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Potentiality of hair growth promoting activity of aqueous extract of *Abrus precatorius* Linn. on Wistar albino rats

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Abstract

Abrus precatorius Linn. popularly known as Crab's eye is a slender, woody perennial climber reported to have anti-oxidant, antibacterial, cytotoxic, anti-diabetic, anti-tubercular and anti-plasmodial activities. The present investigation was carried out to evaluate the hair growth promoting potentiality of aqueous extract of *Abrus precatorius* leaf. Preliminary chemical tests and TLC analysis revealed the presence flavonoids and saponins. Hair growth promoting activity of aqueous extract 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 aqueous extract of *Abrus precatorius* showed a very good hair growth promoting activity at a dose of 300mg/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. A remarkable change in the mineral concentration in blood was not observed for treated group of animals. From the present investigation it was confirmed that *Abrus precatorius* is potent hair growth promoter and can be suggested to be an effective alternative to synthetic hair growth promoters.

Keywords: *Abrus precatorius* Linn, anagen, catagen, telogen, minoxidil

1. Introduction

Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe to treat various kinds of ailments in place of mainstream medicine [1]. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Hair growth is common biological process observed in animals and human beings. Hair on scalp grows about 15 cm or 6 inches every year. The hair fiber is

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composed of a cuticle that is continuous with the root sheath, an intermediate cortex, and an inner medulla. The hair growth and loss is completely random and is not seasonal or cyclic. At any given time, a random number of hairs will be seen in various stages of growth and shedding. There are four stages of hair growth. The stages include anagen (growth phase), catagen (transitional phase), telogen (resting phase) and exogen (shedding phase). Anagen is the active growth phase of hair follicles.

The cells in the root of the hair are dividing rapidly, adding to the hair shaft. During this phase the hair grows about 1 cm every 28 days. The catagen phase is a short transition stage that occurs at the end of the anagen phase. It signals the end of the active growth of a hair. This phase lasts for about 2–3 weeks while a club hair is formed. The telogen phase is the resting phase of the hair follicle.

The club hair is the final product of a hair follicle in the telogen stage, and is a dead, fully keratinized hair. Fifty to one-hundred club hairs are shed daily from a normal scalp [2]. A number of things like illness or a major surgery, hormonal problems, pregnancy, anticoagulants, medicines used for gout, high blood pressure or heart problems, excess of vitamin A, birth control pills and antidepressants, fungal infections may cause hair fall [3, 4].

The main problems associated with hair are pigmentation problems (Fading), dandruff and falling of hair [5]. As such, nutrition has a profound effect on both its quality and quantity. Poor nutrition may produce, and therefore be reflected by, a dull, dry, brittle, or thin hair coat. Pigmentary disturbances may also occur. Nutritional factors that influence hair growth are very complex and can be interrelated [4, 5]. Synthetic drug, minoxidil is a potent vasodilator appears unsafe for long term treatment. It has been reported that after 5 years use of 2% and 3 % topical minoxidil, the improvement has been shown to peak in one year with a slow decline in regrowth over subsequent years. Long term treatment with local side effects may be a problem with continuing use of minoxidil lotion. Potential hairgrowth property is exhibited by the plants possessing antiandrogenic activity (testosterone, 5-alpha reductase inhibition) and antioxidant activity produced by flavonoids like proanthrocyanidines [6]. *Abrus precatorius* have been used for hair growth promotion in Ayurvedic and Tribal medicines.

Abrus precatorius Linn. commonly called as Rosary pea belongs to family Fabaceae. Seeds are bright scarlet-red in color with a large black spot (Fig 1). Seeds are poisonous and contain a toxin called abrin which is protein by nature. Abraline, Abrasine, Abricin, Abrusgenic-acid, Abrusgenic-acid-methyl-ester, Abruslactone, Abrussic-acid, Anthocyanins, Calcium, Campesterol,Choline, Cycloartenol, Delphinidin, Gallic-acid, Glycyrrhizin, Hypaphorine, N,n-dimethyl-tryptophan, N,n-dimethyl-tryptophan-metho-cation-methyl-ester, P-coumaroylgalloyl-glucodelphinidin,Pectin, Pentosans, Phosphorus, Picatorine, Polygalacturonic-acids, Precasine, Precatorine and Protein Trigonelline are determined in the plant.It contains abruquinones B, G, D, E, and F, flavonol glycoside and triterpenoid saponins. In the Ayurvedic medicine, leaves are used as laxative, expectorant, hair growth and aphrodisiac medicines. Other uses of the plant in Ayurveda include acute colic and ephemeral fever (root), skin allergy and diarrhoea (leaves, root), fowl pox, haematuria, colic and wounds (leaves) [7-12]. The present study was undertaken to evaluate the hair growth promoting potentiality of the crude extract of *Abrus precatorius* leaf .

