Available online on 15.03.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Evaluation of hair growth promoting activity of petroleum ether extract of *Abrus precatorius* Linn. on Wistar Albino rats

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ABSTRACT

In present investigation was carried out to screening of hair growth promoting potentiality of petroleum ether extract of *Abrus precatorius* leaf. Preliminary chemical tests and TLC analysis revealed the presence flavonoids and saponins. Hair growth promoting activity of petroleum ether of *Abrus precatorius* was screened by considering different parameters which included time taken for covering bald patch, length of hair produced, percentage of hair follicles in anagen and telogen phases, time of hair growth initiation and completion and level of minerals in blood. The petroleum ether extract of *Abrus precatorius* showed a very good hair growth promoting activity at a dose of 300 mg/kg which was comparable to that of 2% minoxidil. After 30 days of treatment with test and standard drugs it was observed that, time taken for covering the bald patch, hair growth initiation and completion time and quantitative hair growth were found to be comparable to that of the standard drug. An increase in percentage of hair follicles turning from telogen phase to anagen phase was noted. The control treated group of animals showed poor hair growth for all the parameters.

Keywords: *Abrus precatorius* Linn, Anagen, Catagen, Telogen, Minoxidil

Article Info: Received 04 Feb 2019; Review Completed 09 March 2019; Accepted 12 March 2019; Available online 15 March 2019

Cite this article as:

Omhare N, Sahu P, Gautam S, Jain N, Evaluation of hair growth promoting activity of petroleum ether extract of *Abrus precatorius* Linn. on Wistar Albino rats, Journal of Drug Delivery and Therapeutics. 2019; 9(2):330-335
http://dx.doi.org/10.22270/jddt.v9i2.2426

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INTRODUCTION

Herbal medicine has been used in India for thousands of years and is increasingly been used worldwide during the last few decades as evidenced by rapidly growing global and national markets of herbal drugs. The global pharmaceutical market was worth US $550 billion in 2004 and is expected to exceed US $900 billion by the year 2009[1-4].

According to World Health Organization, herbal medicines are lucrative globally and they represent a market value of about US$ 43 billion a year[5]. According to an estimate in 1991, the herbal medicine market in the European countries was about $ 6 billion, with Germany accounting for $ 3 billion, France $ 1.6 billion and Italy $ 0.6 billion while in other countries was 0.8 billion. In 1996, the herbal medicine market in the European countries was about $ 10 billion, in USA about $ 4 million, in India about $ 1 billion and in other countries was $ 5.0 billion[6-7]. In 1997, the European market alone reached about $ 7.0 billion. The German market corresponds to about 50% of the European market, about $ 3.5 billion. This market is followed by France, $ 1.8 billion; Italy, $ 700 million the United Kingdom, $ 400 million; Spain, $ 300million; the Netherlands, about $ 100 million[8-10].

"Ayurveda", the traditional Indian system of medicine reports number of herbs for their purported benefits in treatment of hair loss. These medicaments however suffer from lack of standardization parameters and proper documentation based on scientific screening procedures[11].

Hair is one of the first things other people notice about us and is one of the primary ways we declare our identity to others. Both in our personal relationships and in relationships with the larger world, hair sends an immediate signal that conveys messages about our gender, age, social class, and more[12].

Numerous products or surgical procedures are promoted for the treatment of alopecia as well as improving scalp hair growth. A few of these include vitamins, amino-acids, scalp massage etc. but their efficacy remains dubious, and moreover side effects associated with them cannot be neglected. Herbal drugs therefore are viable alternative to synthetic drugs. Natural products have been quiet prevalent in hair care industry and nearly thousand kinds of plant extracts have been examined with respect to hair growth promoting activity, a few of them have shown tremendous potential as well polyherbal compounds are generally

employed as hair tonic, hair growth promoter, hair conditioner, hair cleansing agent, antidandruff agents, as well as for the treatment of alopecia and lice infection[13-14].

