Evaluation of Hair Growth Activity of Pueraria Tuberosa (Kudzu) by using Chemotherapy Induced Alopecia

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Abstract - This effect was prevented by the oral administration of Pueraria tuberosa (Kudzu) alcoholic extract, contain Pureraria, Daidzein and genistein an isoflavone and phytosterole which promote hair follicle growth and reduce the chances of alopecia condition. Objectives: The present study aimed at alopecic activity of Pueraria tuberosa by using chemotherapy induced hair fall and modulating the mechanisms responsible for this condition. Chemotherapy induced alopecia which is a result of damage of hair follicle given to on day 9 after depilation. Groups of mice received the Pueraria tuberosa extract and standard minoxidile 5% solution administered orally to elucidate the biological activity in vivo. Occurrence of alopecia was evaluated for up to 21 days of Cyclophosphamide, and the Body weight and survival of mice was 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 was increased by chemotherapy. Test drug extract (Pueraria tuberosa) show increase hair density, hair follicle number and testosterone concentration as compared to increased count in toxic group. Conclusions: Increase in testosterone concentration in case of chemotherapy is a good sign of preventing which causes hair loss. Anticancer drug arrest the cell cycle of 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 hair fall due to the presence of isoflavonoid , protein and amino acid, insulin growth factor and may be due to balancing androgen hormone (androgen) which promote hair growth in humans.

Keywords: Alopecia, Pueraria tuberosa, Isoflavonoid, Testosterone, Hair density, Hair follicle number.

I. INTRODUCTION

Hair loss is a distressing condition for number of men & women. It is also a common & ever increasing problem in cosmetics as well as primary health care practice. Presently, Minoxidil & Finasteride are two USFDA approved synthetic drugs widely used for treatment of androgenic alopecia. Hair follicle growth occurs in cycle. Each cycle consists of a long growing phase (anagen), a short transitional phase (catagen) and a short resting phase (telogen). At the end of the resting phase, the hair falls out (exogen) and a new hair starts growing in the follicle beginning the cycle again. It is believed that in Alopecia, an as yet unidentified trigger stimulates an autoimmune lymphocytic attack on the hair bulb. This inflammation is specific for anagen cycle hairs and causes anagen arrest. It has now been widely postulated that alopecia is an organ specific autoimmune disorder with genetic predisposition. Some studies have suggested that emotional trauma contributes to the appearance of alopecia. 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 [1] .

All androgen-dependent follicles require androgen receptors to respond of adult body hair in complete androgen insensitivity. In contrast, the requirement for 5α reductase varies with follicle site. Individuals with 5α reductase type 2 deficiencies only produce female patterns of pubic and auxiliary hair growth, although their body shapes become masculinised. 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. 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 expression 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. 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[2-5].

From the literature review of *Pueraria tuberosa,* commonly known as kudzu, Indian kudzu or Nepalese kudzu and possess, hypolipedemic, antiviral, antibacterial, antifungal, antiandrogenic, Vasodilator , Antiapoptosis, Antihypertensive and anxiolytic as biological activity. Thus from the knowledge of different causes of alopecia we can correlate that all of this activity can lead to hair growth. *Pureria tuberosa* a plant native to the semi-tropical climate of China, India, Asia, and Africa, bears a tubers that is currently the most widely used traditional medicine to treat cardiovascular disorder and is one of the most promising alternative medicines for the disease. A few active components like phytosterole and estrogen-like compounds called isoflavones that prohibits the formation of dihydrotesterone, which is thought to benefit alopecia and investigations suggest that a high diet in phytosterols may inhibit the enzyme, 5-α reductase and block the production of dihydrotesterone (DHT) [6].

II. MATERIAL AND METHOD

The tubers of *Pueraria tuberosa*, was collected from the Bhimtal, Nainital,Utrakhand (India) in the month of April-May. 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 and the plant material was dried and authenticated by Department of Botany, J.H. Govt College, Barkatullah University, Bhopal.

A. Preparation of extract:

The powdered tubers of *Pueraria tuberosa* were extracted with petroleum ether (60-80º) to remove lipid and then against extracted with ethanol in soxhlet extractor. The solvent are distilled to concentrate the extract and dried in vacuum desiccators. Hence Ethanolic extract was selected for hair growth activity screening. All the test suspension (300mg/ml) were prepared in the vehicle i.e. 5% w/v tragacanth mucilage and were administered in the dose of 300mg/kg orally [7-8].

B. Toxicity studies:

Female C57BL/6 mice weighing 100-150 g of either sex, procured, maintained under slandered husbandry condition (Temp 23±2º, relative humidity 55±10% and 12 hours light dark cycle) were used for all set of experiments in group of six animal. Animal were allowed to take slandered laboratory feed and tap water. The extract of *Pueraria tuberosa* Tubers was administered to different group of mice in doses of 300mg/kg [9]. There is no lethality in any of the groups. The experiments were performed after the experimental protocol approved by the institutional Animal Ethics Committee, Technocrate Institute of Technology (Pharmacy) Bhopal Madhya Pradesh.

C. Chemotherapy induced alopecia:

The extract at a selected dose was 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 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 intra-peritoneal route 125 mg/kg of body weight to group III and IV. Group III receives Minoxidile oral treatment from experimental day 15 to experimental day 20. During the stress 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 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 viz tubers of *Pueraria tuberosa* formulation . Individual body weights were registered upon arrival of mice and at daily and weekly interval until the finish of experiment. Survival of mice was also inspected and recorded. After 120 hours, blood was collected by puncturing the retro orbital plexus and was used for determination of lymphocyte count. Hair density was also determined in mm^2[10].

