Original Article

Inefficiency of ethanolic extract of *Glycyrrhiza glabra* and *Ziziphus mauritiana* roots on androgenic alopecia

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Abstract

Aim: To study the air growth promoting effect of roots of *G. glabra* and *Z. mauritiana* in androgen (testosterone) induced male pattern baldness (androgenic alopecia). **Materials and Methods:** Animals were divided into four groups and they were attempted to made alopecic by treatment with testosterone for 3 weeks. Group 1 (control), 2 (standard), 3 and 4 animals (test) were also treated topically with vehicle, finasteride and ethanolic extract of *Z. mauritiana* roots and *G. glabra* roots respectively. Animals were observed for signs of alopecia and after treatment period one animal from each group was euthanized randomly and the skin sections from the treated area was examined for changes in follicular morphology. **Statistical Analysis:** Statistical analysis of data was carried out by one-way ANOVA. Using Instat version 2.1 software. **Results:** The Group 1, 3 and 4 animals develop alopecia and their skin section also shows bulbous and miniaturized hair follicles and prominent sebaceous gland while Group 2 (standard antiandrogenic drug finasteride treated) animals does not develop alopecia and skin samples also shows well-formed follicles and normal sebaceous gland. **Conclusion:** This experiments shows incapability of ethanolic extract of *Z. mauritiana* and *G. glabra* combating androgenic alopecia.

Key words: *Glycyrrhiza*, hair growth, *Ziziphus*

INTRODUCTION

Ziziphus mauritiana also known as ber, chinee apple, Chinese date, cottony jujube, Indian cherry, Indian jujube is a tropical fruit tree species. It is a spiny, evergreen shrub or small tree rise up to 15 m high, with trunk 40 cm or more in diameter; spreading crown; stipular spines and many drooping branches. It is found in tropical region from Africa to Afghanistan and China, Australia and in some pacific regions. It is a rich source of macro cyclic peptides alkaloids lupane and triterpenes, cyclopeptides of *Ziziphus* species showed pharmacological properties, including sedative, analgesic, antibacterial, antifungal and antiplasmodial activity etc., The 13-membered macrocyclic alkaloids are isolated from this plant. These included the 4 (14)-membered ring class: Mauritine C, amphibine F and frangufoline the 5 (14)-membered ring type: Mauritines A and B. It also contains protein, carotene, and vitamin C. It cures vitiated pitta, kapha, obesity, fever, burning sensations, cough, wound, skin disease, ulcers, stomatitis, diarrhea, sexual weakness, and general debility.[1] It is recommended for hair growth promotion in traditional medicine.[2]

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Glycyrrhiza glabra, also known as Yasthi-Madhu in Sanskrit, Jethi-Madhu in Hindi, Jashtimadhuin Bengali (all Indian languages), and licorice in English, is cultivated in Jammu, Kashmir, Punjab and Sub-Himalayan tracts of India. It is a hardy herb or undershrub, attaining a height of 1.8 m. Its roots are thick, having many branches with red or lemon color outside and yellowish or pale inside. It is

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called Keshya (hair growth promoter) in traditional texts of Ayurveda.[3] The decoction of the root is a good wash for falling and graying hair.[4] There is no literature evidence about *Z. mauritiana* to promote hair growth or combat androgenic alopecia but *Ziziphus jujube* is proved as hair growth promoter[5] and in our previous studies petroleum ether extract of *G. glabra* also recommended for hair growth and androgenic alopecia.[6,7] Hence, here experiments were performed in ethanolic extract of *Z. mauritiana* and *G. glabra* for observing their effect in androgenic alopecia.

MATERIALS AND METHODS

Plant material

Roots of *G. glabra* and *Z. mauritiana* were collected in the month of February from Jim Corbett National Park, Ramnagar, Uttrakhand, UP. The samples were authenticated by Dr. D. V. Amla, Scientist D, National Botanical Research Institute, Lucknow UP. A voucher sample no NBRI-SOP-202 was submitted there for further references.

Preparation of extract

Both extract were prepared from defatted roots macerated with ethyl alcohol for 1-day separately and then extract was concentrated under reduced pressure. The yield was 15% w/w for *Z. mauritiana* and 20% w/w for *G. glabra*.

Animals

Male Wistar albino rats, weighing 100-150 g, age 5-6 months were used for hair growth studies. The rats were placed in polypropylene cages and kept in standard animal house conditions in 12 h light and 12 h dark cycle, fed with standard pellet diet *ad libitum* and allowed free access water. The permission for animal work was provided by Animal Ethical Committee of the IFTM University, Moradabad, UP, India.

Testosterone test solution

Marketed preparation of testosterone Testovirion Depot manufactured by German Remedies Pvt Ltd, Mumbai-400018 was diluted up to 5 mL with water for injection. This was able to produce the concentration of 5 mg/mL and 0.1 mL was injected at the thigh of animal (*s.c.*) route in 0.1 mL dose daily.

