"EFFECT OF METHANOLIC EXTRACT OF ROOTS OF PANAX GINSENG IN TESTOSTERONE INDUCED HAIR LOSS IN MICE"

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Abstract:

Present study is associated with the evaluation of anti-hair loss activity of methanolic extract of roots of Panax ginseng in animal models of hair loss induced by testosterone. Androgenic alopecia is most common form of hair loss in men. Panax ginseng on albino mice using a testosterone –induced alopecia model. Five groups of albino mice were studied: (A) Testosterone solution only (n=6); (B) Testosterone + Minoxidil solution (2%) (n=6); (C) Testosterone + *Panax ginseng* (100mg/kg p.o.) (n=6); (D) Testosterone + *Panax ginseng* (200mg/kg p.o.) (n=6); (E) intact control (n=2, without testosterone). Alopecia was induced in all intervention groups by testosterone 2.0 mg subcutaneous. *Panax ginseng* solution was given orally to animals in the respective group. Hair growth was evaluated by visual observation and histological section of skin by various parameters such as follicular density. After 21 days patch of hair loss was seen in animals received testosterone while animals treated with Panax ginseng showed less hair loss as compared with those treated with testosterone only. The follicular density observed in the *Panax ginseng*-treated group was 1.92 ± 0.47, compared to 1.05 ± 0.21 in testosterone-group and 2.05 ± 0.49 in minoxidil-treated animals. According to visual observation and quantitative data (follicular density and anagen/telogen ratio), *Panax ginseng* was found to possess good activity against testosterone-induced alopecia.

Keywords: *Panax ginseng*; Anderogenetic alopecia; Testosterone.

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. [11]

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%. [05]

1.1.2 ANDROGENIC ALOPECIA

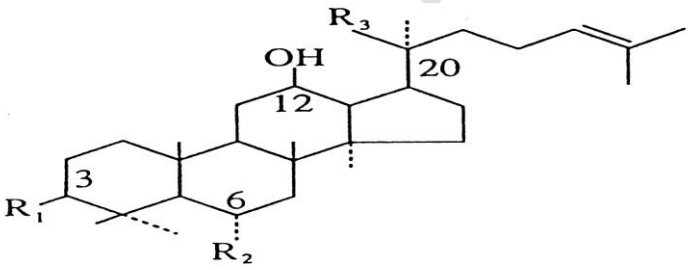
Androgenetic alopecia or simply "balding", the most common form of hair loss in men, involves the progressive hair loss, in response to circulating androgens. In this disease the hair-loss occurs will be more. It may also occur in women. Although, there is racial variation in the incidence of androgenetic alopecia, it affects at least 50% of men by the age of 50 years, and up to 70% of all males in later life. [01]

1. 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. [08]

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. [08]



Ginsenosides	R_1	R_2	R_3
Ginsenoside-Rb_1	-O-Glc^2-Glc	-H	-O-Glc^6-Glc
Ginsenoside-Rc	-O-Glc^2-Glc	-H	-O-Glc^6-Ara (pyr)
Ginsenoside-Re	-OH	-O-Glc^2-Rha	-O-Glc
Ginsenoside-Rf	-OH	-O-Glc^2-Glc	-OH
Ginsenoside-Rg_1	-OH	-O-Glc	-O-Glc

2.3 Pharmacological Indications—

Panax Ginseng (PG) is the agent which has been used in various physiological interventions like common anti hair-loss, aphrodisiac, respiratory disorders etc in Indian and other Asian systems 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.[08]

3. MATERIALS AND METHODS.

3.1 Animals

Swiss albino mice (20-25 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:

Testosterone was obtained from Cadila Healthcare Pvt. Ltd. (Werna, Goa, India). Minoxidil was obtained from Cipla Ltd. (Rorathang, Sikkim, India). Testosterone (2mg/kg) was prepared in saline solution. The extract was reconstituted as suspension in Ethanol and PEG as the ratio of 9:1 at the time of oral administration in the doses of 100 and 200 mg/kg body weight.

