IJBPAS, October, 2016, 5(10): 2474-2484

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EVALUATION OF HAIR GROWTH ACTIVITY OF *MOMORDICA CHARANTIA* BY USING CHEMOTHERAPY INDUCED HAIR LOSS

LIKHITKAR M[1*], PANDE M[2] AND SINGH SK[3]

[1,3]Suresh Gyan Vihar University, Jagatpura, Jaipur – 302025, Rajasthan, (India)

[2]NIMS Institute of Pharmacy, NIMS University, Jaipur-303121 Rajasthan(India)

Received 1st June 2016; Revised 18th July 2016; Accepted 17th August 2016; Available online 1st Oct. 2016

ABSTRACT

Background: We previously demonstrated that chemotherapy induced alopecia in mice. This effect was prevented by the oral administration of *Momordica charantia* alcoholic extract, contain momordicine, charantin and phytosterole which promote hair follicle growth and reduce the chances of alopecia condition. **Objectives**: The present study aimed at alopecic activity of *Momordica charantia* by using chemothrapy induced hair fall and modulating the mechanisms responsible for this condition. Chemotherapy induced alopecia which is a result of damage of hair follicle which causes hair loss. Anticancer drug arrests the cell cycle of the proliferative cell and inhibit the hair growth. In this, we studied the parameters which responsible for hair loss. **Methods:** In adult mice induction of anagen phase developed by depilation treatment and chemotherapy induced alopecia adult mouse model mainly work on cyclophosphamide. A single dose of 150mg/kg cyclophosphamide was given on day 9 after depilation. Groups of mice received the *Momordica charantia* extract and standard minoxidil 5% solution administered orally to elucidate the biological activity *in vivo*. The occurrence of alopecia was evaluated for up to 21 days of cyclophosphamide, and the body weight and survival of mice were calculated with hair density, hair population, and testosterone level, hair follicle number and norchromatic erythrocyte cell in peripheral blood was investigated**. Results**: The hair density, hair follicle number, hair population decrease and lymphocyte count were increased by chemotherapy. Test drug extract (*Momordica charantia*) show increase hair density, hair follicle number and testosterone concentration as compared to increased count in the toxic group. **Conclusions:** Increase in testosterone

concentration in case of chemotheraphy is a good sign of preventing hair fall due to the presence of flavonoid, protein and amino acid, insulin growth factor and may be due to balancing androgen hormone (androgen) which promotes hair growth in humans.

Keywords: Isoflavonoid, Phytosterole, *Momordica charantia*, Testosterone, Hair density, Hair follicle number

1. INTRODUCTION

Chemotherapy induced hair loss (alopecia) is the most feared negative side effect of chemotherapy[1] and chemotherapy induced alopecia is noticeable approximately 65% of all patient[2] and easily effect the patient quality of life , leading to poor result of treatment due to lack of confidence and depression. Chemotherapy induced alopecia also reduced the social activities and sexual behavior[3]. Despite substantial efforts in research of Chemotherapy induced alopecia, no effective treatment has become available due to lack of basic knowledge of Chemotherapy induced alopecia pathogenesis [4]. Cancer treatment drug causes damage the scalp hairs and slow down the hair growth rate. During the hair growth cycle hair follicle structure changes in three phase known as anagen, catagen and telogen in which anagen is known as active growth phase and telogen is known as regression phase in which hair comes out from hair shaft and lead to hair loss [5]. The basic pathophysiology of cancer treatment is to affect the mitotic process of cancer cell as well as normal and rapid metabolic cell such as hair follicles. After chemotherapy cancer drug increase the rapid hair growth and high blood flow rate at the hair bulb leading to drug accumulation which promote the hair follicle to transverse into telogen phase and hairs pulled out [6]. Chemotherapy induced alopecia usually starts after 2 to 4 weeks and complete in 1 to 2 month after chemotherapy. The Chemotheraphy induced alopecia depends on type of drug, dosage regimen as well as route of administration. The intravenous high dose is associated with rapid alopecia whereas oral therapy with weekly interval tends to initiate less alopecia [7]. Some patient treated with combination therapy consisting two or more drug causes higher and severe hair loss and long term therapy may also produce loss of auxiliary and pubic hair [8]. It has now been widely postulated that alopecia is an organ specific autoimmune disorder with genetic predisposition.

