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Short Communication

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Study of protective effect of Glycyrrhetinic acid in androgen induced alopecia

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ABSTRACT

Androgenic alopecia is a common hair disorder of both gender but it is most prevalent in aged male. *Glycyrrhiza glabra* is a herb generally use to treat hair disorders and previous studies showed that petroleum ether extract of *G.glabra* possess hair growth promoting activity. In present study Glycyrrhetinic acid was isolated from *G.glabra* and was studied as curative agent for androgenic alopecia. Male rats were taken for conducting studies on androgenic alopecia as they were made alopecic by injected with testosterone intramuscularly and protective effect was observed by Glycyrrhetinic acid and standard finasteride. The rat group on only testosterone developed alopecia while the animals on finasteride and Glycyrrhetinic acid along with testosterone do not developed alopecia. Thus this study proves protective effect of Glycyrrhetinic acid in androgen induced alopecia.

KEYWORDS: Hair, *Glycyrrhiza glabra*, testosterone, androgens,alopecia

INTRODUCTION

*Glycyrrhiza glabr*a belonging to the family Fabaceae is a well known herb generally known for anti-inflammatory activity and effectiveness against cold and flu in form of antiviral agent. It is an herb found in north India and its roots are used in traditional and folkloric medicines in the treatment of cough, respiratory troubles, hoarseness of voice and its decoction is used for washing of hair to prevent hair fall.

It is found effective in various ailments in pre clinical and clinical trials. In Indian text of ancient system of medicine it is also known as "keshya rasayan". In our previous studies conducted on female wistar albino rats it was found effective in hair growth promotion by prolonging anagenic phase (Upadhyay et al., 2014) and in male albino rats petroleum ether extract of *Glycyrrhiza glabra* showed anti androgenic alopecic activity.(Upadhyay and Singh, 2013) So in recent studies glycyrrhetinic acid or compound GG 1 was isolated and evaluated for its properties on hair growth.

Androgenic alopecia or common baldness is recognized by continuous hair loss, following a typical pattern (either male or female) hair loss from the scalp due to androgen or male hormone (Hamilton, 1942). In this era the pathogenesis and hereditary basis of the hair loss have been understood, the stress is experienced by persons who have lost their hair (Bronough et al, 1983). There have also been breakthroughs in the treatment of androgenic alopecia. The transition of some terminal hairs into vellus hairs is an androgen dependent secondary sexual characteristic but vice versa of that phenomenon cause baldness (Cash, 2001). Androgenic alopecia becomes a medical problem only when the hair loss is seen as excessive, premature, and causing stress. The premature androgenic alopecia occurs due to genetic predisposition as it is a hereditary condition and it requires sufficient circulating androgens or metabolites. Herbs are always used as remedy of androgenic alopecia.

In 2000s Takahashi et al, 2001 reported that procyanidin b-2, selectively inhibit protein kinase C activity, intensively promote hair epithelial cells proliferation *in vitro* and stimulate anagen induction *in vivo*. Other procyanidin with both protein kinases show relatively low activity. Later on Roh et al, (2002) found that extract of dried roots of *Saphora flavescense* has good hair growth promoting activity and exert inhibitory effect on type II 5α- reductase. Later on Matsuda et al, 2002 observed the aqueous extract of spores of *Lygodium japonicum* and found its hair growth promoting and anti androgenic property due to inhibition of 5 α reductase. In 2003 Adhirajan et al, declared that petroleum ether extract of *Hibiscus rosa sinensis* leaves possesses the hair growth promoting potential and its revealed efficacy *in vitro* on isolated neonates hair follicles also.

The 5 α alpha reductase enzyme is a microsomal enzyme that causes biotransformation of 3-oxo - 4 steroidal compounds such as testosterone, progesterone or corticosterone by catalyzing

the reduction of unsaturated steroid into 5 hydroxy saturated derivative. In humans, 5 a reductase enzyme plays a major role in the reduction of testosterone into a more potent androgen, dihydrotestosterone (DHT) which is necessary for normal male growth but excess amount of DHT causes androgen related disorders such as acne, hirsutism, androgenic alopecia, benign prostatic hyperplasia (BPH) and also prostate cancer (Randall,1994; Messenger,2000:Bruchovsky and Wilson,1968,Allali and Mahoudeau,1993).

