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Hair Growth Promoting Activity of *Ganoderma Lucidum* in Testosterone induced Androgenic Alopecia in Rat Model

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ABSTRACT

Alopecia, or hair loss, affects many people all around the world. The goal of the current study was to examine the growth-promoting effects of a preparation of the Ethanolic extract of Ganoderma lucidum and its main ingredients on hair regrowth using a model of alopecia caused by testosterone..Also did the acute toxicity study and phytochemical screening of Ganoderma lucidum.The study has 5 groups. A. negative control group (n = 6); B. positive control group (n = 6); C. testosterone plus minoxidil 2% (n = 6); D. testosterone and Ethanolic extract of Ganoderma lucidum extract 10 mg/kg (n = 6); and E. testosterone and Ethanolic extract of Ganoderma lucidum extract 15 mg/kg were the five groups of rats tested. Daily Inducing agent Testosterone (1mg/kg Sc) was given for 21 consecutive days through subcutaneous route to the all groups accept negative control group. Before 1 hr to apply 2% minoxidil topically to the standard group and EEGL 10mg/kg and EEGL15 mg/kg preparation to treatment group 1 and 2 respectively. The difference in growth of hair in each group was evaluated by visual observation and was recorded by photograph after 21 days. And after completion of study the all animals were sacrificed and the section was then observed for different parameters for evaluating hair growth.. The standard had a higher hair length. when compared to a positive control group, EEGL 10mg/kg and EEGL 15mg/kg treatment group. EEGL exhibited an increase in the hair length.The EEGL was able to stimulate hair growth by restoring normal parameters. The EEGL15mg/kg produced more positive results than EEGL10mg/kg. Its results were comparable to those of the frequently prescribed medication Minoxidil 2% solution, which is used to promote hair growth in people with androgenetic alopecia.

Keywords: Androgenic alopecia, Hair growth, Ethanolic extract of Ganoderma lucidum, testosterone, Minoxidil 2%, follicular density.

INTRODUCTION

Along with sebaceous glands, sweat glands, and nails, hair is one of the essential body parts that develops from the ectoderm of the skin. Hair serves as protective appendages on the body and is regarded as an accessory structure of the integument. Hair loss is a dermatological condition that has been known about for more than 2000 years and affects 0.2% to 2% of people worldwide, according to estimates.[1]

Numerous aggressions are directed at hair, thus there may be various conditions that interfere with its natural health. Alopecia is a dermatological disorder that has been recognized for more than a thousand years is a common problem in cosmetics as well as primary health care practices.[2]

The phrase "androgenetic alopecia" (AGA) is made from of the words "androgen" and "genes" It is frequently used to describe the systematic loss of scalp hair in genetically predisposed men and women. It is the most prevalent type of hair loss. Numerous genetic and environmental factors contribute to the development of AGA. In androgenetic alopecia, genetically susceptible terminal hairs in the afflicted area shrink into vellus hairs, which is primarily driven by androgen.[3]Androgens are thought to shorten the anagen phase, causing more hair follicles to enter the catagen and telogen phases and delaying the transition from the telogen to the anagen phase. Because of the follicular miniaturisation caused by androgens, terminal follicles become vellus-like follicles, producing thinner and shorter hairs.[4]

In this circumstance, both 5"-reductase activity and 5"-dihydrotestosterone (5-"-DHT) levels are elevated. In addition to a number of hereditary and environmental factors, androgens are regarded as one of the most significant causes of alopecia. The hormone androgen gradually shrinks the normally sized scalp hair follicles. There are numerous strategies to influence the growth of androgen-dependent hairs. By reducing androgen synthesis, preventing testosterone's (T) conversion to 5-"-DHT, or by inhibiting androgen receptors [5,6]

When 5-"-DHT attaches to the androgen receptor in scalp hair follicles that are sensitive, the hormone-receptor complex activates the genes that cause the terminal hair follicles to gradually shrink in size.[7] As hair cycles progress, the duration of the anagen phase shortens and hair follicles

miniaturise, resulting in shorter, finer hairs that inadequately cover the scalp11. The distinctive feature of AGA is these tiny hairs in a range of lengths and diameters. [8]

There are presently only 2 USFDA-approved medications for the treatment of AGA in males (for central/vertex hair loss exclusively) and women (for female pattern hair loss), both of which are topical medications like Minoxidil and oral medications like Finasteride. Treatment and management options for androgenetic and biological response modifiers are currently available. On the other hand, their limited clinical use is a result of their low success rate and associated side effects.[9] Numerous plant extracts have been studied for their potential to promote hair development, and natural products are increasingly being used in cosmetics. Numerous plants and herbal preparations have been claimed to encourage hair development and improve the condition of hair in the traditional Indian medical system, but their usage is restricted due to a lack of reliable scientific evidence. [10]