Testosterone, the main male circulating androgen, binds androgen receptors in specific

tissue. Testosterone is metabolized by 5α reductase enzymes to 5-dihydrotestosterone (5DHT), a more potent androgen, which binds more strongly to the androgen receptor. This suggests that 5-dihydrotestosterone is necessary for male specific follicles, including beard, chest and upper pubic diamond, like the prostate, while testosterone itself can stimulate the axilla and lower pubic triangle follicles characteristic of women. Since people with 5α reductase type 2 deficiencies do not show androgenetic alopecia and the 5α-reductase type 2 inhibitor, finasteride, can restore hair growth. Androgen receptors are localized in the dermal papilla and dermal papilla cells derived from androgen-sensitive follicles including beard, balding scalp and deer manes [8]. Most importantly, testosterone metabolism by dermal papilla cells reflects hair growth in 5α-reductase deficiency with beard, but not pubic or non-balding scalp, cells forming 5α dihydrotestosterone (5DHT), 5α reductase type 2 gene expressions also supports this. The dermal sheath, which isolates the follicle from the dermis, now seems to play other important roles. It can form a new dermal papilla and stimulate follicle development. On ageing, our testosterone levels decrease. This is partially due to an increased activity of an enzyme known as 5-alpha reductase as on men age. 5-alpha reductase converts testosterone into dihydrotesterone (DHT). DHT is 10 times more powerful than testosterone in terms of stimulating cellular growth, which contributes to swollen prostate gland and increased risks of developing prostate cancer and alopecia[10]. Thus, the age-associated increase in the activity of 5-alpha reductase simultaneously lowers testosterone levels and increases DHT-associated alopecia and prostate cancer risks.

Sensory neuron such as CGRP and nueropeptide nerves play promising role in hair growth. IGF-I is a basic peptide distributed in various tissues and organs and promoting hair growth [9]. Furthermore we showed that administration of phytosterole increase the insulin growth factor in hair bulb thereby shows the anti-aloepcic activity. Phytoesterole have estrogen like biological activity and referred as phytoestrogen has been shown to increase CGRP and Insulin growth factor. In these context phytosterole increases CGRP and these hypotheses suggest that it might increase CGRP dermal level, thereby increasing hair growth [11]. *Momordica charantia* used traditionally as a antimicrobial and it's oxidative stress protective activity is attributed to its polypeptide and flavonoid content which activate the neurons peptide which distributed all over the body. This nueropetide activated by insulin growth factor which is present in *Momordica*

charantia. These flavonoids are highly present in leaves, flowers and fruits[12].

Therefore, the aim of this study was to evaluate the effect of *Momordica charantia* ethanol extract on the chemotherapy induced alopecia in mice and to asses hair growth for such indication.

2. MATERIALS AND METHODS

The tubers of *Momordica charantia*, was collected from the Satpura plateau region of central India (Betul) in the month of Sep-Oct. The agro-climatic conditions prevailing in the region provide an ideal habitat for the natural growth of a variety of plants and herbs, which provide raw materials for, phytochemical, pharmaceutical and cosmetic industries. The plant material was dried and authenticated by Department of Botany, J.H. Govt College, Barkatullah University, Bhopal.

2.1 Chemicals and reagents

All the reagents and chemicals such as petroleum ether, ethanol, tragacanth, sorbitol and glycerin used were of analytical grade. Cyclophosphamide, Minoxidil was procured from Merck, India.

