

# “EFFECT OF ETHANOLIC EXTRACT OF ROOTS OF PANAX GINSENG IN STRESS INDUCED HAIR LOSS IN ALBINO RAT”

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## Abstract:

Present study is associated with the evaluation of anti-hair loss activity of ethanolic extract of roots of Panax ginseng in animal models of hair loss induced by Stress. Panax ginseng cream formulation was employed in this study. The study observations and results were compared with Minoxidil in stimulating hair growth in stress induced alopecia animal model. Thus on comparison Panax ginseng cream and Minoxidil it has been observed that Panax ginseng cream herbal formulation application shows better growth than the patch with minoxidil. Panax ginseng on albino rat using a stress –induced alopecia model. Rats were divided into five groups. Each group contained six animals. Group I – considered as Normal control which were not undergone the hair-loss induction methods but received only the simple ointment base alone. Group II – considered as Negative control which were undergone the hair-loss induction methods Exposed to sonic stress. Group III– this group was known as the Standard group which received the Std drug i.e. Minoxidil 2 % topically and Exposed to sonic stress. Group IV– Considered as Test 1 group which received Ethanolic extract of *Panax Ginseng* cream (2 % topically.) and Exposed to sonic stress. Group V– Considered as Test 2 group which received Ethanolic extract of *Panax Ginseng* cream (4 % topically.) and Exposed to sonic stress. After 25 days patch of hair loss was seen in animals received sonic stress while animals treated with Panax ginseng showed less hair loss as compared with those treated with stress only. The hair length determinations for Panax Ginseng cream 2% were  $2.51 \pm 0.21$ , and Panax Ginseng cream 4%  $2.85 \pm 0.18$  respectively. The hair density determinations for Panax Ginseng cream 2% were  $1540 \pm 52.23$ , and Panax Ginseng cream 4%  $1730 \pm 53.85$  respectively.

**Keywords:** *Panax ginseng*; Alopecia; Stress.

## 1. Introduction

Alopecia is a complex phenomenon that is not fully understood either in human or non human species. Hair loss can occur as the result of a congenital or genetic disorder, or it can develop during the lifetime of the animal and human being. Hair loss occurring throughout life can be further divided into inflammatory and non-inflammatory types. Pattern of hair-loss can be categorized in various ways, Hair is one of the vital parts of body derived from ectoderm of the skin and it is one of the protective appendages

on the body. It is an important of the overall appeal of the human body. Many people suffer from hair loss or hair thinning (ALOPECIA) despite the development of several medical treatments. Therefore, it is important to develop new therapies that prevent hair loss and increase hair growth. In this respect, alternative medicine has attracted interest, although it has not yet been included into mainstream of medical care, due to the limited scientific proofs and incomplete knowledge of the mechanisms involved. <sup>[1]</sup>

## 1.1 ALOPECIA

Alopecia is a medical term for hair-loss or thinning of hair can be a sign of serious diseases specially if the hair losses rapidly. Alopecia is a dermatological disorder.

Alopecia is mainly divided in to two types

- Alopecia Areata.
- Androgenic alopecia.

### 1.1.1 ALOPECIA AREATA

Alopecia areata is a common, non-scarring, auto-immune disease that can affect hair-bearing area. This disease is generally presented as a single, well-demarcated patch of hair loss, multiple patches or extensive hair loss. It is generally to be the caused by association with auto-immunity, but the pathogenesis is still uncertain. The general population of the alopecia areata (AA) is generally 0.1% to 0.2%. <sup>[2]</sup>

### 1.1.2 ANDROGENIC ALOPECIA

Androgenetic alopecia or simply “balding”, the most common form of hair loss in men, involves the progressive hair loss, in response to circulating androgens. In this disease the hair-loss occurs will be more. It may also occur in women. Although, there is racial variation in the incidence of androgenetic alopecia, it affects at least 50% of men by the age of 50 years, and up to 70% of all males in later life. <sup>[3]</sup>

