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Effect of ethanolic fraction of Hibiscus rosa sinensis l., leaves in androgenic alopecia

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Abstract

Hibiscus rosa sinensis leaves are well known as hair growth promoter in traditional and folklore medicines. In this study, experiments were performed on male albino rats for induction and development of androgenic alopecia by subcutaneous dose of testosterone for three weeks (21 days). Animals were divided into three groups and group one, two and three animals were treated with vehicle, finasteride and ethanolic extract of Hibiscus rosa sinensis leaves topically along with testosterone respectively. Animals were observed for signs of alopecia on dorsal skin. After the treatment period one animal from each group was euthanized and follicular morphology and density were studied. The animals treated with testosterone and vehicle become alopecic from the second week of treatment, while animals treated with finasteride and ethanolic extract of Hibiscus rosa sinensis did not become alopecic and follicular morphology study also supported this. We concluded that ethanolic extract of H.rosasinensis posses significant anti androgenic alopecia activity as proved by this study and it is comparable to standard FDA approved drug for androgenic alopecia finasteride.

Introduction

Alopecia has a great impact on a person's self-respect, mental health, and overall quality of life. The disorder, androgenic alopecia (AGA) is the most common type of hair loss, which affects large numbers of both men and women. [1] It affects at least half of all men by the age of 50, and up to 70% of 70-year-old men. [2] AGA is caused by excessive activity of the 5alpha reductase

enzyme in hair follicles. [3] At present, there are some medicines that are used to treat AGA. For example, 5alpha reductase inhibitors, finasteride and dutasteride, are used to treat androgen-related disorders. [4] But these medicines have several undesirable side effects, for example, impotence (erectile dysfunction), abnormal ejaculation, decreased ejaculatory volume, abnormal sexual function, gynecomastia, testicular pain, impairment of muscle growth, and severe myopathy. [5] Alternatively some herbal extracts are found effective in androgenic alopecia such as that of Cuscuta reflexa [6], Abrus precatorius [7] and Carthamus tinctorius [8] etc. Out of praised plants for hair growth promotion, the herb Hibiscus rosa-sinensis L. (Malvaceae) is native to China but easily available in various part of India, was found to be a promoter of hair growth and its leaves' extract is more potent than the flowers' extract. [9] As The herb is a source of antioxidants like Taraxeryl acetate, sterculic and malvalic acids, quercetin and its glycosides, cyanin and cyanidin chlorides, kaemperol-3-xylosylglucoside, thiamine, riboflavin, niacin and ascorbic acid. [9]

In the current study, we are evaluating potency of ethanolic extract of Hibisus rosa sinensis leaves on androgenic alopecia experiments, conducted in male albino rats. No previous work is reported for this aspect.

Material and Method

Plant material

Fresh leaves were collected from garden in Girls Hostel, U.T.D Sagar in the month of Feb 2008 and identified by Dr Pradeep Tiwari of Department of Botany. Dr H.S. Gaur University, Sagar, and a voucher specimen no. (Bot/H/2792) was preserved there for future references

Chemicals

Finasteride was procured from CIPLA LTD, Sikkim, India while petroleum ether, ethanol and propylene glycol were obtained from Central Drug House, Delhi, India. Testosterone is obtained from German Remedies, Mumbai, India.

Preparation of extracts

Leaves were dried in oven at 35- 37°C and then it was defatted in petroleum ether(60-80)°C in soxhlet apparatus then dried and macerated with ethyl alcohol overnight and then the filtrate was concentrated by vacuum under reduced pressure the yield was 30% w/w.

Animals

Male Wistar albino rats, weighing 120-150 g, 3-4 months old were used for hair growth studies. The rats were placed in 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 to drinking water.

Testosterone test solution

Marketed preparation of testosterone Aquavirion was diluted up to 5 ml with water for injection. This was able to produce the concentration of 5mg/mL

Finasteride, extract solution

The 2% finasteride and extract solution was prepared in vehicle (ethanol/propylene glycol/water, r = 8: 1: 1).

Phytochemical Screening

Ethanolic root extract was screened by performing various microchemical tests for the presence of active phyto-constituents.

Toxicity studies

Toxicity studies of extract on skin of animals were carried out by the application of ethanolic extract in a concentration of up to 10% for seven days on the shaved denuded skin of albino rats. They were further observed for signs of toxicity such as swelling, itching, erythema etc. [10]

Treatment of animals

The animals were divided into three groups of six animals in each group. Group 1M, 2M and 3 M consists of male albino rats. [11] All animals were treated with 0.1 mL of testosterone daily for three weeks and group1M, 2M and 3M receives topical massage of vehicle, finasteride and ethanolic extract of leaf respectively.

