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Research Article

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POSSIBLE QUINCUNCIAL AND SCISSORING INTERVENTION OF ANAGEN GROW IN 5α-REDUCTASE CASCADE AND HAIR GROWTH PROMOTING EFFECT

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ABSTRACT

Alopecia or baldness is although a part of aging process where many bio-chemical aspects in mammalian system undergoes irreversible change, hair root cell activation and inactivation of alpha reductase enzyme may partly decrease the problem of hair loss. Anagen grow, in the present study proves that the phyto-consitiunets in the formulation increases hair follicle cell activation and alpha reductase enzyme inhibition which in-turn hinder DHT conversion the culprit of hair follicle atrophy. We have employed Rat liver microsomal preparation was used as enzyme source wherein finasteride as positive control. The EGCG like constituent in the formulation is implicated for the above activity, details are presented in the paper.

Keywords: Alpha reductase, Anagen grow, herbal hair growth serum, 5 alpha reductase and dihydroxytestosterone, hair loss.

INTRODUCTION

The role of 5α-reductase enzyme in converting the steroid hormone, testosterone to dihydroxytestosterone (DHT) which in turn results in the shrinkage and atrophy of dermal papilla cells in hair root; the principal reason for androgenic alopecia is well recognized.[1] In the recent times, several herbal and chemical preparations are found to have certain effect in arresting/promoting hair loss. The exact underlying mechanism of action of all those preparations in exhibiting such pharmacologic effect is not clearly known. The above scientific query partly also got sustained due to the lack of a cost effective, less time consuming, rapid method for analyzing the mechanism of action of all those lead compounds for hair growth.

Anagen grow is a polyherbal siddha hair growth serum composed of *Murrayakoenigii, Lawsonia alba, Indigoferatinctoria, Hibiscus rosasinensis, Eclipta prostrate* and *Phyllanthusemblica*. Our earlier studies have shown the hair follicular activation, prolonging and sustaining the 'life' of the gelatinous sheet around anagen hair due to anagen treatment in vitro.[7-9] One of the phytoactive compounds present in anagen grow is a distant analogue of EGCG (Epi gallocatachen 3 gallate). EGCG is well proven for its action against 5α-reductase enzyme that convert testosterone to DHT and such biochemical change of the endocrinogic compounds results in hair fall and hair root atrophy. [2]The distant analogue EGCG that is likely to be present in anagen grow also got similar effect like EGCG and is responsible for the hair growth as reported by thousands of patients here in India after use of anagen grow is not clearly understood.

In order to understand the underlying mechanism that is responsible for the above pharmacological effect of Anagen grow, the present study was taken. We employed the latest, simplistic method of assaying the steroid 5α-reductase developed by At Sushi *et al.,* 2013 using NADPH as co-substrate for the enzyme against testosterone. [3]We used Finasteride as positive control and also for reconciling the activity of Anagen grow. Details are presented in the paper.

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MATERIALS AND METHODS

The experiment was performed by Apex laboratory, Chennai as a part of the funding obligation.

Preparation of rat liver and prostate microsomes

The Wistarstrain of male rats (10 weeks old) was chosen for the study. The animals were well fed and were healthy. After decapitation, liver and prostate from all rats were immediately removed. The pooled liver samples were minced and homogenizedin chilled Tris buffer 3 mM pH 7.4, containing 1.15% KCl and 0.1 mM EDTA. The homogenate was then centrifuged at 9000g for 20 min and again at 10000g for 60 min at 4°C. The pellet thus obtained was resuspended in10 mM phosphate buffer (pH 7.4) and used as liver microsomes enzyme fraction.

For prostate microsomes enzyme fraction, we followed the method of Mitamura *et al.,* 2005. The minced prostate was ground in three volumes of ice-cold 40 mM phosphate buffer (pH 6.5) containing 0.3 M sucrose, 1 m Mdithiothreitol (DTT) and then centrifuged at 1500g for 20 min. Thesupernatant was further centrifuged at 10000g for 60 min at 4°C. Thepellet thus obtained was resuspended in 40 mM phosphate buffer (pH 7.5) containing 0.3 M sucrose and 1 mMDTT and used asprostate microsomes. The microsome enzyme source was stored at –80°Cuntil use. The protein content of the microsomes was measured by the method of Lowry *et al.,*1951. The liver and prostate microsomes were used after dilution with 40 mM phosphate buffer of pH 7.0 or pH 5.5 containing 0.3 M sucrose and 1 mMDTT for the assay of liver 5α-SR1 and prostate 5α-SR2, respectively

Assays of 5α-SR activity of rat liver and prostate microsomes and inhibitory activity of finasteride[4]

Rat liver microsomal 5α-SR1-catalyzed reduction oftestosterone was carried out at 37°C for 30 min in 200 µL of40 mM phosphate buffer (pH 7.0) containing 60 µ Mtestosterone, 800 µM NADPH and 55 – 1100 ng mL–1microsomal protein. The reaction was started by adding themicrosomes (20 µL) and stopped by heating at 80°C for 5

min. After cooling to room temperature, a portion (100 µL) of their action mixture was transferred to a cuvette, 850 µL of the cycling reagent (0.1 M potassium phosphate buffer, pH 8.0,containing 1.76 m Mthio-NAD and 0.6 mM NADH) was added and the mixture was preheated at 37°C for 3 min. Enzymatic cycling was initiated by adding 50 µL of 400 U mL–1 3α-HSDand ΔA1–4 min at 400 nm was measured. As a control, the liver microsomes were used after heating at80°C for 5 min. The readings were taken spectrophometrically at 340 nm and the activity of the test material was compared with Finasteride.