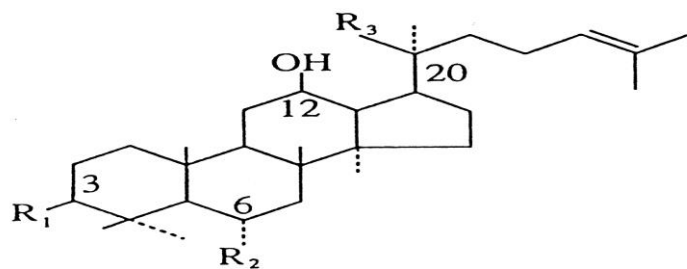
## 2. Plant Profile

### 2.1 *Panax Ginseng*:

*Panax Ginseng* belonging to the family Araliaceae commonly known as *Panax ginseng* and *Panax quinquefolius*. (Synonym: The botanical/genus name *Panax* means "all-heal" in Greek, sharing the same origin as "Panacea" was applied to this genus because Linnaeus was aware of its wide use in Chinese Medicine as a muscle relaxant. <sup>[4]</sup>



*Panax ginseng* contains triterpene glycosides, or saponins most commonly referred as ginsenosides. Many active compounds can found in all parts of the plant including amino acids, phenols, proteins polypeptides, and vitamins B1 and B2.3 Up to 40 distinct ginsenosides have been identified by thin layer chromatography (TLC) and methanol extraction experiments. The two major sub-types of ginsenosides, protopanaxadiol and protopanaxatriol, are classified according to the arrangement and number of sugar residues – glucose, rhamnose, xylose, and arabinose – on the ginsenoside. Rb1, Rb2, Rc, and Rd are examples of protopanaxadiol ginsenosides. <sup>[4]</sup>



Ginsenosides	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
Ginsenoside-Rb <sub>1</sub>	-O-Glc <sup>2</sup> -Glc	-H	-O-Glc <sup>6</sup> -Glc
Ginsenoside-Rc	-O-Glc <sup>2</sup> -Glc	-H	-O-Glc <sup>6</sup> -Ara (pyr)
Ginsenoside-Re	-OH	-O-Glc <sup>2</sup> -Rha	-O-Glc
Ginsenoside-Rf	-OH	-O-Glc <sup>2</sup> -Glc	-OH
Ginsenoside-Rg <sub>1</sub>	-OH	-O-Glc	-O-Glc

### 2.3 Pharmacological Indications—

*Panax Ginseng* (PG) is the agent which has been used in various physiological actions like common anti hair-loss, aphrodisiac, respiratory disorders etc in Indian and other Asian system of medicines. In China it was traditionally used in the treatment of may be found in small doses in energy drinks or herbal teas, such as ginseng coffee. Hair tonics and cosmetic preparations but none has been proved as muscle relaxant. The ethanolic extract of the *Panax Ginseng* root shown significant anti hair-loss activity against androgenic alopecia and alopecia areata. Ethanolic and petroleum ether extract of *Panax Ginseng* roots has shown the anti diabetic action against type1 and type2 diabetes.<sup>[04]</sup>

## 3. MATERIALS AND METHODS.

### 3.1 Animals

Swiss albino rat (225-250 g) of either sex obtained from the animal house of Anuradha college of pharmacy, Chikhli were used. The animals received standard pellet diet (M/s Hindustan Lever Foods, Kolkata, India), water *ad libitum* and were maintained under standard environmental conditions were approved by the Institutional Animal Ethics committee of Anuradha College of pharmacy, Chikhli.

### 3.2 Chemicals and standard drugs:

Stress was given by using epilator device. Minoxidil was obtained from Cipla Ltd. (Rorathang, Sikkim, India). The extract was reconstituted and cream was prepared for topical administration in the doses of 2% and 4% respectively.

### 3.3 Preparation of extract:

The roots of *Panax Ginseng* were collected in December 2015 in the Botanical garden of Department of Botany, Shri Shivaji College of science, Chikhli Dist. Buldana.

The dried roots were extracted at room temperature with the absolute ethanol for 2 hours for the preparation of the extracts for this study. The obtained extract was then concentrated at 50<sup>0</sup> C until a yellow brown coloured solid mass was obtained further the after drying a yellow brown powder of drug is used for study.

