

Effect of petroleum ether and ethanol fractions of seeds of *Abrus precatorius* on androgenic alopecia

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Article

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Abstract: Seeds of *Abrus precatorius* L., Fabaceae, are commonly used as purgative, emetic, aphrodisiac and in nervous disorder in traditional and folk medicines. In present study petroleum ether and ethanolic extracts of *A. precatorius* seeds are evaluated for reversal of androgen (testosterone by *i.m* route) induced alopecia in male albino wistar rats and compared to topical administration of standard antiandrogenic drug finasteride for 21 days. The results were reflected from visual observation and histological study of several skin sections via various parameters as anagen to telogen ratio and follicle density/mm area of skin surface. The animal of group 1 who were treated with only testosterone became alopecic on visual observation. Animals of Group 2, 3 and 4 who were treated with finasteride, petroleum ether and ethanolic extract of seed respectively topically along with testosterone (*i.m*) did not developed alopecia. To investigate the mechanism of observed activity, *in vitro* experiments were performed. Inhibition of 5 α -reductase activity by extracts and finasteride suggest that they reversed androgen induced alopecia by inhibiting conversion of testosterone to dihydrotestosterone (potent androgen responsible for androgenic alopecia). So it may be concluded that petroleum ether and ethanolic extract of *A. precatorius* seed posses anti androgenic alopecia activity due to inhibition of 5 α -reductase enzyme.

Keywords:

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Introduction

Hair cosmetics and medicaments constitute one of the major parts in global market. Hundreds of hair growth products are available in the markets and these are prepared by combination of one or more herbal drugs. (Ali & Ansari, 1997). Natural remedies have been used for centuries in treating alopecia (Ayyanar & Ignacimuthu, 2005; Hoermann & Korting, 1994). In androgenic alopecia 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 (Paus & Costeralis, 1999). It is dihydrotestosterone mediated process, characterized by continuous miniaturization of androgen reactive hair follicles and accompanied by per follicular fibrosis of follicular units in histological examination (Yoo et al., 2006). Androgenic alopecia results in a decrease in hair follicle size accompanied by a decrease in the duration of anagen and an increase in the percentage of hair follicles in telogen (Cotsarelis & Millar, 2001).

Finasteride, a synthetic antiandrogenic drug is marketed for Benign prostrate hyperplasia and androgenic alopecia and the mechanism involved is inhibition of 5 α -reductase activity (Tian et al., 1994) Finasteride also has been used topically to treat androgenetic alopecia (Mazzarella et al., 1997). For the treatment of androgen related disorders like androgenetic alopecia, finasteride has been approved by USFDA (Libecco & Bergfeld, 2004), but there are many side effects associated with finasteride. As *Cuscuta reflexa* Roxb was found effective in androgen induced alopecia (Pandit et al., 1998), the study here was focused on the seed extracts of *Abrus precatorius* L., a leguminous plant of the Fabaceae family that is also called Indian liquorice, jequirity, crab eye, glycyrrhizin glabra and olho de Pombo, among others. The plant grows widely in fairly dry climates of tropical and subtropical regions, such as India, Sri Lanka, Nigeria and the West Indies. The leaves, roots and seeds of *A. precatorius* are used for medicinal purposes, a practice most probably dating back to antiquity (Ross, 2003). The seeds contain campesterol, cholesterol, palmitic acid, linoceric acid, linoleic acid, oleic acid, β -sitosterol, stigmasterol, trigonellin, brassicasterol (Ross, 2003).

Material and Methods

Plant material

Seeds of *Abrus precatorius* L., Fabaceae, were collected in the month of November from Dr HS Gaur University, Sagar (23N50 78E43) campus. The plant was identified by Dr Pradeep Tiwari, at Department of Botany of the same university and a voucher specimen no (Bot/H/3031) has been preserved for further references. The material was dried under sunlight and carefully reduced to coarse powder and stored in airtight container after sieving them through # 40 mesh.

Preparation of extracts

Coarsely powdered drug was taken in a Soxhlet apparatus and extracted with petroleum ether (60-80 °C) till complete extraction. The solvent from the extract was recovered under reduced pressure and its yield was 1.6% w/w. Then successively extracted with 99.9% ethanol and the yield was 1.8% w/w.