2.2. Preparation of extract:

Fruit were chopped and placed in ethanol for 72 hr at 4^0C.The *Momordica charantia* were extracted with petroleum ether (60-80ºC) to remove lipid and then against extracted with ethanol in a soxhlet extractor. The solvent is distilled to concentrate the extract and dried in vaccum desiccater [13]. Hence ethanol extract was selected for hair growth activity screening. All the test suspension (300mg/ml) was prepared in the vehicle. Plant extract was triturated with 5 ml of sorbitol solution was mixed with 25 ml of glycerin. The suspending agent tracanth (1.25%) in aqueous medium with previously added preservative was then added in to wetted masses with contious stirring untill so as get so unifrm product. Now oral suspension is subjected to evaluation of anti-alopecic activity as per offcial standard[14-16].

2.3. Animals

The adult 7-week-old C57BL/6 female mice was used for this experiment as per the chemotherapy induced alopecia model which was first used by Paus et al in 1994. In this animal model total of 48 mice (female C5LBL/6) were purchased from Jahangirabad , Bhopal (M.P.) were procured, maintained under slandered husbandry condition (Temp 23±2º, relative humidity 55±10% and 12 hours light dark cycle) and were used for all set of experiments in a group of six animal. Animal was allowed to take slandered laboratory feed and tap water. The experimental study was approved by Institutional Animal Ethics Committee (Approval no.TIT/IAEC/831/P'col/2015/52).

2.4. Alopecia induction and test drug administration.

The extract at a selected dose of 300mg/kg as evaluated for its effect on chemotherapy induced alopecia by studying biochemical parameters. Mice divided into control, negative control, standard and test groups, each group comprising of six animals. Anagen was induced by depilation method where mice were anesthetized with diethyl ether. Then, a wax mixture was applied to the dorsal skin of all mice, as evidenced by the pink back skin color. Peeling off the wax mixture removes all hair shafts and induces an anagen phase of hair follicle. When the depilated skin show the late anagen phase at day 9 experimental depilation skin color change from pink to black and this phase cyclophosphamide (freshly prepared at 10 mg/mL in phosphate-buffered saline [PBS] pH 7.4) was injected by the intraperitoneal route 125 mg/kg of body weight to group III and IV. Group III receives minoxidil oral treatment from experimental day 15 to experimental day 20. During the cancer induction control group received vehicle (5% tragacanth mucilage 1.5 ml/kg p.o.) at 0.24 and 48 hours intervals for next five days of the first dose of administration.

The mice were inspected daily for general aspect, toxicity sign and adverse effect to exposure of chemotherapeutic drug (cyclophosphamide) and administration of plant extract of *Momordica charantia* oral suspension. Individual body weight was registered upon arrival of mice and at daily and weekly interval until the finish of experiment. Survival of mice was also inspected and recorded. On experiment day of 28^{th} day blood samples were collected by puncturing the lateral tail vein and sent for hematological parameters and was used for determination of testosterone level. Hair density was also determined in $mm^{2.}$ In this evaluation parameter mouse of the group subjected to evaluated to hair density by square area method. Dorsal area of mice firstly made $1mm^2$ of the area and counted the hair in a particular area and measure and recorded the density of hair [17]. Quantitative hair follicle number was determined by using bright field microscopy and calculations were based on an average hair follicle number from microscopy 200x magnification.

2.5. Histopathology studies

After 21 days of chemothareupatic drug treatment, all mice were anesthetized with diethyl ether and killed by cervical dislocation. The sample of skin of was collected from all group such as control, negative control, standard and test drug. Skins of mice from all groups 5mm skin were collected and placed in 10 % formalin solution and histopathological analysis was done. The sections were observed under a

microscope for histopathological change in skin architecture and their photomicrographs were taken [18-19].

2.6. Statistical analysis

For determining the significant intergroup difference each parameter was analyzed separately and one-way analysis of variance (ANOVA) was carried [20].