Results and Discussion

Phytochemical screening

Hibiscus rosa sinensis leaf ethanolic extract shows presence of carbohydrates, flavonoids and phenolics.

Toxicity studies

The extract was considered safe for topical use as no signs of toxicity as erythrema, swellings, irritation were produced in rats for seven days study.

Effect of hair growth

The 1M group of animals on a dose of testosterone and vehicle become alopecic as hair shedding starts from day fourteen from the dorsal skin and alopecia progressed from the cranial to the caudal region while in groups 2M and 3M the animals did not develop signs of alopecia up to 21

days of the study. The finasteride and ethanolic extract treated animals **(Fig 2)** did not develop signs of alopecia and the skin section study shows higher follicle density, anagenic follicles while the vehicle treated animals developed alopecia **(Fig 1)** which shows that the vehicle does not have anti androgenic alopecia activity while finasteride and the extract successfully combat alopecia. The statistical study of hair follicles by occular micrometer also supports the observed results significantly **(Fig 2, 3, 4)**. Skin section of the animal shows hair follicles in anagenic stage and very few follicle in telogenic stage. In **figure 4** the column graph shows a high percentage of anagenic follicles in extract treated animals followed by finasteride treated animals while the animals on vehicle shows least percent of anagenic follicles. The quantitative study of hair follicle density also shows maximum hair density in extract treated animals. This activity in extract may be due to the presence of antioxidant potential of the plant or presence of flavonoids. [12] One of the major causes of androgenic alopecia is the reduced blood supply to the androgen sensitive and genetically predisposed hair follicles. However, a number of investigators have shown that polar compounds like flavonoids possess hair growth promoting activity by strengthening the capillary wall of the smaller blood vessels supplying the hair follicles; improve blood circulation to nourish the hair follicles and thereby promoting hair growth. [13,14,15] . As the extract is rich in flavonoid content, strengthening capillary wall may be the reason for increased blood circulation and combating the effect of androgen supplied by the subcutaneous route.

Fig 1: The dorsal skin of animal on dose of testosterone and vehicle (1M).

Fig 2: The dorsal skin of extract treated animal along with testosterone (3M) does not develop alopecia.

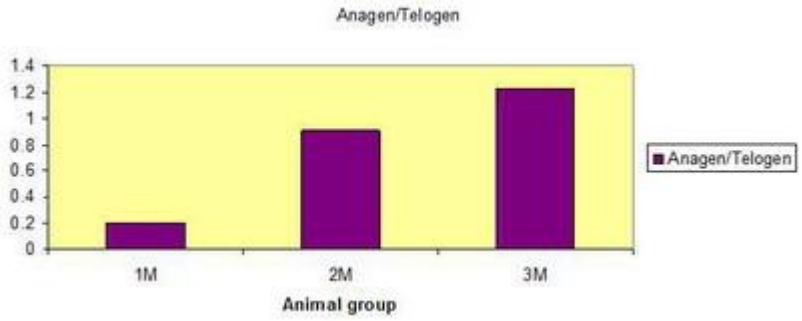

Fig 3: Anagen/ Telogen ratio of different groups of animals.

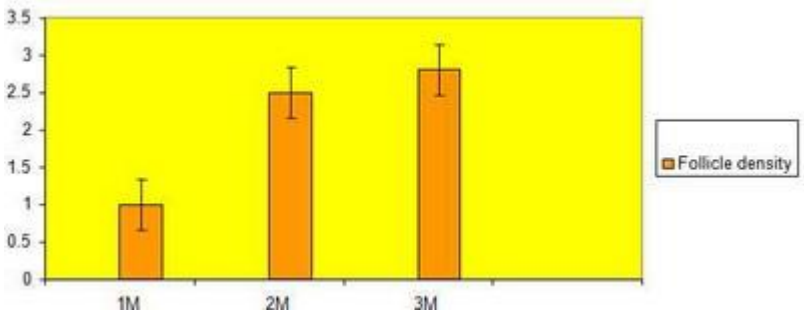

Fig 4: Effect of various treatment along with testosterone on Follicle density of male rats.

Conclusion

Ethanolic extract of leaves posses effect against androgenic alopecia in male rats so conclusively it may be used in the future as a hair restoring agent for males suffering from alopecia.

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