Fig 1: *Abrus precatorius* Linn

2. Materials and Methods

2.1 Plant material

The fresh plant was collected from the month of November to February from the surrounding areas of Nalgonda District, A.P, India. The plant material was authenticated by Mr.Lakshma Reddy, Retd. Professor in Botany Nagarjuna Government College. (Affiliated To Osmania University) Nalgonda. A herbarium was prepared and deposited in the department of Pharmacognosy, Nalanda College of Pharmacy for future references. The plant was identified and certified as Abrus precatorius, Family - Fabaceae, under the voucher no: NCOP-NLG/ ph'cog/2009-10/001.

2.2. Equipments and Chemicals used

Rotary vacuum evaporator (Indosatt Scientific lab Equipments), Dhona electronic balance (Dhona instrument. Ltd/Model no. Dhona 200D, Kolkata), UV-VIS Spectrophotometer (Elico Ltd/ Model no. SL 196, Hyderabad). All the drugs, chemical and reagents used in this study were of analytical grade. Commercially available 2% minoxidil (Dr. Reddy's Laboratory)was used.

2.3. Preparation of plant extracts

The plant material collected was cleaned, shade dried and powdered. Leaf powder weighing 250 g was defatted with petroleum ether and then exhaustively extracted with water at 60°C to obtain the crude aqueous extract. It was then concentrated under reduced pressure at 40°C in a rotary vacuum evaporator to obtain a concentrated mass.

2.4. Preliminary phytochemical screening

The crude extract thus obtained was characterized by preliminary chemical tests and thin layer chromatographic analysis. The TLC analysis was performed on precoated silica gelGF 254 plates using solvent systems (a) Chloroform: Methanol: Water (50 : 30 : 10) and (b) Ethyl acetate: Formic acid: Glacial acetic acid: Water (100:11: 11: 23) which showed the best resolution. The spots were visualized using UV chamber [13-15].

2.5. Experimental animals

Wistar Albino Rats of either sex were procured from National Institute of Nutrition, Hyderabad, A.P, India.The experimental protocol was initially approved from the Institutes animal ethics committee under the reference no. NCOP/IAEC/approval/07/2010 and then experimental studies were undergone according to their rules and regulations. The animals were housed under standard environmental conditions and had free access to standard pellet diet (Goldmohar brand, Lipton India Ltd.) and water *ad libitum*.

2.6. Hair growth promoting activity [6,16-21]

Wistar albino rats of either sex weighing 150 - 250g were taken. They were caged at a temperature of 21 ± 0.5°C and provided with food and water *ad libitum*. The hair on dorsal portion was clipped with scissors and removed with marketed hair removing cream Veet Reckitt Benckise (India) Ltd. in an area of $3cm^2$

diameter. The animals were divided into four groups with six animals in each group. Group I was applied water (negative control). Group II and III were applied 150 mg/kg and 300 mg/kg of test drug (aqueous extract of *A. precatorius* leaf). Group IV was applied 2% minoxidil (positive control). The aqueous extract made into a paste and was applied twice daily for 30 days on depilated area. The qualitative and quantitative evaluations were performed to determine the hair growth promotion. Additional parameters observed were effect of aqueous extracts on hair length of albino rats, effect of aqueous extracts on hair growth and change in concentration of minerals in blood during hair growth.

2.6.1. Effect of aqueous extracts on hair growth

The hair growth in the depilated area was observed and the time taken to cover the bald patch was noted.

2.6.2. Qualitative observation of hair growth

Qualitative hair growth analysis was undertaken by visual observation of two parameters: hair growth initiation time (i.e., minimum time to initiate hair growth on depilated skin region) and hair growth completion time (i.e., minimum time taken to completely cover the depilated skin region with new hair).

2.6.3. Quantitative observation of hair growth

Quantitative observation of hair growth was performed by observing the hair follicles under the microscope for the respective stage of hair growth. The shape and condition of the follicle was observed to determine the stage of hair growth. Anagen and telogen phases were considered and the percentages of follicles in both stages were accounted.

2.6.4. Effect of aqueous extracts on hair length of albino rats

Average length of hair was calculated by plucking 25 hairs randomly from the depilated area using a sterile forceps. The length of hair was noted for every ten days. The completion of hair growth was considered based on the average length of hair.

2.6.5. Change in concentration of minerals during hair growth

The animals were anaesthetized using ketamine (intra-muscular) and blood samples were collected from retro orbital plexus and diagnosed for concentration of iron, zinc and total protein which aid hair growth.

2.7. Statistical analysis

The statistical calculations were performed using the soft ware Graph Pad Instat. The difference between the groups was compared by One way analysis of variance (ANOVA) followed by Dunnett multiple comparison test (Test Vs Control).Values were expressed as mean ± S.D, where n=6,*P<0.05 considered statistically significant.

