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Original Research Article

EVALUATION OF HAIR GROWTH ACTIVITY OF *PUERARIA TUBEROSA* BY USING STRESS INDUCED HAIR LOSS

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Abstract: Background: We previously demonstrated that stress induce alopecia in mice. This effect was prevented by the oral administration of *Pueraria tuberosa* alcoholic extract, an isoflavone and phytosterole containing plants which prevent hair loss alopecia condition. **Objectives:** The present study aimed at hair growth activity of *Pueraria tuberosa* by Using Stress Induced Stress induced alopecia and modulating the mechanisms responsible for this condition. **Methods:** 4 Group of mice were exposed whole-body to sonic stress. 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 exposure, and the hair density, hair population, lymphocyte count and testosterone level in peripheral blood was investigated. **Results:** The hair density and hair population decrease and lymphocyte count was increased by stress; irrespective of treatment with ultrasonic sound. Test drug extract show decreases the lymphocyte count as compared to increased count in toxic group. **Conclusions:** Decrease in lymphocyte count in case of stress is a good sign of preventing hair loss 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.

Keywords: Isoflavonoid, Phytosterole, Pueraria tuberosa, Lymphocyte count, Hair density.

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

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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 organspecific 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 metabolised 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 deficiency only produce female patterns of pubic and axillary 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 deficiency 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)[6-7]

Material and method: The tubers of *Pueraria tuberosa*, 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 and the plant material was dried and authenticated by Department of Botany, J.H. Govt College, Barkatullah University, Bhopal.

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 vaccum desiccater. Hence Ethanolic extract was selected for hair growth activity screening. All the test suspension (300mg/ml) were prepared in the vehical i.e. 5% w/v tragacanth mucilage and were administered in the dose of 300mg/kg orally[8].

Toxicity studies: Female C57BL/6 mice weighing 100-150 g of either sex, procured, maintained under slandered husbandery 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. 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.[9]

Stress induced alopecia: The extract at a selected dose was evaluated for its effect on stress induced alopecia by studying biochemical parameters. Mice divided into control, negative control ,standard and test groups, each group comprising of six animals. The stress was produced by rodent repellent device at 300 hertz for 120 hertz 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 stress and administration of plant Extract viz tubers of *Pueraria tuberosa* ,individual body weight were registered upon arrival of mice and at 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] Hair follicles number was determined by using 10 µm paraffin sectons 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 and the number of hair follicle being expressed per one filed.

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].

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].

Result and discussion: In the study of the Ethanolic extract of *Pueraria tuberosa* on hair growth activity, it was found that in the stress induced 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 V, followed by group III and then group IV.where as the treated groups shows decrease in lymphocyte count as compare to group II and I. Also it was observed that the lymphocyte count was less in group V, followed by group III and then group IV.

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**	22.20±.51	22.41±0.47	22.89±0.25	22.96±0.26	23.13±0.23
2	**Standard**	21.39±0.35	22.02±023	22.38±0.15	22.46±0.10	23.07±0.18
3	**Negative control**	21.40±0.52	21.09±0.31	20.89±0.36	20.87±0.34	20.78±0.30
4	***Pueraria.Tuberosa***	22.74±0.81	22.27±0.67	22.34±0.68	22.25±0.66	22.21±0.62

stress 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 **.**

Percentage Population of Hairs (stress)

Table No. 2:- Percentage Population of Hairs

S.No.	Groups	Anagen	Catagen	Telogen
1.	Control	62	3	35
2.	Negative	40.5	2.5	57
3	Standard	83	5.5	11.5
4.	*P.Tuberosa*	74	4.8	21.2

Lymphocyte count And Hair Density: It was observed table no. 3 that the percentage of lymphocyte count in negative control is high when compared with control and standard which might be due to inflammatory change due to stress treatment, On comparing the data the lymphocytes count of Control , Negative control , Positive control and test drug *Pueraria. Tuberosa.* It was revealed that Positive control (minoxidile) and *P. tuberosa* Preparation decreases the lymphocytes count.

Percentage of Lymphocyte count and Hair density

Table No. 3 :- Percentage of Lymphocyte count and Hair density

S.No.	Treatment	Lymphocyte count(%)	Hair density (mm^2)
1	Control	17.89 ± 0.75	20.16 ± 0.33
2	Negative control	31.10 ± 0.75	10.50 ± 0.65
3	*Positive Control*	08.39 ± 0.71	33.55 ± 0.88
4	*P.Tuberosa*	12.00 ± 0.89	28.33 ± 0.66

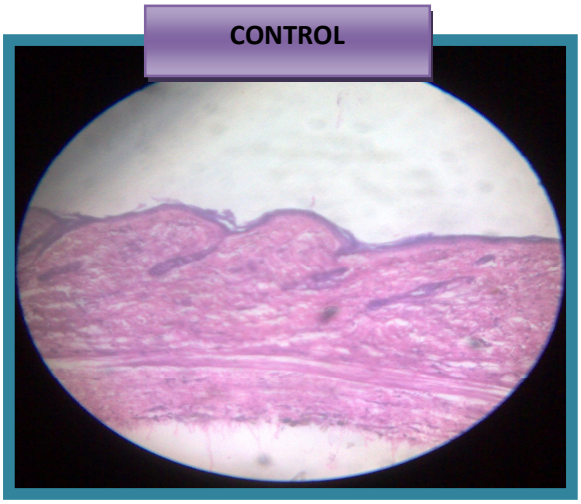
Lymphocyte counts increased and Hair Density is decreased in the case of the animals treated with Sonic sound, whereas the lymphocyte counts got decreased and Hair density is increased in *Pueraria Tuberosa* as compared to minoxidil and other control and Negative control groups after sonic stress treatment.

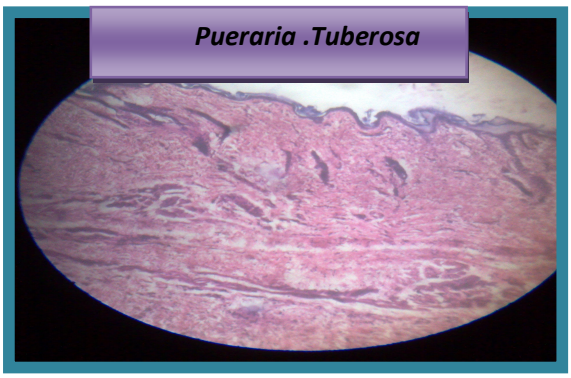
Testosterone Level:-

Table No. 4 Testosterone Level

S.No.	Treatment	Testosterone level(µg/ml)
1	Control	1.20±0.011
2	Negative control	1.14±0.0121
3	Standard	1.64±0.008
4	*Pueraria Tuberosa*	1.35±0.0081

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 sectons 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	26.7±4.7
2	Negative control	12.3±5.2
3	*Positive Control*	45.5±5.3
4	*Pueraria Tuberosa*	32.8±17.2

Conclusion: In stress induced alopecia model, the results shows that the Standard and test drug both decreases the lymphocyte count as compared to increased lymphocyte count in toxic group. Decrease in lymphocyte count in case of stress 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 stress induced alopecia model was investigated by using various parameters like hair density, lymphocyte count with histopathological studies. The plant. *Pueraria tuberosa* tubers shows result in stress induced alopecia. *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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