Finasteride solution

The 2% standard finasteride solution was prepared in vehicle (ethanol: Propylene glycol: Water, 8:1:1). 0.4 mL was applied daily to the animals on the dorsal side.

Extracts solution

The 2% extracts solutions were prepared in vehicle (ethanol: Propylene glycol: Water, 8:1:1).

Toxicity studies

The extract and finasteride solution were applied to animals up to 10% concentration and animals were observed for signs of toxicity for 7 days.[8,9]

Grouping and treatment of animals

The rats were divided into four groups of six rats each. All groups of animals were treated with testosterone solution injection in 0.1 mL (*s.c.*) for continuous 21 days to induce androgenetic alopecia and to evaluate combating effect of finasteride and extracts, back or dorsal massage of 0.4 mL vehicle, 2% finasteride solution and 2% extracts solutions were administered to Groups 1, 2, 3 and 4 animals respectively daily for 21 days. Animal was observed for 3 weeks and then one rat from each group was selected randomly and sacrificed on the 21th day of drug treatment.[10] Skin biopsies were taken from the affected area and fixed in 10% formalin buffer. Tissues were embedded in paraffin wax and sectioned into uniform thickness of 10 μm with microtome and were stained with hematoxylin and eosin. From the sections, the number of hair follicles per millimeter of the skin and the percentage ratio of different cyclic phases, like anagen and telogen, of hair follicles were determined.[11]

Statistical analysis

Data are reported as mean ± standard error of the mean statistical analysis of data was carried out by one-way ANOVA comparing all test groups versus control followed by Dunnet's test using Instat version 2.1 software. A $P < 0.05$ considered as statistical significant.

RESULTS

The animals of different groups were observed for hair loss and skin sections were examined for various parameters as shown in Table 1, Figures 1 and 2.

DISCUSSION

In the animal study of 3 weeks the first, third and fourth group animals observation shows that the alopecia start to visualize from day 15 on the dorsal side of skin, but the Group 2 animals did not showed any signs of alopecia,

Table 1: Hair growth population of 100 hair follicles in (anagen and telogen stage) and follicle density after various treatments

Group	Telogen stage	Anagen stage	Follicle density
1	83.10±0.57	16.90±0.57	1.00±0.38
2	52.40±0.69**	47.60±0.69**	2.50±0.31**
3	90.00±0.57**	9.00±0.56**	0.80±0.15**
4	95.00±0.57**	5.00±0.56**	0.40±0.15**

Values are mean±SEM (*n*=6). **P<0.01

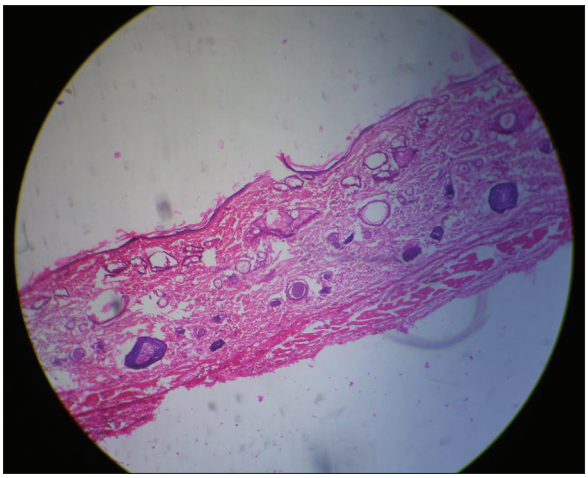

Figure 1: Skin section of animal treated with ethanolic extract of *Z. mauritiana*

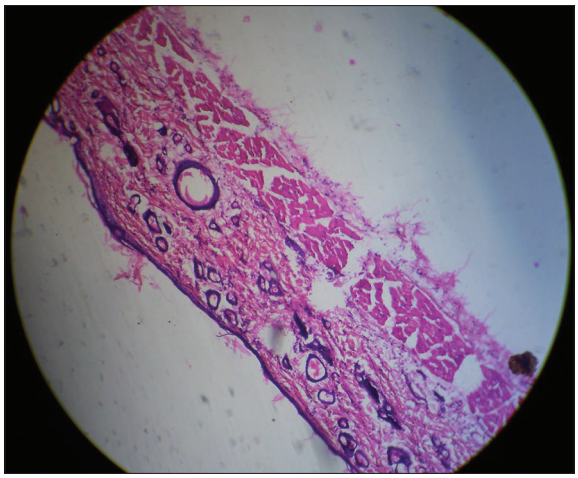

Figure 2: Skin section of animals treated with ethanolic extract of *G.glabra*

ethanolic extract of *Z. mauritiana* [Figure 1] and *G. glabra* roots treated group [Figure 2] showed development of alopecia or male pattern baldness, which was progressed from cranial to caudal region and in histopathological studies of the skin sections the bulbous appearance of follicles and very prominent sebaceous gland confirmed androgenic alopecia and it was compared significantly to control group.[12,13] While Group 2 animals (finasteride treated) remain unaffected [Table 1]. Hence, the results of ethanolic extract of drugs do not support traditional belief about roots of *Z. mauritiana* and *G. glabra* in treatment or reversal of androgenic alopecia.[2]