Recently, various plant extracts have been patented for use in hair growth or hair tonics products, and for prevention of alopecia. the patent claim that the effect are due to stimulation of the hair follicle or scalp metabolism, possibly due to acceleration of blood circulation, activation of dermal papillae, anti testosterone or increased nutrition to the hair follicles through accelerated blood flow but mechanism is not yet not clear[15-16].

Thus it were decided that, in present investigation, phytochemical screening of *Abrus precatorius* and evaluated for hair growth

MATERIALS AND METHODS

Plant Materials

Collection of Plants

Abrus precatorius Linn.(seeds) were purchased from well known supplier from local market, Indore (M.P.).

Authentification of Plants

The collected crude drugs rati, anantmool were identified and authenticated on the basis of macroscopic and microscopic characters at the Department of Botany, Government Agriculture College, Indore. and voucher specimens SCOPE/Phcog/ and SCOPE/Phcog/ have been deposited at the herbarium of the Department of Pharmacognosy of the College for further reference.

The experiments were carried out using air dried plant materials which were reduced to moderately coarse powder using mechanical grinder. The powders were then passed through sieve # 40 and stored in an air tight container for further use.

Preparation of Extracts

The powdered material was extracted by petroleum ether solvent using soxhlet apparatus. The extracts were filtered, concentrated on water bath, dried in vacuum and stored in refrigerator for subsequent experiments. Yield value was calculated for each herb.

Characterization of Extracts

The extracts were characterized by TLC (thin layer chromatographic) studies on silica gel-G plates benzene ethyl acetate (60:30v/v) as mobile phase gave best resolution for petroleum ether extract of both extracts after derivatization with anisaldehyde.

Pharmacognostic Studies

Macroscopy

Color, odor, taste and shape were determined by oraganoleptic evaluation.

Microscopy

The coarse powder was boiled with chloral hydrate to remove the coloring matter and then mounted on a glass slide using phloroglucinol, dilute hydrochloric acid and glycerin, covered with a cover slip. Then the powder was viewed under compound microscope and the microscopic characters of the powder were observed. The mount was made free from air bubbles to determine the types of cells, the nature of cell wall present and cell contents etc[17-19].

Physicochemical Studies

Ash Values

Ash values are useful in determining the quality and purity of the crude drugs, especially in the powdered form. The ash content of a crude drug is generally taken as the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may be also include inorganic matter added for the purpose of adulteration. Hence, ash value furnishes a basis for judging the identity and purity of the crude drug and gives information relative to its adulteration with inorganic matter[20].

Total Ash Value

3 gm of the air dried drug powder was placed in a tarred silica crucible and incinerated at a temperature not exceeding 450^{o}C until free from carbon, cooled and it was again weighed. The percentage of ash was calculated with reference to air dried drug.

Acid Insoluble Ash

The total ash was boiled with 25 ml of 2M HCl for 5 min. The insoluble matter was collected in an ash less filter paper, then it was washed with hot water, ignited and cooled in a desiccator and weighed and the percentage of acid insoluble ash was calculated with reference to air dried drug.

Water Soluble Ash

The total ash was boiled for five minutes with 25 ml of water and the insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temp not exceeding 450^{0}C. The weight of the insoluble matter was subtracted from the weight of the total ash and the difference in weight represents the water soluble ash. The percentage of water-soluble ash was calculated with reference to the air dried drug[21].

Extractive Values

Extractive values of crude drugs are useful for their evaluation. These values indicate the amount and nature of the constituents present in the crude drugs.

Water Soluble Extractive Value

5 gm of the air-dried, coarsely powdered drug was macerated with 100 ml of water in a closed flask for 24 hours, shaken frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered taking precautions against loss of water; 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105^{o}C and weighed. The percentage of water-soluble extractive with reference to the air dried drug was calculated[22].

Alcohol Soluble Extractive Value

5gm of the air dried, coarsely powdered drug was macerated with 100 ml of alcohol (95%v/v) and refluxed for 2 hrs. Then it was filtered and 25 ml of filtrate was transferred in a porcelain dish and evaporated to dryness on a water bath and dried completely in an oven at 105^{o}C and finally weighed. The percentage of alcohol-soluble extractive with reference to the air dried drug was calculated[23].