3. RESULTS AND DISCUSSION

All the treated groups showed increase in hair density and hair follicle number as compared to group II and I. Also it was observed that the hair density was much more in group II (standard), followed by group IV (test drug).

Table no 1 reported the survival and body weights of mice belonging to all experimental mice, as recorded at time 0 and , later on, at weekly intervals. After cyclophpsmide administration body weight decresed in belong to group negative control. Body weight of group III (minoxidil) incresed from days of 14 to 28. No significant difference observed in experimental groups of mice exposed to cancer treatment, as related to oral treatment of test drug of *momordica charantia.*

Cyclophosphamide induced alopecia was given belove it was observed in table no. 2 that all the treated groups show the percentage populations of hair is more than group II where as III and IV show high percentage than group I. Group III (standard) shows a higher percentage than group I (control). It was observed that the hair density was much more in group IV (Test drug), followed by group II.

Table 1: Inspection of body weight and survival

Body weight (g) of mice (mean± S.E.M)as related to treatment and time of experiment						
Treatment		Time (days)				
S.No	Group	0	7	14	21	28
1	Control	24.18±0.49	23.89±0.45	23.89±0.28	22.06±0.16	23.33±0.29
2	Negative Control	22.45±0.30	21.91±033	19.09±0.19	20.08±0.19	20.89±0.22
3	Standard	21.40±0.67	21.89±0.91	22.08±0.86	22.98±0.54	23.78±0.78
4	*Momordica. Charantia* extract	22.56±0.54	21.60±0.30	20.41±0.77	20.99±0.61	21.34±0.27

(Statistical analysis (mean± S.E.M, n=6): *p<0.05,**P<0.01, and ***P<0.001, as compared to control)

Table 2: Effect of *Momordica charantia* extract on population of hair

S.No.	Groups	Anagen	Catagen	Telogen
1.	Control	71±0.011	3.5±0.09	25.5±0.031
2.	Negative Control	30±0.019	8±0.016	62±0.024
3	Standard	82±0.027	5.4±0.029	12.6±0.040
4.	*Momordica charantia* extract	77±0.042	4±0.020	19±0.018

Table: 2 Effect of test drug extract administration on the hair population of anagen, catagen and telogen hair phase in chemotheraphy induced alopecia mice. Each data represent mean±S.D. from six animal experiments. : *p<0.05,**P<0.01, and ***P<0.001, as compared to Standard(Minoxidil)

Table 3:- Effect of *Momordica charantia* extract on hair density and testosterone concentration

S.No.	Treatment	Hair density	Testosterone level(µg/ml)

1	Control	19.16 ± 0.75	1.20±0.011
2	Negative control	11.83 ± 0.75	1.14±0.0121
3	Positive control	37.50 ± 1.049	1.64±0.008
4	*Momordica charantia* extract	32.83 ± 0.57	1.51 ± 0.01271

(Table:3 Percentage of hair density and testosterone concentration in chemotherapy induced alopecia mice. Each data represent mean±S.D. from six animal experiments.:*p<0.01vs Control, *p<0.01vs Negative Control, *p<0.01vs positive Control, *p<0.01vs *Momordica charantia* extract)

The percentage of hair density in negative control is low when compared with control and standard which might be due to cytotoxic effect due to cyclophosphamide treatment, on comparing the data of hair density control, negative control, positive control and test drug *Momordica charantia.* It was revealed that positive control (minoxidil) and *Momordica charantia* preparation increased the hair density.

In group II (standard) administered minoxidil show vasodilation effect and promote the blood circulation at the base of the hair follicle and increase the keratin formation from protein cell and show increased hair growth activity.

At periodical interval i.e. at time of 28 days of treatment, blood samples were collected from tail lateral vein of all mice and testosterone level (µg/ml) of peripheral blood was counted. Testosterone concentration was measured in (µg/ml) when cyclophosphamide administered its show cytotoxic effect on hair follicle and reduce the production of androgen which is directly associated with hair follicle formation.

