

**EFFECT OF *TAMARINDUS INDICA* AND *CURCUMA LONGA*
ON STRESS INDUCED ALOPECIA**

¹Narendra Vyas*, ¹Raj Kumar Keservani, ²Amit Nayak., ¹Sarang Jain, ³Manmohan Singhal

¹Rajeev Gandhi College of Pharmacy, Kolar road, Bhopal-462042, M.P., India

²Pinnacle Biomedical Research Institute, Bhopal-462003, M.P., India

³ School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India

Summary

It has been much disputed whether or not stress can cause hair loss (telogen effluvium) in a clinically relevant manner. Despite the paramount psychosocial importance of hair in human society, this central, yet controversial problem of clinically applied stress research has not been systematically studied in appropriate animal models. It was showed that psychoemotional stress indeed alters actual hair follicle (HF) cycling in vivo, i.e., prematurely terminates the normal duration of active hair growth (anagen) in mice. Further, inflammatory events deleterious to the HF are present in the HF environment of stressed mice (perifollicular macrophage cluster, excessive mast cell activation). Thus hair loss in stress is due to hair cycle manipulation and neuroimmunological events that combine to terminate anagen. The present study is to evaluate the effect of *Tamarindus indica* and *Curcuma longa* on stress induced alopecia.

KEYWORDS: Alopecia, *Tamarindus indica*, *Curcuma longa*, Stress

For correspondence

Narendra Vyas

E mail: manu_research2@rediffmail.com

Contact : +919829153193

Introduction

It has been much disputed whether or not stress can cause hair loss (telogen effluvium) in a clinically relevant manner. Despite the paramount psychosocial importance of hair in human society, this central, yet controversial problem of clinically applied stress research has not been systematically studied in appropriate animal models.¹⁻⁵ It was showed that psychoemotional stress indeed alters actual hair follicle (HF) cycling in vivo, ie, prematurely terminates the normal duration of active hair growth (anagen) in mice. Further, inflammatory events deleterious to the HF are present in the HF environment of stressed mice (perifollicular macrophage cluster, excessive mast cell activation).⁶ Therefore there is a need for the development of a formulation which can prevent stress induced hair loss.

The plants under study are *Tamarindus indica* and *Curcuma longa*. Ethaolic extract of seed coat of *T. indica* and ethanolic extract of rhizomes of *C. longa* are used.

Materials And Methods

Six- to 8-week-old mice were purchased from Bhopal, since mice at this age show the most reliable and profound stress response and are in the telogen stage of the hair cycle. The animals were housed in community cages at the animal facilities with 12-hour light periods, and were fed water and mouse chow ad libitum.^{7, 8}

Anagen Induction

Anagen was experimentally induced by depilation; mice were anesthetized with diethyl ether. Then, a wax/rosin mixture was applied to the dorsal skin of mice. Peeling off the wax/rosin mixture removes all hair shafts and immediately induces a highly synchronized hair growth.⁹⁻¹²

Preparation of formulation

Tamarindus indica and *Curcuma longa* were extracted by using ethanol by soxhlet extraction method. Suspension (2%) was prepared with tragacanth mucilage. Polyherbal formulation contains both the drug in 1:1 ratio.

Groups of animals

Group I: (Control) Group I receives no treatment

Group II: (Negative control) Group II receives sonic stress but with out any drug treatment

Group III: receives suspension of *T. indica* orally (300mg/kg) after sonic stress treatment

Group IV: receives suspension of *C. longa* orally (300mg/kg) after sonic stress treatment

Application of Stress

Group II, III and IV were exposed to sonic stress for the duration of 5 days starting on day 14 post-depilation, when all back skin hair follicles were in late anagen. The sound stress was emitted by a rodent repellent device at a frequency of 300 Hertz in intervals of 15 seconds. The stress device was placed into the mouse cage so that the mice could not escape sound perception. After application of sonic stress Group II receives normal tragacanth suspension while Group III and Group IV receives 300mg/kg test drug extract orally in form of tragacanth suspension of *T. indica* and *C. longa* respectively up to 1.5ml for next 5 days. On 20th day skins of mice from all groups were collected and placed in 10 % formalin solution and histopathological analysis were done. Blood samples were collected from mice of all the groups on day 16th, 18th and 20th day respectively and the samples were subjected to different haematological tests.

