Shanmugasundaram N, Babu M, In vivo and in vitro evaluation of hair growth potential of Hibiscus rosa-sinensis Linn., Journal of Ethnopharmacology, 2003; 88: 235–239.
15. Patni P. et al. Formulation and Evaluation of hair oil for alopecia management. Planta indica, 2006; 2(3): 27-29.
16. Shreejayan and Rao MN, Nitricoxide scavenging by cacuminoids, J. Pharm. Pharmacol., 1997; 49: 105-107.
17. Kale SK, Kale RR, Practical Human Anatomy and Physiology, Nirali prakashan, Edition seventh, 2001; 6-22.
18. Joachim R, Sagach V, Quarcoo D, Dinh Q, Arck P, Klapp B, Effect of stress on eotaxin and expression of adhesion molecules in a murine model of allergic airway inflammation, J. of Neuroimmunology, 2007; 182(1-2): 55- 62.
19. Sawada M, Terada N, Taniguchi H, Tateishi R, Mori Y, Cyclosporin A stimulates hair growth in nude mice, Laboratory Investigation, 1987; 56: 684-686.
20. Singh N, Gupta P, Amit Joshi, Pathak AK. Preliminary pharmacognostic evaluation on leaves of *Hibiscus rosa-sinensis* linn. National journal of "The Pharmacist", 2008, Volume 4, No.2, 57-59.
21. Singh N, Gupta P, Patel AV, Pathak AK. Calotropis gigantea: A review on its Phytochemical & Pharmacological Profile. International Journal of Pharmcognosy (IJP), 2014, vol 1 (1), 1-8.