For thousands of years, Far Eastern traditional medicine has used ganoderma lucidum. The oriental fungus Ganoderma lucidum has a long history of use in China, Japan, and other Asian countries to promote health and lifespan. Lingzhi or Reishi is its common name. It has a massive, black, glossy, and woody mushroom on the outside. The Latin term lucidus, which means "shiny" or "bright," refers to the varnished appearance of the mushroom's surface.[11]

" With its anticancer, antiallergenic, antiviral, hepatoprotective, antioxidant, immunomodulator, hypotensive, hypoglycaemic, anti-inflammatory, antithrombotic, and many other health benefits, Ganoderma lucidum has outstanding therapeutic activity. Included are lectins, polysaccharides, triterpenoids, polysaccharide-peptide complexes, -glucans, and natural germanium (Ge). produced from the mycelia of the ganoderma plant, which also have a variety of beneficial therapeutic properties.[12] Leukaemia, cancer, hepatitis, and diabetes are currently being treated using ganoderma lucidum as an alternate adjuvant. Since the macrofungus is extremely uncommon in nature and not enough to be commercially exploited for life-saving therapeutic emergencies, cultivation on solid substrates, stationary liquid medium, or by submerged cultivation has become a crucial component to meet the driving force towards the rising demands in the international market. [13]

Chemists have recently become interested in a number of triterpenoids including Ganoderic acid that were identified from ganoderma spores and mycelium.[14] Triterpenoid and Ganoderic Acid (Ganoderic Acid TR& B) can inhibit androgen by having inhibitory activity against the enzyme 5'-reductase and the capacity to bind to the androgen receptor (AR). And as we have covered, the steroid enzyme 5-reductase converts the hormone testosterone into the androgen dihydrotestosterone. A membrane-bound NADPH-dependent enzyme called 5-reductase catalyses the conversion of testosterone into the stronger androgen dihydrotestosterone. One natural remedy that lowers high testosterone levels is ganoderma lucidum (GL), which has been shown to have promising hair-growth properties. The goal of the current study is to demonstrate GL's effectiveness in treating T-induced alopecia.[15]

Numerous plant extracts have been studied for their potential to promote hair development, and natural products are increasingly being used in cosmetics. Numerous plants and herbal remedies have been claimed to encourage hair development and improve the condition of hair in the traditional Indian medical system. The reported mechanisms of action for these herbal therapies include enhanced scalp blood circulation, DHT blockers, 5-Reductase blockers, and nutritional support. Since they are natural remedies, employing them has several benefits, including patient compliance, fewer side effects, easy accessibility, low cost, and multiple modes of action for the treatment of alopecia.[16]

MATERIALS AND METHODS

Drugs & Chemicals

Test Drug: The Ganoderma lucidum extracted powder was obtained is collected from Natural Hub, Natural Ingredients Solution Provider, R-1/24A Mohan Garden, Near Gagan Bharti School, Uttam Nagar. New Delhi-110059.

Standard drug: 2% Minoxidil obtained from Angle Biopharma Ltd., Ahmedabad and all other chemicals used for experimental purpose were of analytical arcade used as Standard drug.

Inducing Drug: Testovirone depot injection 100mg/ml from German Remedies Zydus Cedilla Healthcare. was used to induce Alopecia.

Other Chemicals: Fehling's solution A & B, Benedict's Solution, Ferric chloride, HCL, Sulphuric acid, conc. Nitric acid, Mayer's reagent, etc. from the drug store of vidyabharti college of pharamacy Amravati. For Preliminary phytochemical screening of treatment drug i.e. Ganoderma lucidum.

Experimental animal: The experiments were carried out with Wistar albino male rats of 150-200 g bred in the animal house of the Vidyabharti college of pharmacy Amravati. The animals were housed in polypropylene cages at a temp. Almost 24±2EC with a relative humidity of 40-6 0% and 12 h light-dark cycle, with free access to food and water ad libitum during the complete study. Animal were acclimatized to laboratory conditions before the experiment were started. Experiment would perform in accordance with the committee for the purpose and supervision of

experimental animals (CPCSEA) guidelines after the approval of the experimental protocol by the Institutional Animal Ethical committee (IAEC).