G. glabra L. has been used in herbal medicine topically in the treatment of various ailments such as rashes, including dermatitis, eczema, itching and cysts (Saeedi et al 2003). Recently glycyrrhizin (an isolate from *G.glabra*) treatment has revealed its protective effects against UV-radiations in human melanoma cells (Rossi et al 2005). Liquorice extract and its active component, glycyrrhizic acid has been described as effective skin whitener agent and accelerator of skin turnover (Brignati et al 2003).

MATERIAL AND METHOD

Glycyrrhiza Glabra Roots

The plant material *G. glabra* roots were procured in the month of June 2011 from Corbett landscape, Ramnagar (Uttarakhand, India) and identified by Dr. D.V. Amla, Scientist G at National Botanical Research Institute, Lucknow (Uttar Pradesh, India) under voucher specimen no NBRI-SOP- 202 and a specimen was preserved there for further references.

Animals

Male wistar albino rats (Bronough et al 1983), weighing 110–150 g, were-3-4 months old used in androgen induced alopecia studies. The rats were placed in polypropylene cages and kept in standard animal house conditions in 12 hr light and 12 hr dark cycle fed with standard pellet diet *ad libitum* and allowed free access to drinking water.

Approval for Animal Studies

The animals were handled according to CPCSEA Guidelines of Good Laboratory Practice. The research protocol of the animal experimentation (Registration 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.

Statistical Analysis

Data reported as mean (±) SEM.Statistical analysis of data was carried out by one way ANOVA comparing all test group versus control followed by Dunnett's test using Instat v 2.1 software.

Isolation of Glycyrrhetinic Acid (COMPOUND GG 1)

The hydromethanolic extract of roots of *G. glabra* was chromatographed on silica gel column eluting with a gradients of mixture of nButanol: ethanol: water in ratio 10:10:4.1. The compound was confirmed as triterpenoidal saponin, it gives positive reaction with Libberman's Burchard reagent and gives positive saponin test. The compound was recrystallized using methanol: chloroform 50:50. It was obtained as offwhile flakes.Yield 3%, Rf 0.68, melting point 292-294 °C.

IR υmax (KBr):669,769,1094,1216,1402,1627,3019,3429 cm^{-1}.

^{1}H NMR(D2O): δ 5.6(I H,C-19)1.78,1.51(2H,C-17)1.04(3H,C-16),1.32(s,C-13)1.26(C-11),1.49,1.26(2H,C-8),1.0(1H,C-6),1.49(1H,C-4)1.78,1.46(2H,C-3),3.18(1H,C-1)

^{13}C NMR (D2O): δ 150,127,114,93,78,64,40,16

ESIMS *m/z:* 469 $[M-H]^{-}$,

Effect of GG 1 on Hair Growth

Induction of alopecia by testosterone

The rats were divided into three groups of six rats each. One group of animal was serving as negative control. Testosterone solution injection in (0.1ml) was administered to each animal *i.m.* for continuous 21 days to induce androgenic alopecia, inspite of this positive control animal was treated with 2% finasteride solution topically and other groups was treated with 0.4 ml of Isolated compound GG1 solution respectively topically daily for 21 days(Pandit et al 2008,Park et al 2003).

RESULTS AND DISCUSSION

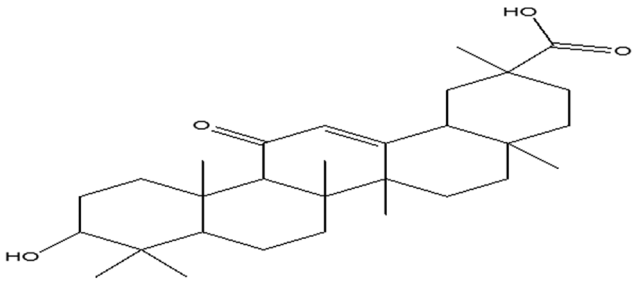
The ESIMS m/z spectrum of compound GG 1 showed a [M-H] - ion at m/z 469, indicating a molecular weight of 470.