### 3.4 Phytochemical screening:

Phytochemical screening of the extract was carried out using convectional protocol (Wagner et al., 1984) for detecting the presence of different constituents like tannins, saponins, unsaturated sterols, triterpenoids, alkaloids, anthraquinones, flavonoids etc. The saponins contents of extract were determined using gravimetric method.

### 3.5 Methods:

#### 3.5.1 Induction of Hair-loss:

Hair loss in animals was produced by various animal models of hair loss by Amjadkhan Pathan et, al. the methods which were used in this study are namely,

##### Sonic stress induced hair-loss

If stress becomes persistent at low-level, however, all parts of body stress apparatus (the brain, heart, lungs, vessels, and muscles) become chronically over or under-activated. This may produce physical or psychologic damage over time. Acute stress can also be harmful in certain situations. A number of studies have shown that subjects under chronic stress have low white blood cell counts and are vulnerable to colds. And once any person catches cold or flu, stress can exacerbate symptoms. People who harbor herpes or HIV viruses may be more susceptible to viral activation following exposure to stress. even more serious, some research has found that HIV-infected men with high stress levels progress more even more serious it will progress more rapidly to AIDS when compared to those with low stress levels.<sup>[05]</sup>

## 4. Results

### 4.1 Evaluation of the anti hair-loss response: -

In this study the responses were taken by the use of various techniques as, skin irritation test, Hair length, Hair density, Biochemical and hematological parameters Qualitative study.

#### 4.1.1 Qualitative study.

The difference in growth of hair in each group was evaluated by visual observations and was recorded by photographs after 25 days.<sup>[03]</sup>

#### 4.1.2 Skin irritation test:

Three healthy female wistar albino rats, weighed 200-250gm were selected for study. Each rat was caged individually food and water given during the test period 24 hrs prior to the test. The hair from the back of each rat of 1 cm<sup>2</sup> was shaved on the side of the spine to expose sufficiently large test areas, which could accommodate three test sites were cleaned with surgical spirit. 1 gm quantity of formulation Panax ginseng cream was applied over the respective test sites of one side of the spine. The test sites were observed for erythema and edema for 48 hrs after application.<sup>[06]</sup>

#### 4.1.3 Hair length

Hair was plucked randomly from the depilated area with the help of electric clipper and measured the hair length with the help of vernier caliper and calculates the mean of hair length.<sup>[05]</sup>

#### 4.1.4 Hair density

A hole of 1cm<sup>2</sup> 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 25 days of depilation. The hair was trimmed of the desired depilated area and the hair was cut with the seizure. The hair was count manually.<sup>[05]</sup>

#### 4.1.5 Biochemical and hematological parameters

##### 4.1.5.1 Total protein estimation

Total serum protein in blood estimated by modified Biuret test method. Biuret is a compound formed by heating urea to 180 degree concentration. When Biuret treated with diluted copper in sulphate in medium, a purple color is obtained.<sup>[07]</sup>

##### 4.1.5.2 Total leukocyte count (White blood cells )

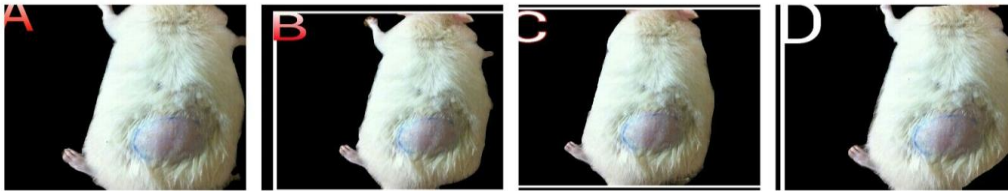
Blood sample was collected after the treatment from retro orbital plexus of rats on the 25th day of treatment. The distal one-half centimeter of the tail was clipped and a capillary pipette containing anticoagulants (EDTA for cell counting) was used to collect 20 µl samples from the bleeding surface. The withdrawn sample was used for cell counting and immediately after collection; the cut surface of the tail was cauterized with styptic powder. The collected blood was transferred to sterile test tube containing anticoagulant at a ratio of 1: 10. The collected blood was used for hematological parameters within two hours of collection. The hematological parameters were determined with the help of with the help of pathology laboratories.<sup>[05]</sup>