The mechanism of hair growth restoration in induced androgenic alopecia is *5 alpha* reductase inhibition and finasteride is known 5 alpha reductase inhibitor so Group 2 animals do not develop alopecia.[14] The ethanolic extract of these herbs may not possess 5 alpha reductase inhibitory activity as alopecia is clearly developed. No major controversies arised by this study, as it is reported in literature that petroleum ether extract of *G. glabra*[7] and volatile oil of *Z. jujube*[5] possess anti androgenic alopecia activity. But chemical constituents were not isolated and identified in above studies. As different solvent use for extraction have different phytoconstituents. Petroleum ether extract of *G. glabra* possess mainly nonpolar phytoconstituents. They supports the traditional claim but the polar compounds present in ethanolic extract of both drugs do not possess hair growth promoting or restoring property.

However, a number of investigators have shown that polar compound like flavonoids possess hair growth promoting activity by strengthening the capillary wall of the smaller blood vessels supplying hair follicles; improve blood circulation to nourish the hair follicles and thereby promoting hair growth.[14-16] However, the polar compound or flavonoids present in ethanolic extract of *Z. mauritiana* and *G. glabra* donot possess hair growth promoting activity in testosterone induced or androgenic alopecia.

As the ethanolic extract of *G. glabra* and *Z. mauritiana* do not possess anti androgenic activity. They may further investigate in future for their androgenic of fertility effect in males.

CONCLUSION

Conclusively in present study ethanolic extract of *Z. mauritiana* and *G. glabra* was found ineffective in combating effect of testosterone in causing androgenic alopecia.

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REFERENCES

1. Upadhyay S, Upadhyay P, Ghosh AK, Singh V. Ziziphus mauritiana: A review on pharmacological potential of this underutilized plant. Int J Curr Res Rev 2012;04:141-4.
2. Suneetha J, Rao K, Reddi T. Herbal remedies for hair disorders by the tribals of East Godavari District, Andhra Pradesh. J Exp Sci 2011;2:30-2.
3. Chatterjee A, Pakrashi S. The Treatise of Indian Medicinal Plants. New Delhi: National Institute of Science Communication; 1992. p. 67-180.
4. Singh J, Tripath A, Gandhi R, Singh SC, Jain SP, Sharma A, *et al*. Traditional uses of Glycyrrhiza glabra (Mulethi/Yastimadhu). J Med Aromat Plant Sci 2006;28:263-9.
5. Yoon JI, Al-Reza SM, Kang SC. Hair growth promoting effect of Zizyphus jujuba essential oil. Food Chem Toxicol 2010;48:1350-4.
6. Upadhyay S, Ghosh AK, Singh V. Hair growth promotant activity of petroleum ether root extract of Glycyrrhiza glabra L (Fabaceae) in female rats. Trop J Pharm Res 2012;11:754-8.
7. Upadhyay S, Singh V. Potentiality of petroleum ether (60-80)°C

extract of Glycyrrhiza glabra on androgenic alopecia. Asian J Pharm Clin Res 2013;6:2-55.
8. A.S.T.M. Standard Practice for Testing Biomaterials in Rabbits for Primary Skin Irritation, A.S.T.M. Designation F719-81. Philadelphia: American Society for Testing of Materials; 1998. p. 178-9.
9. Parasuraman S. Toxicological screening. J Pharmacol Pharmacother 2011;2:74-9.
10. Charan J, Kantharia ND. How to calculate sample size in animal studies? J Pharmacol Pharmacother 2013;4:303-6.
11. Matias JR, Malloy V, Orentreich N. Animal models of androgen-d ependent disorders of the pilosebaceous apparatus 1. The androchronogenetic alopecia (AGA) mouse as a model for male-pattern baldness. Arch Dermatol Res 1989;281:247-53.
12. Yoo HG, Kim JS, Lee SR, Pyo HK, Moon HI, Lee JH, *et al*. Perifollicular fibrosis: Pathogenetic role in androgenetic alopecia. Biol Pharm Bull 2006;29:1246-50.
13. Ebling FJ. The action of testosterone and oestradiol on the sebaceous glands and epidermis of the rat. J Embryol Exp Morphol 1957;5:74-82.
14. Awe EO, Makinde JM. The hair growth promoting effect of Russelia equisetiformis (Schclect and Chan). J Nat Prod 2009;2:70-3.
15. Kobayashi N, Suzuki R, Koide C, Suzuki T, Matsuda H, Kubo M. Effect of leaves of Ginkgo biloba on hair regrowth in C3H strain mice. Yakugaku Zasshi 1993;113:718-24.
16. Kawano M, Han J, Kchouk ME, Isoda H. Hair growth regulation by the extract of aromatic plant Erica multiflora. J Nat Med 2009;63:335-9.

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