Chromatographic characterization

For experiment, petroleum ether seed extract of *A. precatorius* was characterized by TLC on precoated silica gel G plate (10×10) (E. Merk, Germany) developed in toluene:ethyl acetate (95:5 v/v) as mobile phase gave best resolution after derivatization with Lieberman Burchard reagent. Steroidal component was present in this as proved by phytochemical analysis. Ethanolic extract was characterized by giving best resolution in n-butanol:ethanol:water (10:10:4.2v/v) as mobile phase after derivatization with vanillin-sulphuric acid reagent. Flavanoid, alkaloid, carbohydrate, phenolic, protein and amino acid were present in this as proved by phytochemical analysis.

Preliminary skin irritation test

This test was carried out by protocol mention in ASTM (1998). The petroleum ether and ethanolic extracts of *A. precatorius* applied in a concentration of up to 10% for seven days on shaved skin surface of wistar rats, did

not show any irritation or erythema on skin surface. Thus the prepared extracts were considered safe for topical administration (Roy et al., 2008). *A. precatorius* seeds are known for their toxicity but both petroleum ether and ethanolic extract do not contain toxic principles (Humphreys, 1969; Niyogi & Rieders, 1969).

In vivo studies on hair growth against testosterone induced alopecia

Animals

The protocol for experimentation was approved by Institutional Animal Ethics Committee of Dr H S Gour University, Sagar, India in form #08/3870. Male albino rat (2-3 months) were housed in cages at room temperature (26±2 °C) and were fed on standard diet with free access to water.

Preparation of finasteride, testosterone and extract solutions

Marketed preparation of testosterone suspension named Aquavirion (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 finasteride solution and 1% extract solutions was prepared in vehicle (ethanol:propylene glycol:water 8:1:1).

Treatment of animal for study

The method reported by Matias et al. (1989) 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 and 4 were also given topical application of 0.4 mL finasteride, petroleum ether and ethanolic extract of seed respectively on dorsal skin surface once a day for 21 days (Table 1). 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.

Table 1. Percent hair cycle (%) stage of animals on 21st day after treatments.

Group No	Treatments	Percent hair cycle Stages		
		Telogen(Te)	Anagen(A)	A/Te
1	Testosterone (i.m.) only	83.1±0.5667	16.9±0.5667	0.2034
2	T (i.m.) finasteride (2%) topically	52.4±0.6864***	47.6±0.6864***	0.9084
3	T (i.m.)+petroleum ether seed extract in 1% vehicle topically	45.2±0.5735***	55.8±0.5774***	1.2168
4	T (i.m.)+ethanolic seed extract ether seed extract in 1% vehicle topically	34.4±0.5207***	65.3±0.5175***	1.8983

All four groups contain six animals; n=skin sections number=10; T: testosterone; values are mean±SEM, with significant ****p*<0.001 value as compared to control.

In vitro studies on enzymatic activity

Preparation of enzyme solution

Human prostate (about 195 mg) supplied from a local hospital was cut in small pieces and homogenized in 10 mL of medium A (20 mM sodium phosphate, pH 6.5, containing 0.32 g sucrose and 1 mM EDTA). The homogenate was centrifuged at 4000 x g for 15 min. The supernatant was used as a source of enzyme. The concentration of enzyme in the supernatant was determined by Bradford Method of Protein estimation (Bradford, 1976).

Preparation of test materials

Testosterone (1 mM), petroleum ether extract (1 mg/mL), ethanolic extract (1 mg/mL) and finasteride (10 µg/mL) solution were prepared in ethanol 95% with gentle heating wherever necessary. The EDTA solution (10 mg/mL) was made in distilled water.

Determination of optimum concentration of enzyme

It was determined by keeping the concentration of substrate constant and varying the concentration of enzyme. Testosterone solution (1 mM) was prepared in ethanol. Reaction mixture (1 mL) was prepared by adding testosterone solution (0.1 mL), enzyme solution (0.1-0.9 mL), and sodium phosphate buffer (20 mM). The reaction mixture was incubated at 37 °C for 1 h. The reaction was terminated by addition of 2 mL of ethyl acetate. The reaction mixture was then shaken vigorously for 1 min and the ethyl acetate layer was separated. It was evaporated to dryness, and the residue dissolved in 2 mL of methanol. Testosterone content in methanolic solution was estimated by high performance liquid chromatography (HPLC, Shimadzu, Column C 18).

