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Research Article

POTENTIALITY OF PETROLEUM ETHER (60-80) °C EXTRACT OF *GLYCYRRHIZA GLABRA* ON ANDROGENIC ALOPECIA

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ABSTRACT

Objective: *Glycyrrhiza glabra* is well known herb in Indian and Chinese traditional medicines. In our previous study it shows hair growth promoting property in female rats so here experiments were performed for evaluating its effects in androgenic alopecia in males.
Methods: In present study alopecia is induced in three groups(each contain six animals) of male wistar albino rats by intramuscular dose(0.1mL) of testosterone .One group was rendered devoid of any other treatment while other two groups animals are treated with finasteride and petroleum ether extract of *G. glabra* root topically once daily. The animals were observed during treatment period of 21 days then one animal from each group was euthanized for histoarchitecture study.
Results: The study revealed that petroleum ether and finasteride treated animals do not developed alopecia while alopecia was observed in only testosterone treated animals.
Conclusion: Thus it is concluded from this study that petroleum ether extract of *G. glabra* posses anti androgenic alopecia activity which is comparable to that of standard drug recommended for androgenic alopecia finasteride.

Keywords: Hair, alopecia,G.glabra

INTRODUCTION

Male pattern baldness or Androgenetic alopecia (AGA) term consists of two terms Androgen (Andro) and genes (genetic).It run in families. It is inherited condition associated with a gene or genes. Both the testosterone metabolite DiHydroxyTestosterone and the genes for hair loss must be present for AGA to occur. The hair loss is heritable, androgen-dependent, and occurs in a defined pattern. It is assumed that the genetically predisposed hair follicles are the target for androgen-stimulated hair follicle miniaturization, leading to gradual replacement of large, pigmented hairs (terminal hairs) by barely visible, depigmented hairs (vellus hairs) in affected areas. [1] In US 35 to 40 million are affected by AGA. It affects at least 50% of men by the age of 50 years, and up to 70% of all males in later life. [2]

Glycyrrhiza glabra, licorice is a well known herb in traditional Indian and Chinese medicine cultivated in Jammu and Kashmir, Punjab and Sub-Himalayan tracts. It is known as Yasthi-madhu in Sanskrit, Jethi-madh in Hindi, Jashtimadhu in Bengali, and Liquorice in English. It is a hardy herb or undershurb, attaining a height of 1.8m. Roots are thick, having many branches with red or lemon colour outside and yellowish or pale inside.

The main chemical constituents found are Glycyrrhizin, glycyrrhizic and glycyrrhetinic acids, liquiritin, isoliquitirin, neoisoliquiritin, liquiritigenin, isoliquiritigenin, rhamnoliquiritin, glabrine, glabranine, formononetin, licuraside, lichalcones a and b, hispaglabridin A and B, licoricidin, glabrene, pinocembrin, prunetin, saponeretin, 11-deoxoglycyrrhetic and 24-hydroxyglycyrrhetic acids, 24-hydroxyliquiritic and liquiridolic acids, olean-12-en-3beta-ol-3o-oic and olean-11,13(18)-dien-3beta-ol-3o-oic acids, methylolean-11,13 (18)-diene-3, 24-diol-3o-oate, glycyrrhetol2, 21alpha-hydroxy-isoglabrolide, glabrolide, deoxoglabrolide, deoxoglabrolide, isoglabrolidde, licoflavonol. kumatakenin, glycyrol, licoricone, glabridin, glabrol, 7-acetoxy-2-methylisoflavone, 7-methoxy-2-methylisoflavone, 7-hydroxy-2-methylisoflavone, glyzarin, glyzaglabrin, licoisoflavones A, B and licoisoflavone, glycyrin, sugars and aspaargin are also reported in this plant. [3]

The roots are prescribed in coughs, hoarseness and in respiratory trouble, mixed with citrus juice efficacious in catarrhal affections and with honey in jaundice; in combination with ginger and milk, act as a good tonic during convalescence, infusion, decoction and extract is laxative and a useful medicine in urinary disease, bronchial and gastric troubles. [4]

G.glabra is also known for promoting hair growth in traditional and folklore medicines .It is one of the ingredient of Sesa oil which is recommended for hair growth promotion. [5]

Hence, experiments were performed for evaluating effects of *G.glabra* in androgen induced alopecia.

MATERIAL AND METHOD

Plant material

The plant material was collected by Jim corbett national park, Ramnagar, Uttra khand , India. It was authenticated by Dr DV Amla Scientist G, NBRI, and voucher no NBRI-SOP-202 was provide to it and it was kept there for further references.