Preliminary Phytochemical Screening:

Qualitative Phytochemical Investigation: To identify the numerous phytoconstituents, qualitative chemical tests were carried out. Tests include the Biuret Test, Keller-Killiani Test, Salkowski Test, Molisch's Test, Fehling's Test, and the Soap Formation with Water Test. The secondary metabolites were identified using phytochemical testing because they had biological activity.

Acute Dermal toxicity study

Rats' dorsal regions had all of their fur removed the day before the EEGL was given. During a 24-hour exposure period, EEGL15mg/kg and EEGL20mg/kg were applied as evenly as possible across the exposed region of skin with a porous gauze bandage and no irritating tape. Animals were monitored daily for the following 14 days starting two to six hours after the start of the exposure period and at least once every 30 minutes for the following 30 minutes. Observations were also made frequently during the first 24 hours. Changes in the eyes, mucous membranes, skin, and fur are among the toxicity study's observations. In comparison to the EEGL15mg/kg group, which received no treatment, the 20mg/kg group saw one death (OECD 402). After 24 hours, the animal rapidly noted its usual activity and growth. Throughout the observation period, there were no differences in the general look of any of the animals. This outcome shows LD50>20mg/kg.[17,18]

Preparation of Doses:

Sample preparation: During each study protocol drugs were freshly prepared The extracted powder of Ganoderma lucidum were incorporated into Solution was made with ethanol base and adding propylene glycol in proportion of 90:10.

Testosterone test solution: Testosterone solution was prepared in the vehicle ethanol/propylenglycol (90:10).

Minoxidil solution: the standard of Minoxidil (2%) was diluted with ethanol and propylene glycol were applied topically.[19]

Experimental design

Animals divided into 5 groups of 6 rats each. The following treatment given to animals of different groups:

Sr.no	Groups	Treatment
1	I Negative control	Distilled Water (po)
2	II Positive control	Testosterone (1mg/kg sc)
3	III Standard	Testosterone (1mg/kg sc)+ 2%Minoxidil(Topical)
4	IV EEGL10mg/kg	Testosterone (1mg/kg sc)+EEGL(10mg/kg Topical)
5	V EEGL15mg/kg	Testosterone (1mg/kg sc)+EEGL (15mg/kg Topical)

Table1. Different groups of animals and their treatment.

Experimental procedure

1) Animal were obtained from Vidyabharti college of pharmacy, Amravati and were

acclimatized for one week.

2)The rats were dived into 5 groups; each group has 6 rats. All the rat's hairs depilated by

cream in $2cm^2$ area of dorsal portion of skin. The Negative control group was left for natural recovery for 21 days.

3) (Induction and treatment of alopecia) Daily Inducing agent Testosterone (1mg/kg sc) was given for 21 consecutive days through subcutaneous route to the all groups accept negative control group. Before 1 hr to apply 2% minoxidil topically to the standard group and EEGL 10mg/kg and EEGL15 mg/kg preparation to treatment group 1 and 2 respectively.

4)The difference in growth of hair in each group was evaluated by visual observation and

was recorded by photograph after 21 days. And measures the hair length.

Evaluation parameters

Skin irritation test: The Wistar albino male rats' hair was removed, and a test to measure how much the produced formulations (EEGL 15 mg/kg and 10 mg/kg) irritated the rats' undamaged skin was carried out. The dorsal region of the Wistar albino male rats had hairs removed; this area was then washed with spirit, and medication was topically given to the rodents. After treatment, the areas were monitored for erythema and oedema for 48 hours.

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

Measurement hair length: On the 21st day of treatment, hair was randomly removed using sterile forceps from the depilated area. Hair length was measured by scale, and the results were calculated as mean length SEM of 25 hairs, two parameters: (a) hair growth initiation time, which is the shortest amount of time required to start observable hair growth, and (b) hair growth completion time, which is the shortest amount of time required to completely cover the denuded skin region with new hair. For each group of animals, the time between the start and finish of hair development was noted.

Statistical analysis: Statistical analysis. The data were expressed as mean ± SEM. Results were analysed statically Two-way ANOVA followed by Bonferroni multiple comparisons $p<0.0001$ compared to Positive control. For measurement of hair length.

RESULTS

Observation of Phytochemical Test

Sr. No.	Natural Product	Test Performed	Inference
1	Alkaloid	Wagner's reagent	+
2	Steroids	Sakowaski test	+
3	Phenolic compounds	Ferric chloride Test	+
4	Terpenoied	Salkowiski Test	+
5	Carbohydrate	Molisch's Test	+
		Fehling's Test	+
7	Saponin	Soap Formation with water	-
8	Glycoside	Keller-killiani test	+
9	Protein and Free Amino Acids	Biuret Test	+

Table 2. Phytochemical Test of Ethanolic extract of Ganoderma lucidum.