The ^{1}H NMR spectrum showed presence of two methyl groups at d 1.11(5-CH3) and (6-CH3), hydroxyl signals at δ 2.0 ppm (C-18, C-2). Presence of methine, methylene, and hydroxyl protons and its showed presence of terpenoidal structure in the region δ 0.7-2.0 ppm. The proton NMR of the compound GG 1 share similarity to that of Glycyrrhetinic acid (marker compound for *G.glabra*). It is a saponin and various medicinal properties are attributed to it. The ^{13}C NMR revealed presence of carbonyl carbon δ 200,181 ppm, unsaturated carbon δ124, 169ppm and methylene carbon (δ61,50,42,35,23 and 19 ppm. FTIR of the compound GG 1 revealed characteristic absorption band of triterpenoids at 3429 cm^{-1} (OH),1216 cm^{-1}(CO), 669 cm^{-1}(CH),and 1402 cm^{-1}(C-C) bands The chemical tests revealed the presence of hydroxyl and ketonic groups. Melting point, proton NMR, MASS data of compound GG 1 are similar to triterpenoidal saponin, Glycyrrhetinic acid in reported data (Young et al 2012,Ohtake et al 2001).

Table 1: Activity of the isolates on hair growth promotion in testosterone induced alopecia

Groups all groups contain six animals	Induction of alopecia
Control (vehicle)	15th day onwards
Finasteride (2% in vehicle)	No signs of alopecia were observed
GG1 (2%in vehicle)	No signs of alopecia were observed

Vehicle is ethanol/propylene glycol/water in ratio 4:0.5:0.5. All animals were treated with *i.m.* dose of testosterone 0.1mL



Structure of Compound GG 1

Chemical name;- 1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a, 12b,13,14b—icosahydro-10—hydroxy-2,4a,6a,6b,9,9,12a-heptamethyl-130xopicene-2-carboxylic acid

Toxicity Studies

The compound GG1 were applied to the dorsal denuded skin of rats for a week at concentration up to 10%. No signs of toxicity such as erythema, redness or swelling were produced so compound GG1 were considered safe for topical use.

On day 21 all animals had developed alopecia besides animals treated with compound GG 1

The excellent hair growth promoting activity of petroleum ether extract of *G.glabra* may be due to presence of compound GG 1 or Glycyrrhetinic acid as it showed excellent hair growth promoting and hair growth restoring activity in testosterone induced alopecia (Table 1). Although in a study its glycosidic form showed hair growth depleting effect of (Ivosevic et al 2014). So activity may be contributed to terpenoidal molecule as observed in this study.

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REFERENCES

Adhirajan, N., Ravikumar, T., Shanmugasundaram, N. and Babu, M., 2003. *In vivo* and *in vitro* evaluation of hair growth potential of *Hibiscus rosa-sinensis* Linn. *J Ethanopharmacol*, Vol. 88, pp. 235-239.

Allali, Z. V., and Mahoudeau, J. L., 1993. Hirsutisme. *Rev. Prat.*,Vol. 43,Issue 18, pp. 2355-62.

Briganti, S., Camera, E., and Picardo, M. 2003. Chemical and instrumental approaches to treat hyperpigmentation. *Pigment Cell Res,*Vol.16, pp. 101–110.

Bronough, R. L., Stewart, R. F.,Congdon, E. R.1983.Differences in permeability of rat skin related to sex and body site.*J.Soc.Cosmet. Chem.*Vol. 34, pp.127-135.

Bruchovsky, N. and Wilson, J. 1968. The conversion of testosterone to 5-alpha-androstan- 17-beta-ol-3-one by rat prostate *in vivo* and *in vitro*. *J. Biol. Chem.*, Vol. 243, pp. 2012-2021.

Cash, F. 2001. The psychology of hair loss and it's implication for patient care. *Clin. Dermatol,* Vol. 19, Issue 36, pp. 161-166.