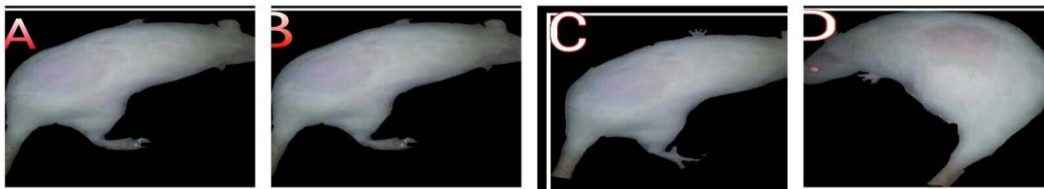
**A] Qualitative study:**

The difference in growth of hair in each group was evaluated by visual observations and was recorded by photographs after 25 days.

Day 1



Day 8



Day 18



Day 25

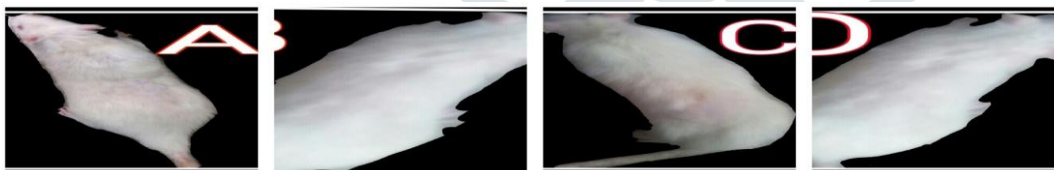


Figure1. Comparison of hair growth patterns in various groups after 25 days: (A) animal treated with cream base (B) animal treated with Minoxidil 2%, (C) animal treated with Panax Ginseng 2%, (D) animal treated with Panax Ginseng 4%,

**B] Primary skin irritation test:**

Three healthy female wistar albino rats, weighed 200-250gm were selected for study. Each rat was caged individually food and water given during the test period 24 hrs prior to the test. The hair from the back of each rat of 1 cm<sup>2</sup> was shaved on the side of the spine to expose sufficiently large test areas, which

could accommodate three test sites were cleaned with surgical spirit. 1 ml quantity of formulations *Panax ginseng* was applied over the respective test sites of one side of the spine. The test sites were observed for erythema and edema for 48 hrs after application.

Sr. No.	Ginseng 2%		Ginseng 4%	
	Erythema	Edema	Erythema	Edema
1	NO	NO	NO	NO
2	NO	NO	NO	NO
3	NO	NO	NO	NO

Table 1: skin irritation test

### C] Hair length:

Hair was plucked randomly from the depilated area with the help of electric clipper and measured the hair length with the help vernier caliper and calculated the mean of hair length.

Sr. No.	Groups	Treatment	Dose	Hair length mm (mean $\pm$ s.d.)
1	Control	Cream base	Cream base	2.16 $\pm$ 0.11
2	Standard	Minoxidil	2 % topically	3.06 $\pm$ 0.15
3	Test 1	Panax ginseng	2% Topically	2.51 $\pm$ 0.21
4	Test 2	Panax ginseng	4% Topically	2.82 $\pm$ 0.18