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 methanol for 8 hours for the preparation of the extracts for this study. The obtained extract was then concentrated at 50^0 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 Maryam Noubarani et al. The methods which were used in this study are namely,

- **Testosterone induced hair-loss**
- Androgenetic alopecia is a heritable and androgen-dependent disorder occurs in a defined pattern. Testosterone is required, along with a genetic predisposition, for Androgenetic alopecia. Hair follicles are the targets for androgen stimulated hair follicle miniaturization, leading to replacement of large, pigmented hairs (terminal hairs) by barely visible, depigmented hairs (Vellus hairs). The result is a progressive decline in visible scalp hair density. The current model for androgen action in the hair follicle focuses on the mesenchyme derived, regulatory dermal papilla at the base of the follicle. This responds to the circulating hormones and coordinates the rest of the follicular cells by altering the paracrine signals it produces. [01]

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 evaluation of hair growth and Quantitative evaluation of hair growth.

4.1.1 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 cm2 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 HRSF, CGF, HCF 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.

4.1.2 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.

4.1.3 Hair density

A hole of 1cm2 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. [06]

4.1.4 Qualitative evaluation of hair growth

The difference in growth of hair in each group was evaluated by visual observations and was recorded by photographs after 21 days.

4.1.5 Quantitative evaluation of hair growth

The skin of each animal from the dorsal area was dissected after shaving the long hair and preserved in 10% formalin. After fixation, vertical sections of the skin were prepared. The sections were stained with hematoxylin and eosin. The sections were then observed for different parameters for evaluating hair growth. The number of hair follicles in 2 mm area was recorded and reported as follicular density (number of follicles/mm). The number of follicles in anagen phase (active growth phase) and those in telogen phase (resting phase) were also counted, and anagen/telogen ratios were determined. [01]

A] Qualitative study:

The animals of groups A, C, and D showed a patch of diffuse hair loss. Loss of hair from dorsal portion of mice was clearly visible after a 21-day treatment with testosterone. After 21 days, the visual observations suggested that the animals treated with Panax Ginseng showed less hair loss as compared to those received testosterone. The hair growth pattern in Panax Ginseng treated groups was comparable to that for the standard drug minoxidil (Figure 1). [01]

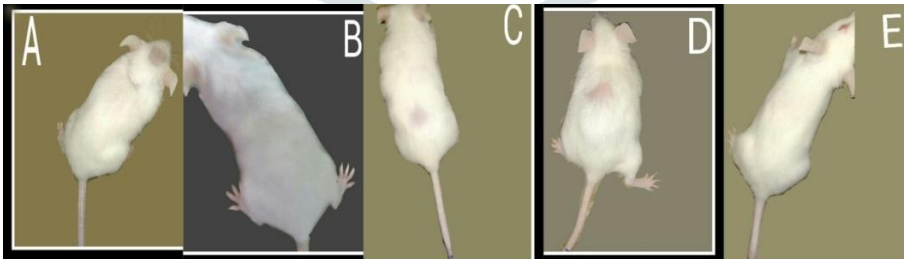


Figure1. Comparison of hair growth/loss patterns in various groups after 21 days: (A) animal treated with testosterone (B) animal treated with testosterone plus Minoxidil 2%, (C) animal treated with testosterone plus Panax Ginseng 200mg/kg, (D) animal treated with testosterone plus Panax Ginseng 100mg/kg, (E) control animal

B] QUANTITATIVE STUDY:

Microscopic photograph of skin sections of animals treated with testosterone and those with testosterone plus Ginseng revealed that testosterone treatment caused miniaturization of hair follicles. The follicles showed bulbous appearance and were short. The effect of testosterone on miniaturization of hair follicle was blocked by administration of topical minoxidil and Panax Ginseng in B, C, and D animal groups, respectively (Figure 2). The histological observations showed an increase in the length as well as number of hair follicles in Panax Ginseng treated group. The follicular density (number of follicles/mm) was calculated. [01]