Hamilton, J. B. 1942. Male hormone stimulation is a prerequisite and an incitant in common baldness. *Am J Anat,* Vol. 71, pp. 451–480.

Ivosevic-Z. J., Hofmann, M., Kakadjanova, A., Valesky, E., Meissner, M., Bereiter-Hahn, J., Kaufmann, R., Bernd, A., Kippenberger, S. Topically applied glycyrrhizic acid causes hair removal in rats. Pharm Biol. 2014 Oct;52(10):1362-5.

Matsuda, H., Yamazaki, M., Naruto, S., Asanuma, Y. and Kubo, M. 2002. Antiandrogenic and hair growth promoting activities of *Lygodii spora* (spore of *Lygodium japonicum*) I. Active constituents inhibiting testosterone 5 α -reductase. *Biol. Pharm. Bull.*, Vol. 25, Issue 5, pp. 622-626.

Messenger, A. G. 2000. Medical management of male pattern hair loss. *Int. J. Dermatol.*, Vol. 39, pp. 585-586.

Ohtake N., Akiko O, Kido, A., Kubota, K., Tsuchiya, Y., Toshimi, M., Kase, Y. and Takeda, S. 2007. A possible involvement of 3-monoglucuronyl glycyrrhetinic acid, a metabolite of glycyrrhizin (GL), in GL-induced pseudo aldosteronism. *Life Sciences.* Vol. 80, pp. 1545-1552.

Pandit, S, Chauhan, N. S. and Dixit, V. K., 2008. Effect of *Cuscuta reflexa* Roxb on androgen induced alopecia. *Journal of Cosmetic Dermatology,* Vol. 7, pp. 199–204.

Park, W., Lee, C., Lee, B. and Chang, I. 2003. The extract of *Thujae occidentalis* semen inhibited 5-reductase and androchrogenetic alopecia of B6CBAF1/j hybrid mouse. *J. Dermatol. Sci.,* Vol. 31, pp. 91-98.

Randall, V. A. 1994. Androgens and human hair growth. *Clin Endocrinol.*,Vol 40, pp.439–457.

Roh, S. S., Kim, C. D., Lee, M. H., Hwang, S, Rang, M. and Yoon, Y. K.,2002. The hair growth promoting effect of Sophora flavescens extract a*nd it's* molecular regulation. J. Dermatol Sci, Vol. 30, pp. 43-49.

Rossi, T., Benassi, L., Magnoni, C., Ruberto, A. I., Coppi,A. and Baggio, G. 2005. E*ffects of glycyrrhiz*in on UVB-irradiated melanoma cells. I*n Vivo, Vol. 9,* Issue 1, pp. 319-22.

Saeedi,M. K., Morteza-Semnani, K. and Ghoreishi, M. R. 2003. The treatment of atopic dermatitis with licorice gel. *J Dermatolog Treat,* Vol. 14, Issue 3, pp.153-157.

Takahashi, T., Kamimura A., Yokoo, Y., Honda, S. and Watanabe, S., 2001. The first clinical trial of topical application of procyanidin B-2 to investigate its potential as a hair growing agent, *Phytotherapy research*, Vol. 15, Issue 4, pp. 331-336.

Upadhyay S., Ghosh A. K., Singh V 2014. Inefficiency of ethanolic extract of *Glycyrrhiza glabra* and *Ziziphus mauritiana* roots on androgenic alopecia. J Pharm Negative Results; 5: 25-8.

Upadhyay S., Singh V.Potentiality of petroleum ether (60-80)°C extract of Glycyrrhiza glabra on androgenic alopecia. Asian J of Pharmaceutical and Clinical Research 2013; 6(3):52.

Yang Li. C., He-Shui-Y,Hong Z H,Li-ping K, Man C,Jiang –ming C,Li-Yan Y, Xin- bo S,Pin MB. 2012.Preparation of Glycyrrhetinic acid monoglucouronide by selective hydrolysis of Glycyrrhetinic acid via biotransformation.Chinese Herbal Medicine Vol. 4 Issue 4 pp.324-328.