Determination of inhibitory concentrations of petroleum ether and ethanolic extract of *A. precatorius*

The reaction mixture (1.5 mL) was made by adding 0.1 mL of testosterone solution, 0.1 mL of EDTA solution, 0.1-0.5 mL of petroleum ether extract/ethanolic extract/finasteride solutions groups, optimum amount of enzyme solution (*i.e.*, 0.5 mL), and sodium phosphate (20 mM), to a final volume of 1.5 mL. Reaction mixture was incubated at 37 °C for 60 min, and reaction was terminated by addition of 3 mL of ethylacetate. The mixture was vortexed for 1 min; was separated and evaporated to dryness, residue was dissolved in methanol and volume made up to 2 mL with methanol. The residual testosterone content in methanol was determined by HPLC. The

column was eluted isocratically with a mobile phase of methanol:water (80:20) at a flow rate of 1.0 mL/m (Purdon et al., 1997).

Statistical analysis

Data are reported as mean±SEM. Statistical analysis of data was carried out by one way ANOVA comparing all test group (group 2, 3 and 4) versus negative control (group 1) followed by Dunnett's test using Instat v 2.1 software.

Results

In vivo hair growth studies against testosterone induced alopecia

Qualitative study

Alopecia proceeds via cranial to caudal region in rodents (Parthasarathy et al., 1992) The animals of group 1 showed diffuse alopecia. Loss of hair from dorsal portion of rat was clearly visible after 21 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. Animal of group 4 also showed no sign of alopecia as their hair did not become thin on dorsal skin (Figure 1).

Quantitative study

In the present investigations alopecia was induced in rat by administration of testosterone. Testosterone is required, along with a genetic predisposition, for androgenetic alopecia to develop in men (Hamilton, 1942) 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 2). Several hair follicles were in telogen phase. The effect of testosterone on miniaturization of hair follicle was blocked by administration of topical finasteride, petroleum ether and ethanolic extract of seed in group 2, 3 and 4 animals respectively. The number of follicles in anagen phase was considerable and fewer follicles in telogen phase were observed. A/Te ratio was significantly affected by finasteride and extracts which was observed in skin sections of various groups (Table 1) (Figure 2A, B and C).

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 (Figure 3).

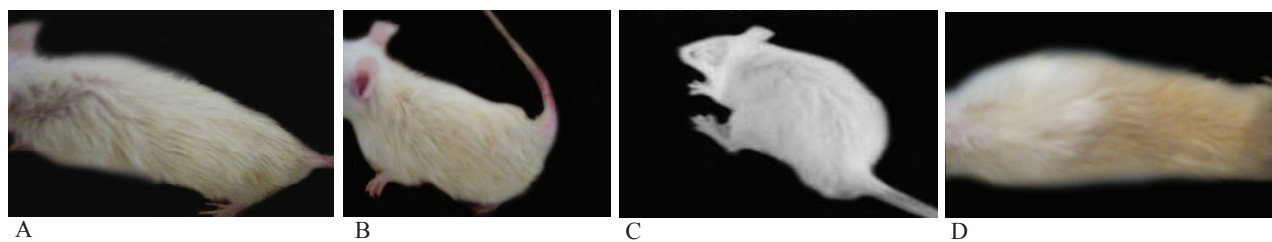


Figure 1. A. The animal shows hair loss from cranial region and hair become thin on dorsal skin, i.e. alopecia condition is visible in group 1 animal due to testosterone administration; B. Animals of group 2 do not develop alopecia on 21 days treatment with testosterone due to simultaneous treatment with finasteride; C. Animals of group 3 do not become alopecic as petroleum ether extract of seed combat testosterone effect; Animals of group 4 also do not develop alopecia as ethanolic extract of seed successfully antagonize testosterone effect.