- Indicates absence

+ Indicates presence

The information in the above table shows that the ethanolic extract of Ganoderma lucidum contained terpenoid alkaloids, flavonoids, carbohydrates, glycosides, phenolic compounds, proteins, and amino acids.

1. Skin irritation test: As can be seen in Fig. 1, rats did not exhibit any erythema or oedema, which suggests that EEGL 10 mg/kg and EEGL 15 mg/kg solution did not irritate the skin of rats. According to results of a skin irritancy test, EEGL solution is safe for topical use(fig1). The produced solution did not cause any dermatological reactions, and rats tolerated it well, proving that it was safe to use.

Fig. 2: Observation of skin irritation test

Solution	**Visual observation**	
	Erythema	**Oedema**
1.EEGL 15mg/kg solution With ethanol and propylene glycol	No	No
2. EEGL 10mg/kg solution With ethanol and propylene glycol.	No	No

Table 3. Observation of erythema and oedema in skin irritation test.

2. Morphologic observation (visual observation): Animals in group I had normal hair, but those in group II had a patch of scattered hair loss. After 20 days of testosterone therapy, there was a noticeable loss of hair in the dorsal region of the rats. In group III (standard) animals, testosterone is provided concurrently with groups IV (EEGL 10 mg/kg) and V (EEGL 15 mg/kg). The absence of the alopecic state in this group of animals indicates that the testosterone-blocking effects of EEGL10 mg/kg and EEGL15 mg/kg and Minoxidil 2% prevented their activity. (fig3)

Fig3. Comparison of baldness pattern in each group. (A) Normal animal without any drug (B)Animal treated with testosterone showing diffuse alopecia (C)Animal treated with testosterone and 2%Minoxidil showing more hair growth. (D) Animal treated with testosterone and EEDL10mg/kg showing less hair growth (D) Animal treated with testosterone and EEDL15mg/kg showing more hair growth.

3.Measurement hair length: Up until the end of the treatment cycle, hair length started to grow. When compared to 2% minoxidil solution (standard), the ethanolic extract of Ganoderma lucidum (15 mg/kg) produced a virtually same effect on hair length, with the other group of animal hair being 9 mm at the conclusion of the course (22 days). In comparison to Ganoderma lucidum 10mg/kg extract, Ganoderma lucidum 15mg/kg extract had a larger impact on hair length. This can be because the follicles switched around too soon. This indicates that the minoxidil 2% and

ganoderma lucidum 15 mg/kg extracts include a higher number of hair follicles that are in the anagen phase of the hair development cycle (Table 4). Both of them have important effects.

Groups	**Time taken for Hair growth in (11 days/mm)**	**Time taken for hair growth completion (22 days/mm)**
I (Negative control) Normal	2.320 ± 0.062	4.250±0.226
II (Possitive control) Inducing	0.783±0.047	2.433±0.049
III (Standard)	3.433±0.066	10.27±0.146
IV (EEGL10mg/kg)	2.667±0.033*	6.233±0.049*
V (EEGL 15mg/kg)	3.183±0.070	9.417±0.104*

Table 4: Observation of hair growth

Value expressed as mean ± SEM (n=6), Two-way ANOVA followed by Bonferroni multiple comparisons *p< 0.0001 compared to Positive control.