D. Histopathology studies:

One animal from the treated group showing maximal activity as indicated by improved biochemical parameters from each test, control and negative control group. skins of mice from all groups 5mm skin was collected and placed in 10 % formalin solution and histopathological analysis was done .the section were observed under microscope for histopathological change in skin architecture and their photomicrograph were taken[11].

F. Statistical analysis:

The mean value±SEM are calculating for each parameter. For determining the significant intergroup difference each parameter was analyzed separately and one way analysis of variance (ANOVA) was carried [12].

III. RESULT AND DISCUSSION

In the study of the Ethanolic extract of *Pueraria tuberosa* on hair growth activity, it was found that in the alopecia model All the treated groups shows increase in hair density and hair follicle number as compare to group II and I. Also it was observed that the hair density was much more in group (standard), followed by group IV (test drug)

Table No 1: Inspection of body weight and survival

Body weight (g) of mice (mean± S.E.)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	*P.Tuberosa*	**23.64±0.71**	**22.97±0.67**	**21.78±0.43**	**22.25±0.66**	**21.21±0.62**

Cyclophosphamide induced alopecia histopathology and figure given belove ,it was observed in table no. 2 that all the treated groups shows 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 higher percentage than group I (control). Also it was observed that the hair density was much more in group IV(Test drug), followed by group II **.** It was observed table no. 3 that 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 *Pueraria. Tuberosa.* It was revealed that Positive control (minoxidile) and *P. tuberosa* Preparation increase the hair density.

Table No. 2 : 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**

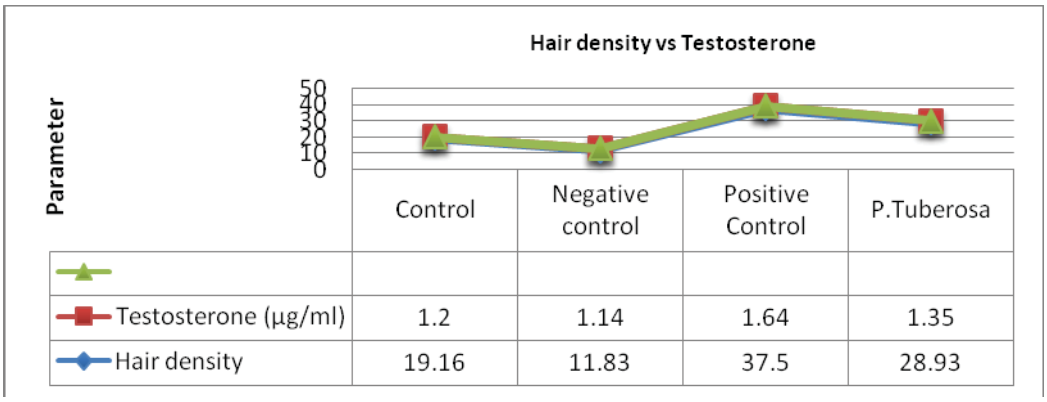
3	Standard	**82±0.027**	**5.4±0.029**	**12.6±0.040**
4.	*P.Tuberosa*	**72±0.022**	**4.8±0.030**	**23.2±0.036**

Table No. 3 :- Percentage of 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	*P.Tuberosa*	28.93 ± 0.81	1.35±0.0081

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 (uµg/ml) of peripheral blood was counted Testosterone concentration was measured in (µg/ml) when cyclopshomide administered its show cytotoxic effect on hair follicle and reduce the production of androgen which is directly associated with hair follicle formation .

Graph 1 : Hair density vs Testosterone



In group II (standard) administered minoxidile show vasodilatation effect and promote the blood circulation at the base of hair follicle and increase the keratin formation from protein cell and show increased hair growth activity. When data was compared with standard drug testosterone concentration was high in test group as compared to negative control but less to standard.

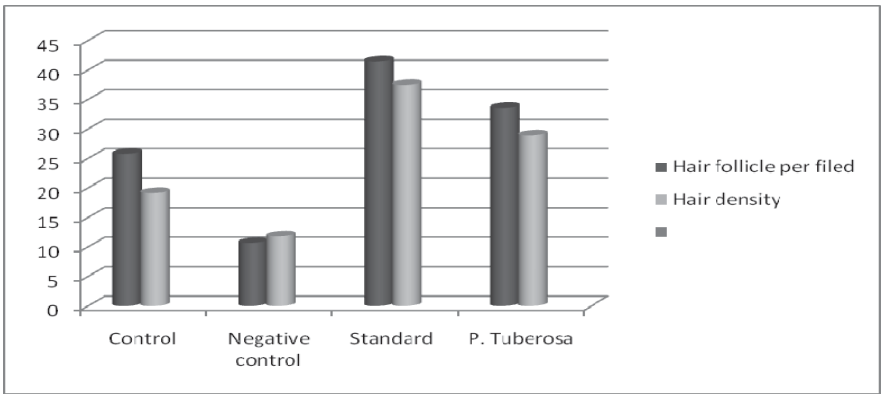
Histopathology:
Paraffin-embedded 5-µm sections were stained with hematoxylin and eosin (H&E). Hair growth was evaluated microscopically in the H&E-stained sections of dorsal skin.

Quantitative hair follicle number :
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 then standard group.

Table No. 5 : Number of Hair follicle 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	***Pueraria Tuberosa***	33.6±12.8

Graph 2: Hair density and Hair follicle per filed



IV. 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 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 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. *Pueraria tuberosa* Tubers may shows it activity due to the presence of isoflavonoid , protein and amino acid, insulin growth factor and may be due to increase blood circulation which promote hair growth in Humans.

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