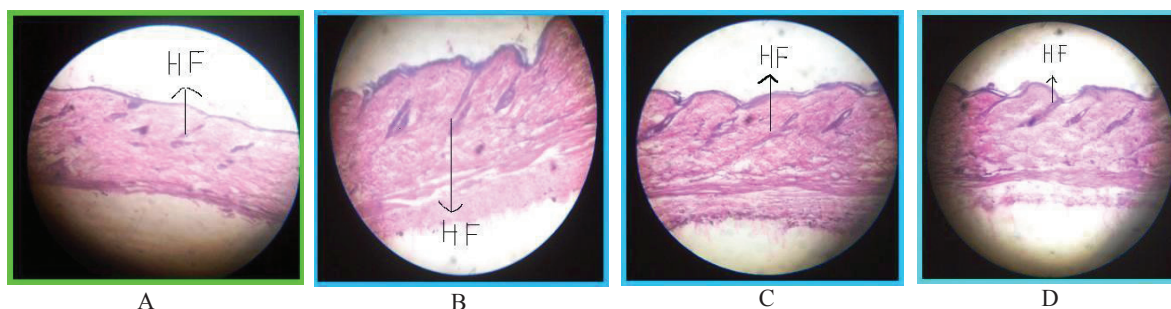


Figure 2. A. Skin section of Group 1 animals showed maximum telogenic hair follicle leading to alopecia; B. Skin section of Group 2 animal shows hair follicles in anagenic (hair growing stage); C. Skin section of Group 3 animal shows more hair follicles in anagenic stage than finasteride treated group 2 animals; D. Skin sections of Group 4 animals showed maximum no of anagenic follicles. HF=hair follicle.

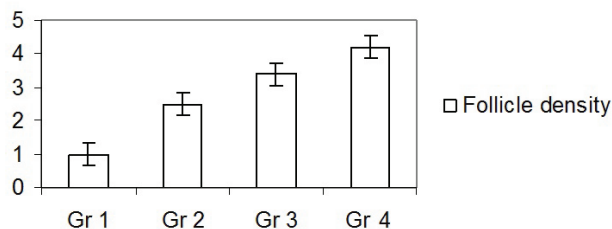


Figure 3. Follicle density is maximum in group four animals followed by group 3 and 2 while group 1 animals showed minimum follicle density. Gr: group.

Determination of IC₅₀

The optimum concentration of the enzyme was found at 0.5 mL (230.9 µg). Varying concentrations of test substances were incubated with a constant amount of testosterone and enzyme in reaction mixture, and the residual testosterone content was determined after termination of reaction with ethyl acetate. The residual testosterone content in reaction mixture increased with increasing concentration of petroleum ether extract, ethanolic extract and finasteride. The IC₅₀ values calculated for petroleum ether extract, ethanolic extract, and finasteride were 1.78 mg, 1.70 mg and 0.72 µg showed enzyme inhibitory activity if these compounds.

Discussion

As evident from above data, the activity of petroleum ether and ethanolic extract of *A. precatorius* is better than finasteride. The alopecia, induced in the rat by testosterone was counteracted when finasteride, petroleum ether or ethanolic extract of seed were administered. The predominance of hair follicle in anagenic growth phase indicates reversal of androgen induced hair loss in finasteride petroleum ether and ethanolic extract treated groups. Conversion of testosterone to dihydrotestosterone, which is a more potent androgen, results in miniaturization of hair follicle and change in cyclic phase of hair growth cycle, which leads to androgenic alopecia. The enzyme 5 α -reductase type 2 is the key enzyme responsible for conversion of testosterone to dihydrotestosterone. (Kaufman, 2002). The prostate is rich in enzyme 5 α -reductase type 2, and prostate homogenate demonstrates conversion of testosterone to dihydrotestosterone in reaction mixtures (Steers, 2001). Both extracts successfully blocks the conversion of testosterone into dihydrotestosterone on comparison with standard finasteride. As dihydroxy testosterone is responsible for androgenic alopecia so it may concluded that both extracts may function similar to finasteride in combating testosterone effect. The ethanolic extract of seed on intraperitoneally administration also lowers the testosterone levels (Jahan et al., 2009). Thus

A. precatorius seed ethanolic and petroleum ether extract is considered a useful preparation for topical use in commercial formulations for androgenic alopecia and other androgen related disorders.

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