When data was compared with standard drug testosterone concentration was high in test group as compared to negative control but less to standard.

Table 4: Effect of *Momordica charantia* extract on hair follicle number per filed

S.No.	Treatment	Hair follicle no. per field
1	Control	25.8±4.2
2	Negative control	10.7±4.9
3	*Positive Control*	41.5±5.1
4	*Momordica charantia* extract	29.8±4.2

(Table:4 Number of hair follicle in chemotherapy induced alopecia mice. Each data represent mean±S.D. from six animal experiments.*p<0.01vs Control, *p<0.01vs Negative Control, *p<0.01vs positive Control, *p<0.01vs *Momordica charantia* extract)

Hair follicles number was determined by using 10 µm paraffin sections under bright field microscopy, and the calculations were based on an average hair follicle number from microscopy 200x magnification. The hair growth was evaluated microscopically in the section of dorsal skin, it was observed that in negative control after sonic stress number of hair follicle decrease due to apoptosis and group I number of follicle is maintained at normal condition, on the other hand minoxidil show (Group III) show increased number of hair follicle due to increased blood circulation and

test drug show satisfactory result as compared to control group but less than standard group.

Histopathology:

Paraffin-embedded 5-µm sections were stained with hematoxylin and eosin (H&E). Hair growth was evaluated microscopically in the H&E-stained sections of dorsal skin.