3. Results

The concentrated extract by Soxhlet extraction was found to be a sticky greenish brown mass with a percentage yield 16.7 %w/w. Preliminary chemical tests revealed the presence of carbohydrates, flavonoids, alkaloids and saponins. Thin layer chromatographic analysis exhibited five spots with the Rf values of 0.18, 0.65, 0.71, 0.84, 0.94 for the solvent system (a) another five spots with the Rf values 0.26, 0.30, 0.39, 0.54, 0.76 for the solvent system (b).These results indicated the presence of flavonoids and saponins.

The test animals did not show erthyema or edema with the hair removing cream applied on their body (Fig 2). The effect of aqueous extract on the depilated area exhibited a better performance as compared to the standard minoxidil. As the treatment proceeded it was observed that hair growth was facilitated from the periphery and

depilated circumference reduced. But since there was substantial variants in this refoliation a suitable alternative to find the surface area of the depilated region was required. Hence a tracing paper was used to trace out the circumference of the bald patch. This was over casted on a graph paper and the total area was calculated. It was observed that bald patch was completely covered on the 8th day for the aqueous extract with a dose of 300 mg/kg and standard drug (Table 1). The control animals showed poor hair growth (Fig 3). Initiation of hair growth time wassame for aqueous extract with a dose of 150 mg/kg and 300 mg/kg and the standard minoxidil. Time taken for hair growth completion reduced considerably for aqueous extract 300 mg/kg and standard minoxidil (time taken for complete covering of bald patch and attain the length of hair before the experiment was carried) which was noted to be 16 and 17 days for respectively (Table 2). The control treated group of animals and the test concentration of 150 mg/kg treated group of animals took 13 and 10 days respectively to completely cover the bald patch. A significance difference was found in the cyclic phase on the 20th and 30th day of observation for the aqueous extract 300 mg/kg and standard minoxidil. In the control treated group most of the hair follicles were in the telogenic phase whereas the treated groups majority of the hair follicles were in the anagenic phase. A prominent and substantial increase in the percentage of hair follicles on 20th day and 30th day were found for aqueous extract at 300 mg/kg and standard 2% minoxidil (Table 3). A remarkable change in the length of hair was observed for the treated groups and the standard minoxidil. The control treated group of animals did not show a prominent action. On the day 10 and 20 the length of hair for test treated animals showed a better response than minoxidil (Table 4), and on the 30th day hair length was found to be similar for test and standard treated animals. Hence the hair lengthening properties were found to be similar for the test and the standard drug. There were no marked changes in the mineral levels for test and standrad treated groups which suggest that these compounds do not alter the mineral contents present in the blood (Table 5).

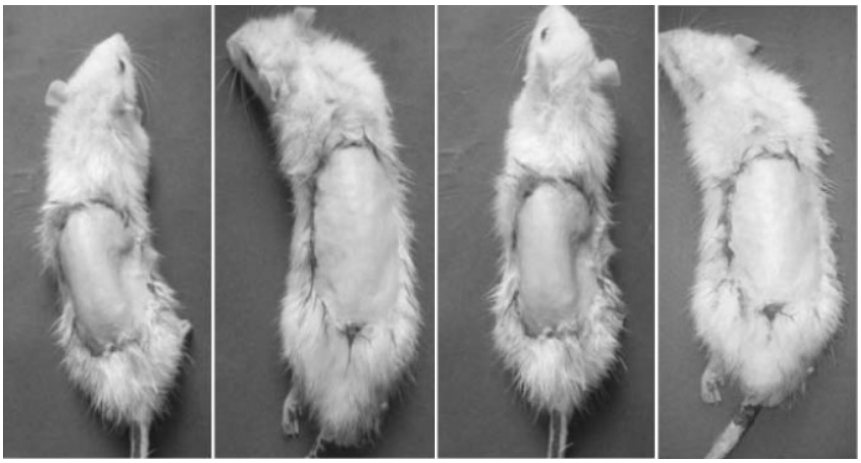

Fig 2: Wistar albino rats on 1st day
A-Control(water), B- Test(150 mg/kg A.P), C-Test(300 mg/kg A.P), D- Standard(2%minoxidil)
A.P=*Abrus precatorius*