Table 2:hair length in mm

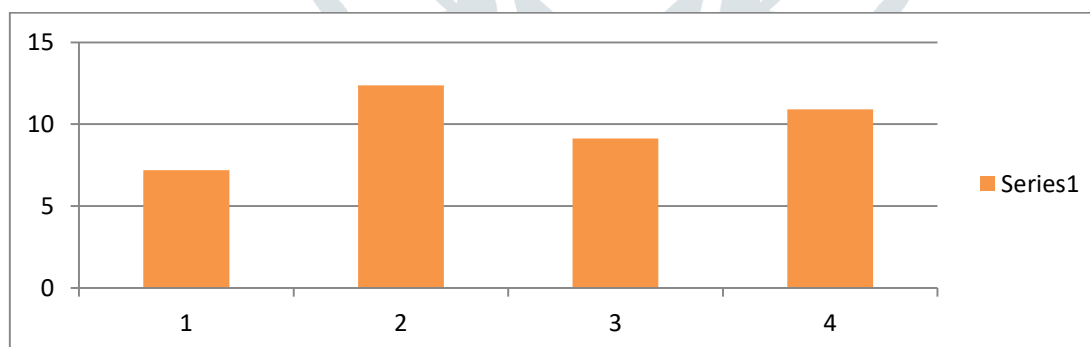


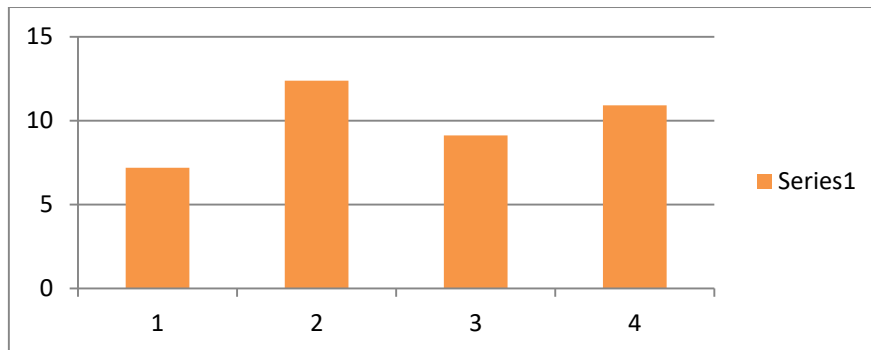
Figure no.2 Graphical representation of hair length mm mean  $\pm$  SD. Values are statistically significant.

### D] Hair density:

A hole of 1cm<sup>2</sup> 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 25 days of depilation. The hair was trimmed of desired depilated area and the hair was cut with the seizure. The hair was count manually.

Sr. No.	Groups	Treatment	Dose	Hair count per cm <sup>2</sup> (mean ± s.d.)
1	Control	Cream base	Cream base	1071 ± 42.11
2	Standard	Minoxidil	2% Topically	1875 ± 51.21
3	Test 1	Panax Ginseng	2% Topically	1538 ± 49.18
4	Test 2	Panax Ginseng	4% Topically	1709 ± 52.23

Table 3: hair density

Figure no.3 Graphical representation of hair density (Hair count per cm<sup>2</sup>) mean ± SD. Values are statistically significant.

## E] Biochemical parameters

### Total protein estimation

Total serum protein in blood estimated by Modified Biuret method. Biuret is a compound formed by heating urea to 180 degree concentration. When biuret treated with diluted copper sulfate in medium, a purple colour is obtained.

Sr. No.	Groups	Treatment	Dose	Total serum protein g/dl (mean ± s.d.)
1	Control	Cream base	Cream base	5.07 ± 1.11
2	Standard	Minoxidil	2% Topically	7.85 ± 1.15
3	Test 1	Panax Ginseng	2% Topically	5.79 ± 0.21
4	Test 2	Panax Ginseng	4% Topically	6.18 ± 1.64

Table 4: Total serum protein g/dl



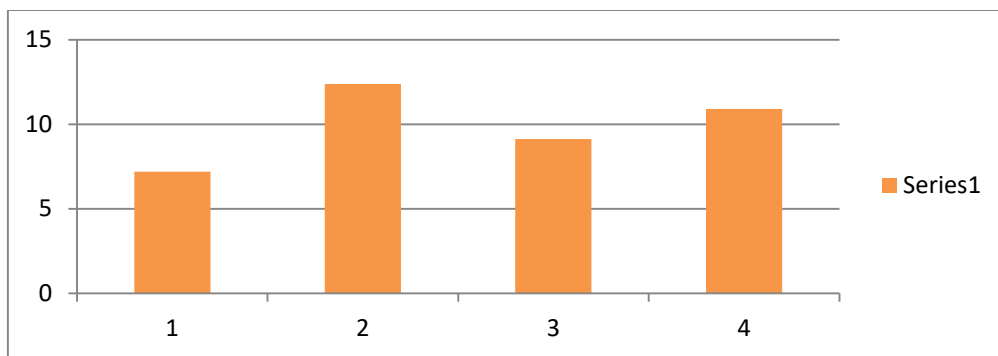


Figure no.4 Graphical representation of total serum protein mean ± SD. Values are statistically significant.

