Study of protective effect of Glycyrrhetinic acid in androgen induced alopecia

Sukirti Upadhyay*, Prashant Upadhyay¹ A K Ghosh¹ and Vijender Singh²

¹Department of Phytochemistry and Pharmacognosy, IFTM University, Moradabad (UP) India, ²Department of Phytochemistry and Pharmacognosy, Sharda University, Greater Noida (UP) India

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 useful 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 glabra 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, 5α-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. 05/837/ac/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 Glycyrrhetic Acid (COMPOUND GG 1)**

The hydromethanolic extract of roots of *G. glabra* was chromatographed on silica gel column eluting with a gradient 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 offwhite flakes. Yield 3%, Rf 0.68, melting point 292–294 °C.

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

$^1$H NMR (D2O): $\delta$ 5.6(1H,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)

$^1$C NMR (D2O): $\delta$ 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 δ 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 Glycyrrhetic acid (marker compound for *G. glabra*). It is a saponin and various medicinal properties are attributed to it. The $^1$C NMR revealed presence of carbonyl carbon δ 200,181 ppm, unsaturated carbon δ124, 169ppm and methylene carbon (δ61,50,42,35,25 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 ketone groups. Melting point, proton NMR, MASS data of compound GG 1 are similar to triterpenoidal saponin, Glycyrrhetic acid in reported data (Young et al 2012, Ohtake et al. 2001).

**References**

1. Pandit et al. 2008
2. Park et al. 2003
4. Ohtake et al. 2001
Table 1: Activity of the isolates on hair growth promotion in testosterone induced alopecia

<table>
<thead>
<tr>
<th>Groups all groups contain six animals</th>
<th>Induction of alopecia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>15th day onwards</td>
</tr>
<tr>
<td>Finasteride (2% in vehicle)</td>
<td>No signs of alopecia were observed</td>
</tr>
<tr>
<td>GG1 (2% in vehicle)</td>
<td>No signs of alopecia were observed</td>
</tr>
</tbody>
</table>

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

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-130oxopocene-2-carboxylic acid

Toxicity Studies

The compound GG1 was 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 GG1

The excellent hair growth promoting activity of petroleum ether extract of Glycyrrhiza glabra may be due to presence of compound GG 1 or Glycyrrhetic 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.

ACKNOWLEDGEMENTS

This research work is based on PhD dissertation work of author. So she is grateful to Vice Chancellor Dr A P J Abdul Kalam Technical University, Lucknow and Chancellor IFTM University, Moradabad for providing facilities for conducting research work.

REFERENCES