Loss on Drying

The zero % coincidence and 100 % were set by rotating the knob in such a way that the marked line, the pointer and the 100% mark, all coincided each other. Then scale was brought to zero and coincided with line marked on it. The lid cover was opened and the test material put in the pan, till the

pointer slide down and came to zero mark. The lid was closed and the temperature started by the thermostatically controlled knob. This knob was adjusted to get constant temperature and the material allowed drying. The pointer remained stable when the drying was over to constant weight and reading on scale was noted.

Fluorescence Analysis

In the near- violet region of the spectrum (3000–4000 Aº) some of the phytoconstituents show more or less brilliant colouration when exposed to radiation. This phenomenon of emitted visible wavelengths as a result of being excited by radiation of a different wavelength is known as fluorescence. Sometimes the amount of Ultra-violet light normally present with visible light is sufficient to produce the fluorescence, but more often a more powerful source of ultra-violet light is necessary, e.g. a mercury vapour lamp. It is often to make use of this phenomenon for the qualitative examination of herbal drugs[24].

Procedure: The powdered samples were exposed to Ultraviolet light at wavelength of 254nm and 366nm. 1mg of powdered drug was placed on a micro slide and observed under UV 366, UV 254 and in daylight to observe the fluorescent characteristics of the powder. One mg of the powdered drug was placed on a micro slide and treated with 1ml 1N HCl, 1N NaOH , 1N nitric acid, 1 ml Iodine water, 1ml ammonium solution and water separately and observed under UV 366, UV 254 and in daylight while wet[25].

Pharmacological Studies

Animals

Male Wistar rats (100-120g) were used in experiments. The animals were housed in polypropylene cages under standard conditions (12 h light; 12 h dark cycle; 25± 5^0 C; 35-60% humidity). They were fed with standard rat pellet diet (Pranav Agro Industries Ltd., Maharashtra, India) and water *ad libitum*. Experimental protocol was approved by the Institutional Animal Ethical Committee. Animal ethical norms were strictly followed during all experimental procedures.

Preparation of Animals

Approximately 24 hours before the study, fur was removed from the dorsal area of the trunk of the test animals by clipping or shaving. At least 10 % of the body surface clears for the application of the test substance.

Administration of Doses

The test substance was applied uniformly over an area which is approximately 10 % of the total body surface area. Test substance was held in contact with the skin with a porous gauze dressing and non-irritating tape throughout a 24-hour exposure period. The test site was further covered in a suitable manner to retain the gauze dressing and test substance and ensure that the animals cannot ingest the test substance.

Animals were observed immediately after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention gave during the first 4 hours and daily thereafter, for a total of 14 days.

After studies it was observed that the petroleum ether extracts of both extracts when applied in a concentration of up to 5% did not show any toxic side effects or erythema on skin surface. Thus, the prepared extracts were considered safe for topical administration.

Treatment

Animals were divided into 6 groups: Group I was applied no treatment and served as control, Group II was applied 2% petroleum ether extract of Abrus precatorius in liquid paraffin, Group III 5%. Petroleum ether extract of *Abrus precatorius* in liquid paraffin, Group IV served as positive control and applied alcoholic solution of minoxidil (Tugaine).

Application of Test Samples

Hairs on dorsal side of the animals were removed using marketed hair remover (Anne French) to assure complete denual of 4 sq cm area .Finally denuded skin was wiped of with surgical spirit. Equal quantity (1 ml) of prepared and 1 ml of standard minoxidil solution was applied to the denuded area of albino rats once a day for 21days[26].