**Total leucocytes Count (White Blood Cells)**

Blood sample was collected after the treatment from retro orbital plexus of rats on the 25th day of treatment. The distal one-half centimeter of the tail was clipped and a capillary pipette containing anticoagulants (EDTA for cell counting) was used to collect 20 µl samples from the bleeding surface. The withdrawn sample was used for cell counting and immediately after collection; the cut surface of the tail was cauterized with styptic powder. The collected blood was transferred to sterile test tube containing anticoagulant at a ratio of 1: 10. The collected blood was used for hematological parameters within two hours of collection. The hematological parameters were determined with the help of pathology laboratories.

Sr. No.	Groups	Drug given	Formulation	Total WBC count mm <sup>3</sup> (mean ± s.d.)
1	Control	Cream base	Cream base	7.19 ± 0.65
2	Standard	Minoxidil	2% Topically	12.37 ± 2.52
3	Test 1	Panax Ginseng	2% Topically	9.12 ± 1.21
4	Test 2	Panax Ginseng	4% Topically	10.91 ± 2.31

Table 5: Total wbc count mm<sup>3</sup>

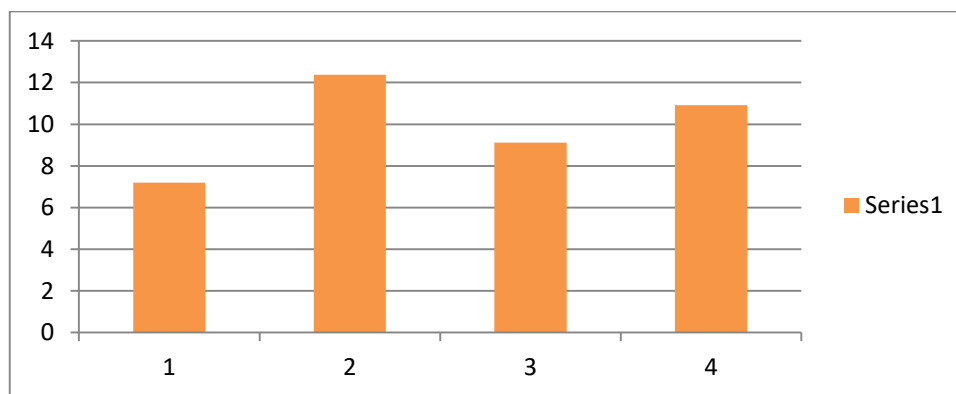


Figure no.5 Graphical representation of total wbc count mm<sup>3</sup> mean ± SD. Values are statistically significant.

## 5. DISCUSSION

The results of the present study indicated that twenty five days treatment of ethanolic extract of the dried roots of *Panax Ginseng* (PG) cream possesses anti hair-loss activity. on and sonic stress induced hair loss. The hair length determinations for Panax Ginseng cream 2% were  $2.51 \pm 0.21$ , and Panax Ginseng cream 4%  $2.85 \pm 0.18$  respectively. The hair density determinations for Panax Ginseng cream 2% were  $1540 \pm 52.23$ , and Panax Ginseng cream 4%  $1730 \pm 53.85$  respectively. Thus on comparison Panax Ginseng cream 4%, and Panax Ginseng cream 2%, and Minoxidil it has been observed that Panax Ginseng cream 4%, as herbal formulation application shows better growth with minoxidil.

## 6. CONCLUSION

In this study the experimental design was made to obtain the anti hair-loss activity of the ethanolic extract of Panax ginseng cream on sonic stress exposed animals rat. The animal model which were used in this study, sonic stress induced hair-loss. The cream formulation of 2% and 4% of Panax Ginseng shows significant anti hair-loss in sonic stress induced hair-loss.