\

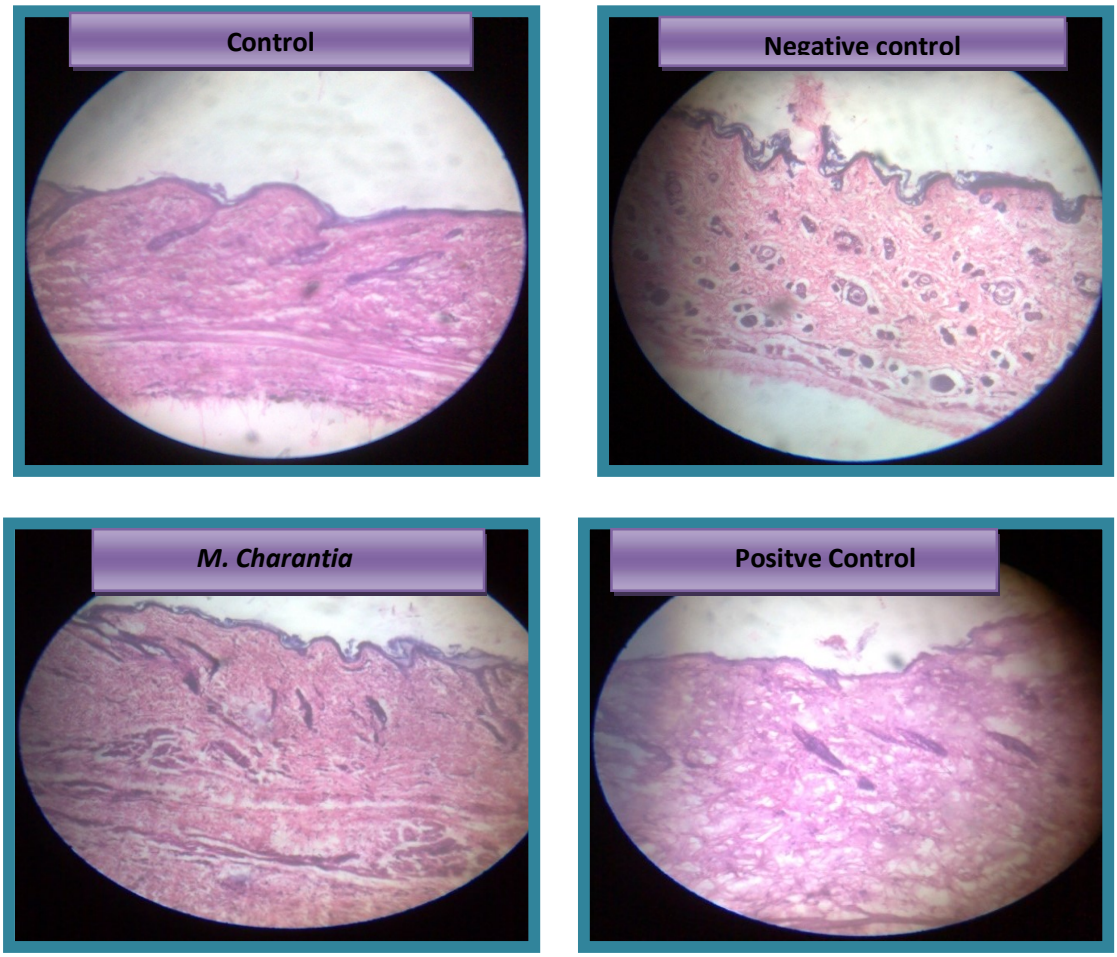

Fig 1: (Histological presentation of mice skin treated with cyclophosphamide .The healthy area of control group shows hair follicle with subcutaneuous hair bulb. The alopecia area of negative control shows small follicle with thinned and devoid of hair follicle. The test drug extract of *Momordica charantia* show hair follicle growth when compared with minoxidile treated group (positive control)

4. DISCUSSION

Alopecia is autoimmune disease in which lymphocyte autoreactive cytotoxic T cell and hair follicle immure privilege is most important factor hair follicle grown present in anagen phase [21] and HFIP is contribute to be development by alopecia in which pigment producing anagen hair bulb are attached to inflammatory cell as well as accumulation of lymphocyte around hair bulb leading to an hair bulb inflammation which lead to conversion of anagen to telogen and lead to

hair fall [22]. In this experimental group of chemotherapy induced alopecia, chemotherapeutic drug (cyclophosphamide) arrests the cell growth of melonocyte cancerous cell [23]. Group II which is treated with cyclophosphamide shows the lowest hair density with highest telogen count which is associated with highest hair fall, where as minoxidil receiving group III shows highest hair density with anagen count as well as lowest hair loss. Testosterone is responsible for hair growth [24-25], in this experiment minoxidil treated group showed highest testosterone level and second highest conc. in blood was observed in test drug which is associated with hair growth activity. The result of our analysis suggest that chemotherapy induced alopecia in group IV contain *Momordica charantia* fruit extract shows best result with high hair follicle per filed. This may play effective role in inhibition of accumulation of mast cell at the base of hair follicle which lead to prolongation of anagen phase and promote hair growth.

5. CONCLUSION:

In induced alopecia model, the results shows that the Standard and test drug both increase the Hair density, hair follicle and testosterone concentration as compared to the toxic group. Increase in hair density and testosterone concentration in case of chemotherapy treatment is a good sign of preventing hair loss. Hair densities in case of both drugs were also higher in comparison to toxic. Histopathology finally clears the results, as a maximum of the Hair follicles were in anagen phase in both test drugs. The hair growth activity that was worked on cytotoxic drug induced alopecia model was investigated by using various parameters like hair density, hair follicle number, testosterone with histopathological studies. *Momordica charantia* fruit may show it activity due to the presence of isoflavonoid, protein and amino acid, insulin growth factor and may be due to increasing blood circulation which promotes hair growth in humans.

6. ACKNOWLEDGEMENTS

The authors are thankful to Dr. Navin Raj, Technocrats Institute Technology-Pharmacy (Bhopal) and Globus college of Pharmacy (Bhopal) for providing all facilities to carry out this research work. Thank to Mr. Ashish Toppo, research scholar for encouragement to carry out this research in time.