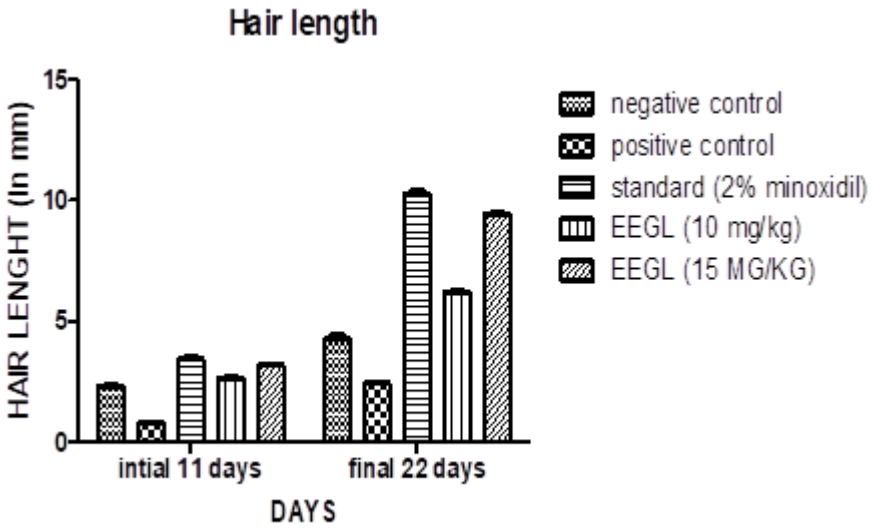

Fig.4 Observation of hair length

Discussion

All over the body, androgens act as mediators for the growth of terminal hair. Androgen-dependent and heritable, androgenetic alopecia manifests in a specific manner. Androgenetic alopecia requires testosterone in addition to a genetic susceptibility. The androgen-stimulated miniaturisation of hair follicles, which results in the replacement of thick, pigmented hair, targets hair follicles. According to histological analysis, androgen-responsive hair follicles continuously shrink and are accompanied by perifollicular fibrosis in androgenetic alopecia (AGA), a process that is driven by the hormone dihydrotestosterone (DHT)[18]. testosterone administration causes alopecia in rats.[23] Androgenic alopecia is caused by dihydrotestosterone, a stronger androgen than testosterone, which also causes the hair follicle to shrink and changes the cyclic phase of the hair growth cycle. DHT triggers the genes responsible for follicular miniaturisation in balding men by binding to androgen receptors in weak hair follicles. The dermal papilla at the base of the follicle is the target of current androgen action-based therapy. [24,25]Testosterone affects hair follicles by attaching to androgen receptors either directly or after being transformed to dihydrotestosterone by the enzyme 5a reductase. Instead of the androgen metabolic pathway, testosterone hair loss may be caused by the death of hair follicles. The current investigation seeks to identify substitutes for the steroid-based medications now in use.[26]

To the best of our knowledge, this study is the first to describe the potential for hair development of EEGL at the two dose concentrations of EEGL10 mg/kg and EEGL 15mg/kg that are indicated. It evaluate the hair growth activity of this plant using the Rat as a model. The earliest approach, biopsy, is still a useful tool. The easiest and least painful way to obtain the most anatomical and histological characteristics of a hair follicle is through a biopsy.

Alopecia caused by testosterone (1 mg/kg) was prevented by simultaneously administering Minoxidil 2% to the rats in the Standard group and topically administering EEGL 10 mg/kg and EEGL 15 mg/kg solution to the Treatment group. The androgen has an effect on hair follicles either

directly or after being converted to dihydrotestosterone, a more potent androgen that binds to androgen receptors in hair follicles, by the enzyme 5a reductase. A synthetic anti-androgenic medication called minoxidil is sold to promote hair growth.

Animals given EEGL and testosterone did not exhibit any signs of baldness. In addition to hair length, visual assessment and histopathological findings (follicular density and anagen/telogen ratio) also point to EEGL 10mg/kg and 15mg/kg as potential inhibitors of androgenic activity. As a result, EEGL15mg/kg is thought to be a more beneficial preparation for topically applying in commercial herbal drugs for androgenic alopecia and other conditions connected to androgens. Triterpenes, flavonoids, sugars, and alkaloids are only a few of the chemicals found in this plant, according to phytochemical screening of EEGL. Because of the 5a reductase inhibitory effect provided by the terpenoids ganoderic acid TR and ganoderic acid B, EEGL can prevent the conversion of testosterone to DHT.[27]

Therefore, it is reasonable to believe that terpenoid have a role in this plant's capacity to produce hair growth. Ethanolic extract of Ganoderma lucidum have a safe topical effect on testosterone-induced baldness. A hair growth promoter should have a high follicular density and anagen to telogen ratio. Therefore, we can draw the conclusion that EEGL can be used to promote hair growth in people who have androgenetic alopecia. These may function by impeding 5-a reductase's ability to carry out its function.

Conclusion and Future prospectives

The results of the current investigation suggest that androgenic alopecia may be treated with Ganoderma lucidum ethanolic extract. The results of this study will aid the researcher in understanding the function of EEGL10 mg/kg and EEGL15 mg/kg, which are substances used to promote hair growth in androgenetic alopecia, a condition brought on by an increase in androgen levels in the scalp. It also showed that EEGL 15mg/kg is superior to EEGL 10mg/kg in treating androgenic alopecia. As a result, the current study will assist the researcher in discovering significant fresh methods of treating androgenic alopecia.

Acne, prostate cancer, and other androgen-dependent diseases like benign prostatic hyperplasia were also brought on by dihydrotestosterone. The extract and its isolates, ganoderic acid TR and ganoderic acid B, both of which include chemical compositions with 5a reductase activity, have been found to block this enzyme, making them prospective candidates for further research in the treatment of these disorders.

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