Statistical Treatment

Data are reported as mean + SEM. Statistical analysis of data was carried out by one way ANOVA comparing all test groups versus control followed by Dunnet's test

Qualitative Hair Growth Study

Qualitative hair growth was evaluated by visual observation of two parameters: hair growth initiation time *i.e.* minimum time to initiate perceptible hair growth and hair growth completion time i.e. minimum time taken to cover the denuded skin region with new hair completely. The investigator was blinded about the treatments to various groups under investigation. Hair growth initiation and completion time was recorded for each group of animals and compared with positive control Tugaine (standard 5% ethanol solution of minoxidil) and control (with no treatment).

Quantitative Hair Growth Study

The method described by Uno was followed for the quantitative evaluation of drug extract. One rat from each group was authenticated after the 21 days of treatment, skin biopsies were taken from the shaved area, and specimen preserved in 10% formalin. The specimen was fixed on paraffin wax and blocks prepared for microtomy. After fixation, vertical sections of the skin were cut with the help of semiautomatic rotary microtome (Remi- 1871, India). The sections were stained with hematoxylin and eosin. The number of hair follicles per millimeter area of skin and ratio of hair follicles in different cyclic phases i.e. anagen (active growth phase) and telogen (resting phase) were determined using the microscope. Hair folliculogram was prepared by observing growth cycle of 100 hairs and length of hair follicle[28].

RESULTS AND DISCUSSION

Macroscopic Characteristics

The crude drugs were examined and following results were found.

Table 1: Macroscopic Characteristics of Crude Drugs.

S. N.	Characteristics	*Abrus precatorius*
1.	Colour	Scarlet with black patch
2.	Odour	Odourless
3.	Taste	bitter
4.	Shape	oval
5.	Size	5-8mm long &4-5mm broad
6.	Surface	Smooth

Physicochemical Characteristics

Table 2: Results of Physicochemical Parameters of *Abrus precatorius* Seeds

S. No.	Physicochemical Parameters	Result (%w/w) of *Abrus precatorius*
1.	Total ash	2%w/w
2.	Acid insoluble ash	1.3%w/w
3.	Water soluble ash	2%w/w
4.	Loss on drying	2% w/w
5.	Water soluble extractive value	25%
6.	Alcohol soluble extractive value	19%

Table 3: Florescence Analysis of Powder of Seeds of *Abrus precatorius*

Powdered drugs	Visible /daylight	Short UV 254nm	Long UV 365nm
Powder as such	Yellowish brown	Greenish yellow	Black
Ammonia Solution+ powder	Yellow	Dark green	Dark blue
Iodine solution +powder	Yellow	Dark green	Dark blue
1N NaOH+ powder	Yellow	Light green	Black
conc.HCL+ powder	Yellow	Fluorescence	Dark blue
Distilled water+ powder	Yellow	Greenish yellow	Black

Preliminary Phytochemical Screening

The petroleum ether extracts of *Abrus precatorius seeds* were subjected to phytochemical tests to detect the presence of phytosterol as described earlier in methodology and the results obtained, are shown in Table 4.

Table 4: Results of Preliminary Phytochemical Screening of Extracts of *Abrus precatorius* seeds.

Tests for Phytosterols	Petroleum ether extract of *Abrus precatorius*
Salkowski reaction	+
Liebermann Burchard reaction	+
Liebermann reaction	+