Extraction

Plant material was extracted in soxhlet apparatus with petroleum ether (60-80) °C for eighteen hours. Then the extract was concentrated under vaccum. The yield was 1.6%

Phytochemical screening

The extract was evaluated for presence of various phytochemicals by standard procedures.

Preparation of various dosage forms

Marketed preparation of testosterone Testoviron depot (German Remedies) (1 mL) was diluted up to 5 mL with water for injection this was able to produce the concentration of 5 mg/mL. The 2% standard solution of crushed finasteride tablet (Fincar, Cipla Ltd.)solution and 1% extract solutions was prepared in vehicle (ethanol: propylene glycol: water in ratio of 8:1:1).

Animals

Male albino rats were used of weight 120-150 g, 3-4 month age were used for studies. The animals were handled according to CPCSEA Guidelines of Good Laboratory Practice. [6] The research protocol of the animal experimentation (Reg no. 837/ac/04/CPCSEA; Resolution no. 05/837ac/PH/10 of December 12, 2010) was approved by the 'Institutional Animal Ethical Committee' of College of Pharmacy, IFTM, Moradabad- 244001, Uttar Pradesh, India. The rats were placed in cages and kept in standard environmental conditions of 12h light and 12h dark cycle, 23 ± 2 °C and 35 – 60 %RH. They were fed with standard diet *ad libitum* with free access to water.

Preliminary skin irritation test

This test was carried out by protocol mention in ASTM. [7] The petroleum ether extract of *G. glabra* applied in a concentration of up to 10%for seven days on shaved skin surface of wistar rats, didnot show any irritation or erythema on skin surface. Thus the prepared extracts were considered safe for topical administration . [8]

Experimentation

The method reported by Matias et al. [9] was followed with slight modification. In brief, the rats were divided in four groups of six rats each. Rats of all the groups were administered testosterone dose (0.1 mL) intramuscularly. Animals of group 2, 3 were also given topical application of 0.4 mL finasteride and petroleum ether extract of root respectively on dorsal skin surface once a day for 21 days . After this period, one rat from each group were selected randomly and sacrificed. The difference in growth of hair in each group was noticed by visual observations and was recorded by photographs. Skin biopsy was also undertaken from balding site and the cyclic phase of hair follicles (Anagen,Telogen) and follicular density was determined with the help of ocular micrometer.

RESULTS AND DISCUSSIONS

Phytochemical screening

The petroleum ether extract of *G. glabra* shows presence of Glycosides, terpenoid, phenolics and flavonoids.Its Chromatographic characterization shows presence of twelve compounds using mobile phase Toluene: Ethyl acetate in 97:03 ratio and derived with Libbermenn burchard reagent.(Fig 1A)

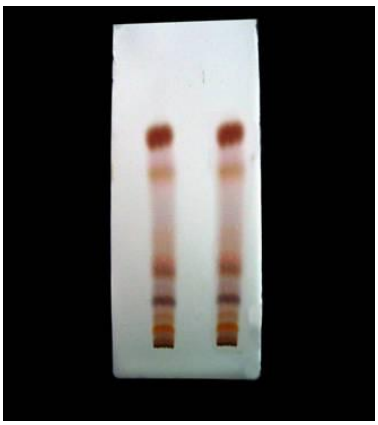

Fig.1A: Separation bands of Petroleum ether extract on thin layer chromatogram

***In vivo* hair growth studies against testosterone induced alopecia**

Qualitative study

Alopecia proceeds via cranial to caudal region in rodents. [10]The animals of group 1 showed diffuse alopecia. Loss of hair from dorsal portion of rat was clearly visible after 15 days treatment with testosterone. The observation was better in animals of group 2; no signs of alopecia were developed. In animals of group 3 the alopecic conditions was not visible and showing that the extract successfully prevented and blocked testosterone induced hair loss. (Figure 1, 2 and 3).

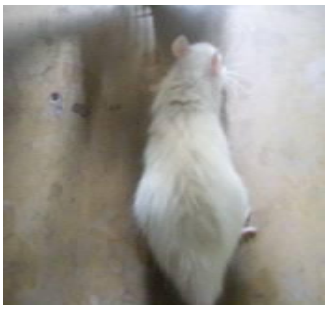

Fig 1

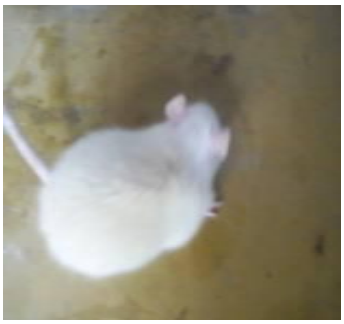

Fig 2

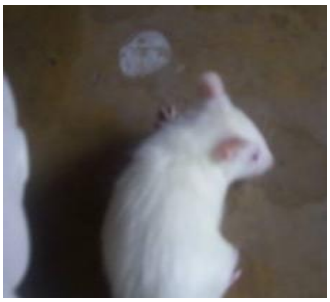

Fig 3

Fig 1. . The animal shows hair loss from cranial reason and hair become thin on dorsal skin, *i.e.* alopecia condition is visible in group 1 animal due to testosterone administration;