7. REFERENCES

[1] Carelle. N, Piotto. E., Bellanger. A, changing patient perceptions of the side effects of cancer Chemotherapy, 95, 2002, 155-163

[2] Wang J, Lu Z, and Au J.L.S., Protein against chemotherapy induced alopecia, 23, 2001,34-37.

[3] Batchelor, D., Hair and cancer chemotherapy: consequences and nursing care a literature study. European Journal of Cancer Care, 10, No.3, 2001, 147-163.

[4] O'Leary, A., Stress, emotion, and human immune function. Psychological Bulletin, Vol.108, 3, 1990,363-382

[5] Cash, T.F., The psychosocial consequences of androgenetic alopecia: a review of the research literature, 1999, 141(1), 398–405.

[6] Chase, H.B., The physiology and histochemistry of hair growth, 6, 1955, 9–14

[7] Wilkes, G. M., Potential toxicities and nursing management, In: Cancer Chemotherapy: A Nursing Process Approach, 1996, 130-135

[8] Randall V.A., Sheppard M., Stewart P, Alopecia in women -the role of 5α reductase in health and disease, 8(1), 1994, 405–31.

[9] Carroll P.V., Treatment with growth hormone and insulin-like growth factor-I in critical Illness,15,2001, 435–451.

[10] Su H.Y., Hickford J.G., Bickerstaffe R., Insulin-like growth factor 1 and hair growth ,5,1999, 1.

[11] Shimozawa N., Okajima K.,Harada N., Estrogen and isoflavone attenuate stress-induced gastric mucosal injury by inhibiting decreases in gastric tissue levels of CGRP in ovariectomized rats, 292, 2007 ,615–619

[12] Cunnick J.E., Sakamoto K., Induction of tumor toxic cytotoxic immune cells using a protein From Bitter melon, 126, 2, 1990, 278

[13] Mukherjee P.K. "Quality control of herbal drug",1 , 2002, 186-192.

[14] Gennaro R Alfonso, Remington- the science and practice of pharmacy, Lippincott Williams and Wilkins, 2(20) ,1999, 487-848.

[15] Dahanukar S.A. and Thatte U.M., Ayurveda Revisited, Popular Prakashan, 1, 1994,1-28.

[16] Aulton, M. E., Pharmaceutics: The science of Dosage form, Churchill Livingstone,1996,304.

[17] Fielder V, Gray A, Diffuse alopecia: telogen hair loss, Disorders of Hair Growth: Diagnosis and Treatment. New York,, McGraw-Hill, 2003,303-320.

[18] Whiting D A, Histopathology of alopecia areata in horizontal sections of scalp biopsies, J Invest Dermatol , 1995, (210) , 26-27.

[19] Lee Y, J, Alopecia syphilitica, a simulator of alopecia areata-Histopathology and differential diagnosis,12, 1991, 87-92.

[20] Armitage, P, Statistical Methods in Medical Research, Blackwell Scientific Publications, London,1, 1971, 217.

[21] Alonso,L., Fuchs,E., The hair cycle, Journal of cell science, 2006, 119(3), 391-393.

[22] Corsarelies,G., Millar,S.E., Towardsa molecular understanding of hair loss and it's treatment, 2001, 7 970, 293-301

[23] Paus, R., Handjiski, B.; Eichmuller, S., Czarnetzki, B. M., Chemotherapy-induced alopecia in Mice induction by cyclophosphamide, inhibition by cyclosporine A and modulation by dexamethasone, American Journal of Pathology ,144, 4, 1994, 719-734.

[24] Messenger, A.G.,Rundengren, Minoxidile:Mechanism of action on hair on hair growth, British journal of Dermatology, 2004, 150(2), 186-194

[25] Ohnemus U, Uenalan, M.; Inzunza, J.; Gustafsson, J. A. & Paus, The hair follicle as an estrogen target and source, Endocrine review, 2006, 27(6). 677-706.