RESULTS

Table 1 5-α reductase enzyme activity

All the herbs used in the formulation of anagen grow exhibited activity against the enzyme 5-α reductase and the extent of activity directly correlate with the concentration. The complete formulation – anagen grow also exhibited activity in a concentration dependent manner. The EGCG extracted from green tea showed significant activity however the activity of Finasteride was the highest at lowest concentration possible in comparison with various compounds tested. The details are given in the table below

Compounds	Concentration in µg/% reduction of 5α reductase activity	
	1µg	2 µg
Murrayakoenigii	5	7
Lawsonia alba	3	6
Indigoferatinctoria	6	9
Hibiscus rosasinensis	11	16
Eclipta prostrate	5	8
Phyllanthusemblica	4	6
Anagen grow	8.55	22.86
EGCG from green tea	11	15
Finasteride	18 (at 0.01 µg)	26 (at 0.01 µg)

DISCUSSION

Although hair loss cannot be described strictly as a medical condition that warrant treatment intervention, the aesthetic complexities due to hair loss can make the problem quite serious and hence the entire world is running after developing a promising treatment intervention to arrest the hair loss. Due to the obvious role of aging etiology in hair loss, the exact mechanism that governs the process of hair loss, otherwise called as androgenic alopecia is still elusive and remains enigma to the modern day science. [5]One of the reasons implicated in the above biological aspect is the conversion of the steroid hormone, testosterone to DHT which subsequently binds to the dermal papilla cells of hair follicle. Continuous apposition of DHT over dermal papilla cells make the dermal papilla cells non-functional and in due course such situation would push the dermal papilla cells to go atrophy. The enzyme that converts the steroid hormone to DHT is 5-α reductase and therefore partial inactivation of the enzyme is being approached to reduce and retard hair loss.

The Finasteride is widely used for the above purpose but such treatment certainly come with serious side effects which make the choice of Finasteride more cautious and sparing. Several herbal preparations are reported to promote hair growth but the underlying mechanism of action of most of such herbal preparations is not clearly understood. Mere anagen hair activation or increasing its life expectancy is not going to address the problem because telogen conversion of hair and loss of regeneration capacity of the hair follicle beneath under most of the telogen hair is the reason for hair loss and hence protecting and promoting the hair follicle beneath every telogen hair is inevitable. More than promoting the active phase of such hair follicles, protecting them from the endocrinal biochemistry during aging is necessary and 5-alpha reductase enzyme is the most important factor in causing near permanent hair loss. Although the enzyme is only a catalyzing agent and the substrate, testosterone is the principal culprit. The substrate has got several other functional significances and hence targeting the enzyme activity may be the safest approach than the substrate.

The assay method for 5-alpha reductase activity is either expensive or require radiolabelled substances and hence such studies cannot be conducted easily. As a result, many herbal preparations have to remain with tall claim without any substantive scientific proof. Anagen grow is a siddha product prepared as hair serum. The product has been studied further for its hair growth promoting benefit in vitro by basic method which has shown the activity only indicatively. However, thousands of people who have been using Anagen grow in India have reported significant hair growth per unit area, reduction in hair loss and hair thickening. Interestingly, most of those reported such benefit was suffering from androgenic alopecia where the reversion of hairline is not that easy. On our subsequent phytochemical analysis has led us to an understanding that Anagen grow has the phytochemical constituent which has got distant structural analogue with EGCG. EGCG is proven to have activity against 5-alpha reductase enzyme and hence we initially presumed that the structural analogue of the compound in anagen grow with that of EGCG may be responsible for the reported hair growth benefit. Our present study has clearly demonstrated that all the medicinal plants individually and Anagen grow collectively has activity against the enzyme alpha-reductase. We employed a simple but at the same time highly sophisticated method for evaluation where the enzyme uses the substrate NADPH to convert testosterone to DHT and therefore the status of NADPH in the experiment may predict the activity/denudation of the enzyme as NADPH can be read easily by spectophotometrically at 340 nm.[6]

Our present study has revealed that the herbs in the Anagen grow has activity against the enzyme. Whether they are preventing the NADPH in the reaction process or attenuating the enzyme we could not establish clearly because some of the herbal extracts when incorporated in the experiment system (enzyme pre-treated with herb) or (NADPH pre-treated with herb), both showed activity reduction. We presume that some herbal constituents may be blocking NADPH from participating in the chemical reaction. We led to this conclusion only due to the status of NADPH that remained same. Therefore we strongly believe that some of the herbs in Anagen grow may be acting both at enzyme and co-substrate level. Our present study clearly show that the Anagen grow has definite activity against the cascading events of 5 alpha reductase conversion of steroid and associated hair loss. We have brought out the possible underlying mechanism of Anagen grow and more in depth studies are required to equate Anagen grow with Finasteride.

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