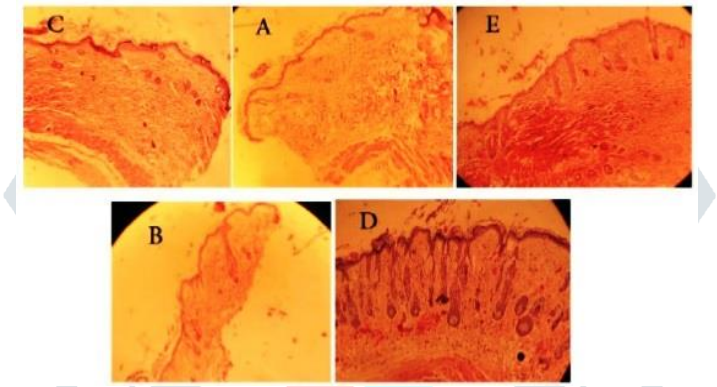


Figure2. Histology of skin sections: (C) Skin of animal treated with Testosterone, (A) Skin of animal treated with testosterone and Panax Ginseng 100 mg/kg, (E) Skin of controll animal, (B) Skin of animal treated with testosterone and minoxidil solution, (D) Skin of animal treated with testosterone and Panax Ginseng 200 mg/kg

Table1. Follicular density in sections of skin of different groups of study animals.

Sr. No.	Group	Follicular density (no/mm) mean ± SD
1	Control (E)	2.36 ± 0.34
2	Testosterone (A)	1.05 ± 0.21
3	T + minoxidil (B)	2.05 ± 0.49
4	T + ginseng 200 mg/kg (C)	1.92 ± 0.47
5	T + ginseng 100 mg/kg (D)	1.18 ± 0.41

C] Skin irritation test

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 No.2: Skin irritation

D] Hair length

Sr. No.	**Groups**	**Drug given**	**Formulation**	**Hair length mm (mean ± s.d.)**
1	Control	Cream base	Cream base	17.3 ± 0.11
2	Negative control	Testosterone	2 mg/kg S.C.	11 ±0.15
3	Standard	Testosterone + Minoxidil	2 mg/kg S.C. + 2%Topically	16.66 ± 0.21
4	Test 1	Testosterone + P.G. extract	2 mg/kg S.C. + 100 mg/kg P.O.	11.66 ±0.18
5	Test 2	Testosterone + P.G. extract	2 mg/kg S.C. + 200 mg/kg P.O.	12.66 ± 0.23

Table No.3: Hair length

E] Hair density

Sr. No.	**Groups**	**Drug given**	**Formulation**	**Hair count per cm^2 (mean ± s.d.)**
1	Control	Cream base	Cream base	1571 ± 42.11
2	Negative control	Testosterone	2 mg/kg S.C.	859 ±35.15
3	Standard	Testosterone + Minoxidil	2 mg/kg S.C. + 2%Topically	1820 ± 51.21
4	Test 1	Testosterone + P.G. extract	2 mg/kg S.C. + 100 mg/kg P.O.	1195 ±49.18
5	Test 2	Testosterone + P.G. extract	2 mg/kg S.C. + 200 mg/kg P.O.	1280 ± 52.23

Table No.4: Hair Density

5. ACKNOWLEDGMENT

I would like to express my gratitude to my guide DR. Chavare sir as well as principal Dr. Biyani sir and Principal Dr. Hukkeri sir who gave me the golden opportunity to do this wonderful work on the topic of Effect of ethanolic extract of roots of panax ginseng in stress induced hair loss in albino rat, which also helped me in doing lot of research and I came to know about so many things I am really thankfull to them

which also helped me in doing a lot of Research and i came to know about so many new things I am really thankful to them.

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