Fig 2. Animals of group 2 do not develop alopecia on 21 days treatment with testosterone due to simultaneous treatment with finasteride;

Fig 3. Animals of group 3 do not become alopecic as petroleum ether extract of root combat testosterone effect;

Quantitative study

In the present experiment alopecia was induced in rat by administration of testosterone. Testosterone is required, along with a genetic predisposition, for androgenetic alopecia to develop in men. [11] Microscopic examination of skin sections of group 1 animals revealed that testosterone treatment cause miniaturization of hair follicles. The follicles had bulbous appearance and were short (Figure 4). Several hair follicles were in telogen phase. In skin sections of group 2 and 3 animals the effect of testosterone on miniaturization of hair follicle was blocked by administration of topical finasteride and petroleum ether extract of root. The number of follicles in anagen phase was considerable and fewer follicles in telogen phase were observed. A/T ratio was significantly affected by finasteride and extracts which was observed in skin sections of various groups (Table 1)(Figure 4, 5 and 6).The follicular density i.e. no of follicles in mm of skin surface. The follicular density also showed that both seed extracts treated animal had denser hair follicle (Table 1).

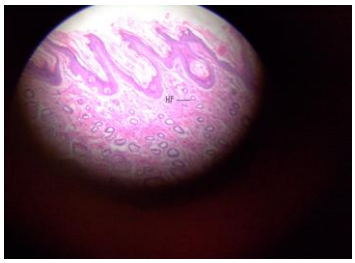

Fig 4

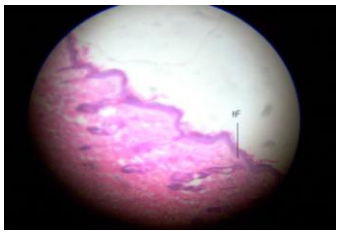

Fig 5

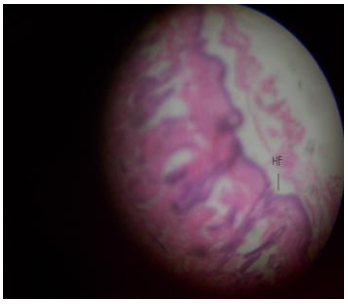

Fig 6

Fig 4. Skin section of Group 1 animals showed maximum telogenic hair follicle leading to alopecia;

Fig. 5. Skin section of Group 2animal shows hair follicles in anagenic (hair growing stage);

Fig 6. Skin section of Group 3 animal shows more hair follicles in anagenic stage than finasteride treated group 2 animals;

Table 1 Grouping and treatment of animals and variation in there Anagenic and Telogenic hair percent

Group	Telogen	Anagen	A/T	Follicle density
1	83.1±0.5667	16.9±0.5667	0.2034	1.0±0.3371
2	52.4±0.6864***	47.6±0.6864***	0.9084	2.5±0.3128
3	34.0±0.4714***	66.0±0.4714***	1.9412	2.9±0.2599

Values are mean ± SEM, n=10, *p<0.05, **p<0.01, ***p<0.001, significance Vs control

DISCUSSIONS

Roots of *G.glabra* have there value in traditional system of medicines since ancient time . Recently hair growth promoting activity of roots are explored. [12] Here it was investigated for its effect in antagonizing testosterone effect in testosterone induced alopecia. Previously studies were conducted which shows that herbs able to reduce serum testosterone level are used in androgenic alopecia. [13] The roots of *G.glabra* is able to reduce serum testosterone level.[14,15] Previous studies shows that testosterone along with 5 Dihydroxy Testosterone is required for androgenic alopecia to occur. Phytosterols that may inhibit the conversion of testosterone into the more active dihydrotestosterone via inhibition of some17ß-HSD isozymes. Licorice (*Glycyrrhiza glabra*) is reported to inhibit some of the steroid dehydrogenases. [16] So it is useful in androgen driven disorders like androgenic alopecia, acne, Benin prostrate hyperplasia and prostrate cancer and polycystic ovary syndrome etc. The topical application of extract on scalp is able to combat the androgenic alopecia **.**

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