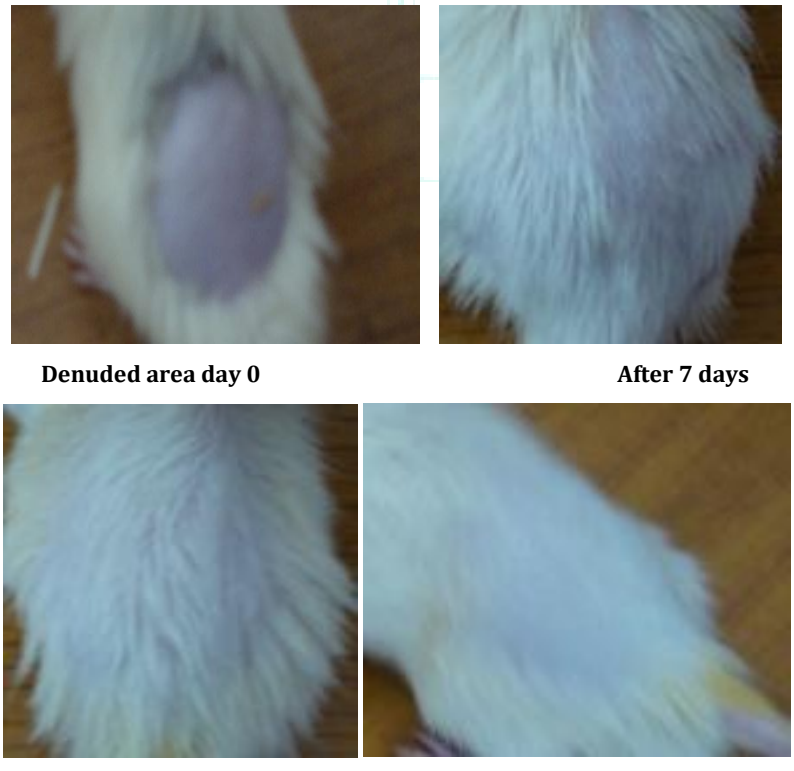
(+): Present, (-): Absent.

Pharmacological Activity

All experimental data are expressed as mean ± SEM. Statistical analysis were carried out by using one way ANOVA using Dunnett's t-test.

Result of Acute Dermal Toxicity Studies

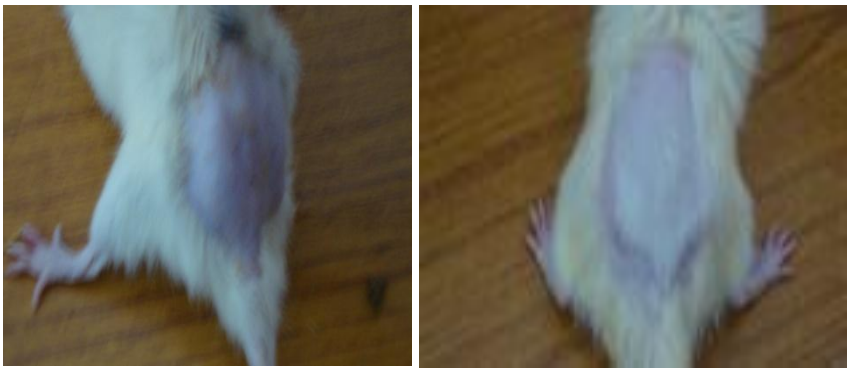
Petroleum ether extracts were applied on the surface of denuded skin for 14 days at 5% concentration and observation made for skin rashes, inflammation, or allergic reactions thus, the prepared extracts were safe for topical administration. Permission from the institutional ethical committee was obtained for animal experimentation.



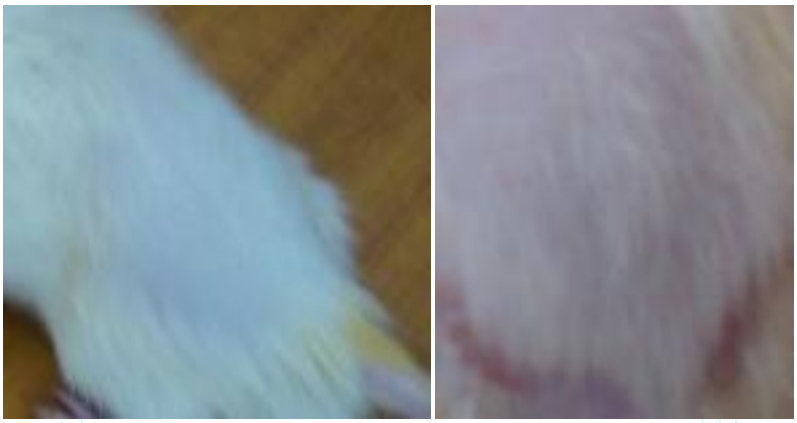
Denuded area day 0 **After 7 days**

After 14 days **After 21 days**

Figure 1: *In vivo* hair growth study showing hair growth initiation day photographs of rats treated petroleum ether extracts of *Abrus precatorius* on 0 ,7 ,14,21day.