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## REFERENCE-

1. Melinda A Novak,\* and Jerrold S Meyer. Alopecia: Possible Causes and Treatments, Particularly in Captive Nonhuman Primates, Vol 59, No 1 February 2009 Pages 18–26
2. Ga Na Oh and Sang Wook Son Efficacy of Korean Red Ginseng in the Treatment of Alopecia Areata Research Article J Ginseng Res(2012) Vol. 36, No. 4, 391-395
3. Maryam Noubarania, Hossein Rostamkhanib, Mohammad Erfanb, Mohammad Kamalinejadb, Mohammad Reza Eskandaria, Effect of Adiantum Capillus veneris Linn on an Animal Model of Testosterone-Induced Hair Loss Iranian Journal of Pharmaceutical Research (2014),13 (supplement): 113-118
4. Sarah Khalid, Uzma Akhtar And Mohammad Tahir, Panax Ginseng, Its Effects On Development Of Hair Follicles – An Experimental Study, Biomedica Vol. 28 (Jan. – Jun. 2012), 18-22

5. Pathan A. , Pathan M., Garud N., Garud A., effect of some novel medicinal plants and polyherbal formulation on stress induced alopecia pharmacology On Line December 30, 2012 vol.3 • 150 – 157
6. Uno H, Kurata S, Chemical agents and peptides affect hair growth, Journal of Investigative Dermatology 1993; 101;143S-147S.
7. Thorat R. M, Jadhav V. M. Kadam V.J. Development and evaluation of polyherbal formulations for hair growth promoting activity International Journal of PharmTech Research 2009; Vol.1, No.4, pp 1251-1254.
8. Shahnaz Begum,<sup>1</sup> Mi Ra Lee, <sup>1</sup>Li Juan Gu,<sup>1</sup> Md. Jamil Hossain,<sup>2</sup> Hyun Kyoung Kim,<sup>1</sup> and Chang Keun Sung<sup>1</sup>, Comparative Hair Restorer Efficacy of Medicinal Herb on Nude (Foxn1nu) Mice. Hindawi Publishing Corporation Bio Med Research International, Volume 2014, 1-9 , doi.10.1155/2014/319795
9. Patil SM\*, Sapkale GN, Surwase US, Bhombe B.T. Herbal medicines as an effective therapy in hair loss – A review. Research Journal of Pharmaceutical, Biological and Chemical Sciences. April – June 2010 RJPBCS Volume 1 Issue 2 Page No. 773
10. S.Tripathy,\* Ravi Kumar.Musunuri, D.Rajesh Babu,G.Murali. AN MEDICINAL PLANT GINSENG, International Journal of Research and Reviews in Pharmacy and Applied science, IJRRPAS, 2(2). 212-217,
11. Kwang-tae CHOI<sup>1</sup>, Botanical characteristics, pharmacological effects and medicinal components of Korean Panax ginseng C A Meyer, Acta Pharmacol Sin 2008 Sep; 29 (9): 1109–1118
12. Upadhyay Sukirti \*<sup>1</sup> And Singh Vijender, <sup>2</sup> Potentiality Of Petroleum Ether (60-80) °C Extract Of Glycyrrhiza Glabra On Androgenic Alopecia, Asian J Pharm Clin Res, Vol 6, Suppl 3, 2013, 52-55
13. Dong In Keum, Long-Quan Pi, Sungjoo Tommy Hwang, Won-Soo Lee. Protective effect of Korean red ginseng against chemotherapeutic drug-induced premature catagen development assessed with human hair follicle organ culture model. Journal of ginseng research, 2015, 1-7
14. Alastair J.J, Wood,M.D. Treatment of hair loss The New England Journal Of Medicine .1999 September 23 vol. 341, p.no. 964-973
15. Adhirajan N. Ravi Kumar T. Shanmugasundaram N. In vivo and in vitro evaluation of hair growth potential of Hibiscus rosa-sinensis Linn. J Ethnocol 2003; 88:235–239