Result and Discussion

In stress induced alopecia 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 keratinocytes apoptosis. At the same time, it induces neurogenic inflammation characterized by perifollicular mast cell degranulation and accumulation of antigen-presenting cells, e.g., activated macrophages. Thus the lymphocyte count in stressed mouse also increased.^{13,14} It was observed that the lymphocyte count in control group was around 15.83 % while that of negative control was around 24.16 % which might be to inflammatory change due to stress treatment, while the lymphocytes count of *C. longa* was around 20.16 % and that of *T. indica* was around 18.50 % respectively. On comparing the data it was revealed that *T. indica* decreases the elevated lymphocytes count better in comparison to *C. longa*.(Table 1)

Alkaline phosphatase (ALP) is a zinc-metallo enzyme. Its activity was detected in dermal papilla. Its localization and strength dramatically changed during the hair cycle. Activity in the dermal papilla (DP) was moderate in very early anagen, reached a maximal level in early anagen, decreased at the proximal region of DP after mid anagen, and was kept at a low level during catagen. The bulbar dermal sheath showed intense ALP activity only in early anagen. It helps to maintain anagen phase for long time periods and helps to induce anagen phase by its activity in dermal papilla.³ Alkaline phosphatase level in control group was 93.50 U/L and that of negative control was raised to 114.17 U/L may be due to stress treatment. *C. longa* shows decrease in alkaline phosphatase level 97.16 U/l as compared to toxic and *T. indica* shows decrease in alkaline phosphatase level to 94.00 U/L as closer to control group. (Table 1)

The hair density of the control group in 1mm² was 19.16 and that of negative control was 12.83 this might have happen due to inflammatory changes brought by stress in dermal papilla. The hair density in *T. indica* was found to be 17.66 mm² in comparison with toxic group, the *C. longa* also significantly increases the hair density by 15.16 mm² but low in comparison to *T. indica*. (Table 1)

The result of histopathological analysis clearly shows that in control group maximum hair follicles (HFs) were in anagen phase of hair cycle while that in negative control group were in telogen phase which may be due inflammatory events that were deleterious to HFs, were present in the HFs environment of stressed mice. While in *C. longa* and *T. indica* maximum of HFs were in anagen phase of hair growth cycle.

Also in negative control lymphocyte migration around the hair bulb was clearly seen which was less or none in case of test and control group. The percentage population of hairs in different phases of hair cycle was given Table 2.

Table: 1 Effect of ethanolic extract of *T. indica* and *C. longa* on parameters

STRESS INDUCED ALOPECIA MODEL				
S.No.	Treatment	Lymphocyte count (%)	Alkaline phosphatase level (U/L)	Hair density (mm ²)
1	Control	15.83±0.75	93.50±2.34	19.16±0.75
2	Negative control	24.16±1.16	91.17±1.16	12.83±1.16
3	<i>C. longa</i>	20.16±0.75	97.16±0.75	15.16±0.75
4	<i>T. indica</i>	18.50±0.54	99.00±0.89	17.66±0.81

All the values were expressed as mean ± standard deviation (S.D.). The difference was considered significant when *P*-values < 0.01.

Table: 2 Percentage population of hairs in stress model

Percentage Population of Hairs				
S.No.	Groups	Anagen	Catagen	Telogen
1.	Control	65	3	32
2.	Negative Control	40.5	2.5	57
3.	<i>C. longa</i>	57	2	41
4.	<i>T. indica</i>	60	2.5	37.5