Denuded area on 0 day After 7 day



After 14 day After 21 day

Figure 2: In vivo hair growth study showing hair growth initiation day photographs of rats treated petroleum ether extracts of *Hemidesmus indicus* on 0, 7, 14, 21day.

Hair Growth Promoting Activity

Table 5: Effect of *Abrus precatorius Linn.* extracts on Qualitative hair growth

S. N.	Treatment	Hair growth initiation time (days)	Hair growth completion time (days)
1	Control	10±0.70	17±0.44
2	Standard	6±0.70	10±0.44
3	petroleum ether extract(2% A*brus precatorius)*	6±0.44	10±0.44
4	petroleum ether extract(5% A*brus precatorius)*	5±1.54	8±0.54

Table 6: Study of %population of hair follicles in *Abrus precatorius*

S. N.	Groups	% Population of Hair Follicles		
		Anagen	Catagen	Telogen
1	Control	29	6	65
2	Standard	65	3	32
3	Petroleum ether extract of Abrus precatorius2%	61	4	35
4	Petroleum ether extract of Abrus precatorius 5%	71	2	27

DISCUSSION

The plant *Abrus precatorius* belonging to the family Fabaceae were studied for its pharmacognostical, physicochemical, phytochemical studies and hair growth promoting activity. Pharmacognostical studies of plant namely: Macroscopy, Microscopy is valuable source of information and provide suitable standards for the authentification of this plant material for future investigation.

Qualitative Studies on Hair Growth

Hair growth initiation and completion time was significantly reduced upon treatment with petroleum ether extract of *A .precatorius*. In control group animals, hair growth was initiated in denuded area in the second week, whereas it was noted in the first week in both the petroleum ether extracts treated groups and minoxidil treated groups. Hair growth was initiated on 5th &6th day with 5% of petroleum ether extracts of *A .precatorius & H. indicus* respectively. Hair growth initiation was recorded on 7th day with 2% solution of *A. precatorius* same, it was with minoxidil treated control group.

The time taken for complete hair growth on shaved area was also affected with petroleum ether extract of. *A .precatorius & H.indicus* Complete hair growth was observed on the 10th day with 2% and 7th day with 5% petroleum ether extract treatment in case of *A.precatorius* on 19th day with minoxidil treatment. In, other extract and control treated group

animals complete hair growth was noted after17th &18th days respectively.

It was further observed that in petroleum ether extract treated and minoxidil 5%treated group the hairs appeared soft and silky on touching. The results clearly suggest that the extract was successful in reducing the time taken for hair growth initiation and completion.

Quantitative Studies on Hair Growth

Cyclic phase of hair growth was markedly affected in groups treated with minoxidil and petroleum ether extract of *A. precatorius.* In vehicle control group animals most of the hair follicles were in telogenic phase, only one or two are in catagenic phase. I. There is a complete reversal of picture in petroleum ether extract and minoxidil treated group where majority of hair follicles are in anagenic phase with only few catagenic hair follicles. Telogenic hair follicles are almost negligible.

Appreciable increase was noted in anagenic population A low anagenic population was found in vehicle control group, whereas with 2% petroleum ether extract of *A. precatorius* it was 61 and with 5% petroleum ether extract 71hair follicles were found in anagenic phase. With minoxidil treatment, 65% hair follicles were observed in anagenic phase.

Length of Hair Follicles

The treatment with 2 and 5% petroleum ether extract of *A. precatorius* had a remarkable effect on length of hair follicle. In control group only 29% had average length of 0.5 mm, whereas in extract treated groups 61% and 71%hair population with more than 0.5 mm was observed with 2 and 5% petroleum ether extract of *A. precatorius* treatment. The results of treatment were comparable with minoxidil group where 65%hair population had length of 0.5 mm and above.

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