HISTOPATHOLOGICAL PARAMETERS

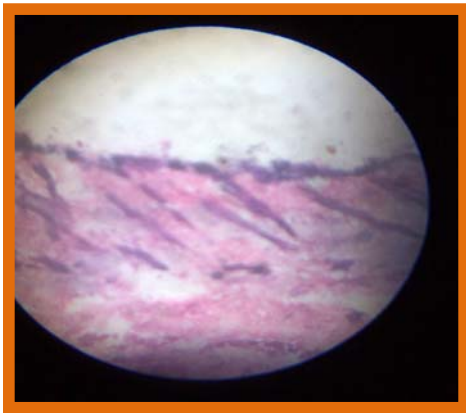


Figure 1 Control Group

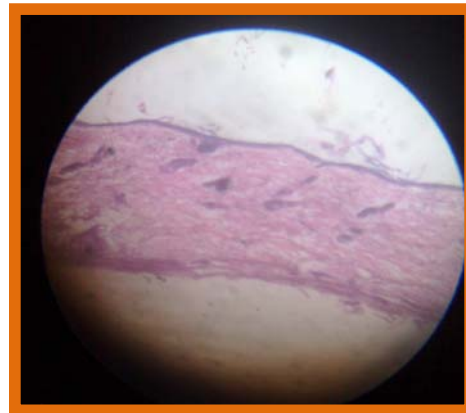


Figure 2 Negative control group

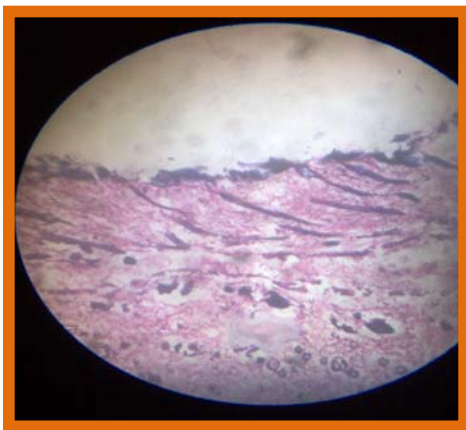
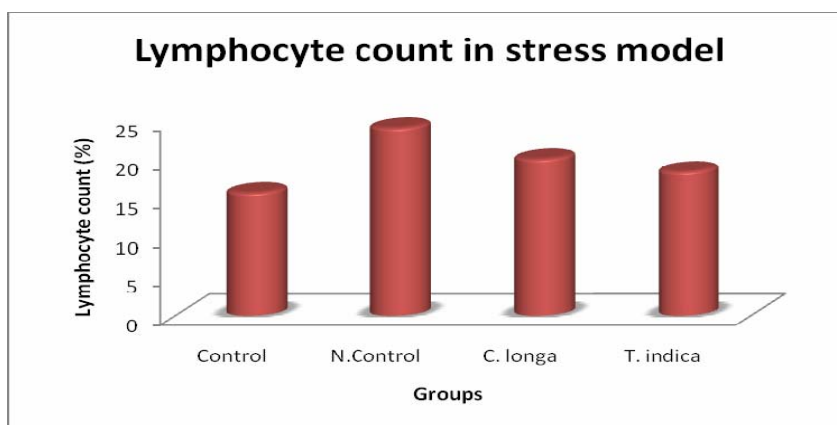


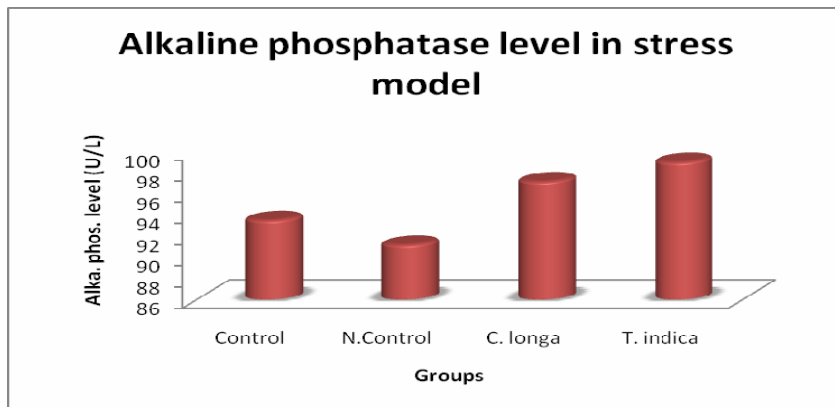
Figure 3 Test drug *Curcuma longa*



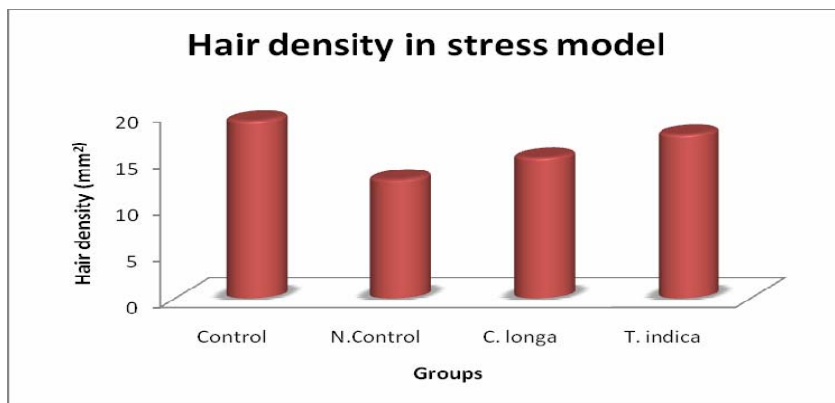
Figure 4 Test drug *Tamarindus indica*



Graph: 1 Lymphocyte count of all groups



Graph: 2 Alkaline phosphatase level of all groups



Graph: 3 Hair density count of all groups

References

1. Arck PC, Handjiski B, Peters EMJ, Peter AS, Hagen E, Fischer A., Klapp BF, Paus R. Stress Inhibits Hair Growth in Mice by Induction of Premature Catagen Development and Deleterious Perifollicular Inflammatory Events via Neuropeptide Substance P-Dependent Pathways. *American Journal of Pathology* 2003; 162 (3): 803–814.
2. Arck PC, Handjiski B, Hagen E, Joachim R, Klapp BF, Paus R. Indications for a “brain-hair follicle axis (BHA)”: inhibition of keratinocytes proliferation and up-regulation of keratinocyte apoptosis in telogen hair follicles by stress and substance. *FASEB J.* 2001; 15(13):2536– 2538.

3. Arck PC, Handjiski B, Peters EMJ, Hagen E, Klapp BF, Paus R. Topical minoxidil counteracts stress-induced hair growth inhibition in mice. *Exp Dermatol* 2003; 12:580–590.
4. Arck PC, Handjiski B, Peters EMJ, Peter A, Hagen E, Fischer A, Klapp BF, Paus R. Stress inhibits hair growth in mice by induction of premature catagen development and deleterious perifollicular inflammatory events via neuropeptide substance P-dependent pathways. *American Journal of Pathology* 2003; 162:803–814.
5. Paus R, Heinzelmann T, Schultz KD, Furkert J, Fechner K, Czarnetzki BM. Hair growth induction by substance P. *Lab Invest* 1994, 71:134-140.
6. Peters EM, Botchkarev VA, Botchkareva NV, Tobin DJ, Paus R. Haircycle-associated remodeling of the peptidergic innervation of murine skin and hair growth modulation by neuropeptides. *J Invest Dermatol* 2001; 116:236–245.
7. Paus R. Stress and the skin. *Exp Dermatol* 2001; 10:367.
8. Ader R, Cohen N. Behaviorally conditioned immunosuppression. *Psychosom Med* 1975; 37:333–340.
9. Arimura A, Takaki A, Komaki G. Interactions between cytokines and the hypothalamic-pituitary-adrenal axis during stress. *Ann NY Acad Sci* 1994; 739:270–281.
10. Chrousos GP. Stressors, stress, and neuroendocrine integration of the adaptive response: The 1997 Hans Selye Memorial Lecture. *Ann NY Acad Sci* 1998, 851:311–335.
11. Ader R. On the development of psychoneuroimmunology. *Eur J Pharmacol* 2000, 405:167–176.
12. Padgett DA, Glaser R. How stress influences the immune response. *Trends Immunol* 2003; 24:444–448.
13. Peters EMJ, Handjiski B, Kuhlmei A, Hagen E, Bielas H, Braun A, Klapp BF, Paus R, Arck PC. Neurogenic inflammation in stress-induced termination of murine hair growth is promoted by nerve growth factor. *American Journal of Pathology* 2004;165(1); 259-271.
14. Iida M, Ihara S, Matsuzaki T. Follicular epithelia and dermal papillae of mouse vibrissal follicles qualitatively change their hair-forming ability during anagen. *Develop. Growth Differ* 2007; 49: 185-195.