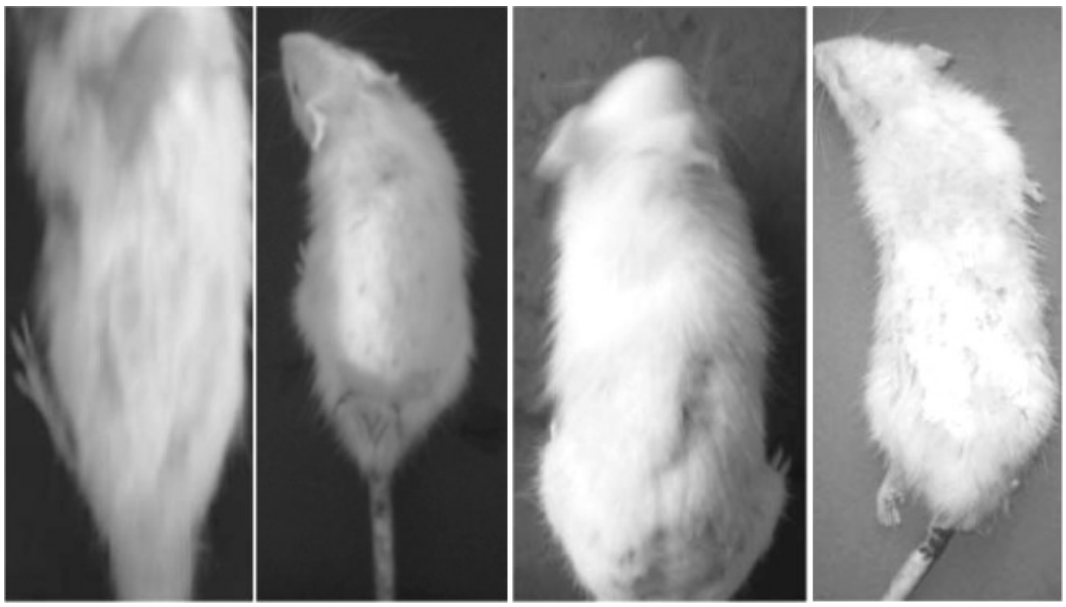

Fig 3: Wistar albino rats on 5th day
A-Control(water), B- Test(150mg/kg A.P), C-Test(300 mg/kg A.P), D- Standard(2%minoxidil)
A.P=*Abrus precatorius*

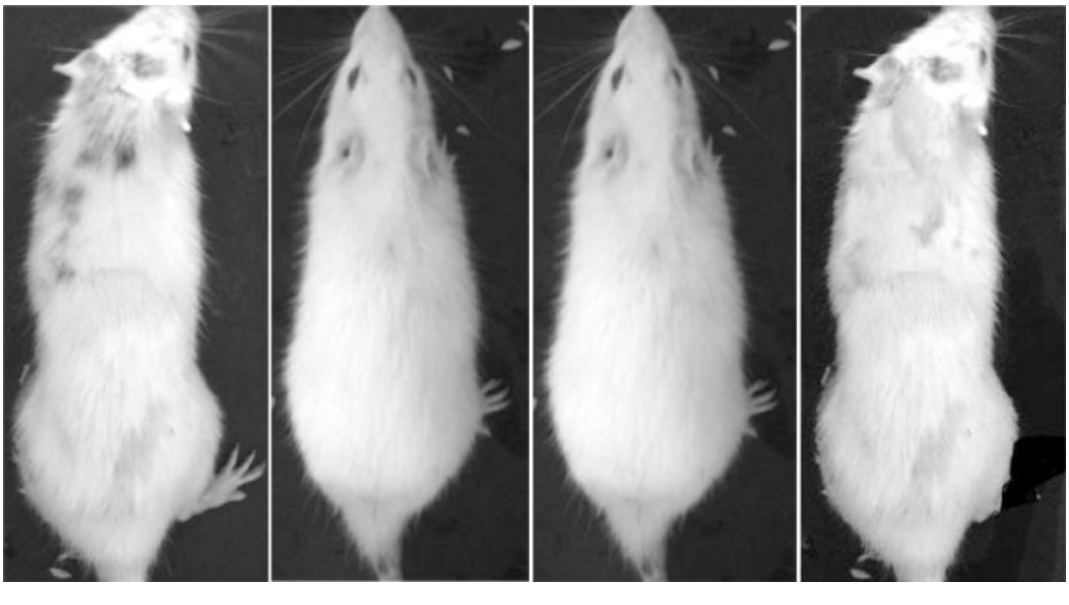

Fig 4: Wistar albino rats on 8th day
A-Control(water), B- Test(150mg/kg A.P),C-Test(300 mg/kg A.P),D- Standard(2%minoxidil)
A.P=*Abrus precatorius*

Table 1: Effects of aqueous extract of *A.precatorius* on hair growth

Treatment group	Dose	Number of days							
		0	1	2	3	4	5	6	7	8
		Diameter of shaved area(cm)								
Group I	2ml	3±0	3±0	3±0	3±0	2.7±0.10*	2.1±0.1*	1.6±0.14*	1.0±0*	0.6±0.16*
Group II	150 mg/kg	3±0	3±0	2.7±0.11*	2.4±0.10*	1.9±0.13*	1.4±0.16*	0.9±0.11*	0.5±0.09*	0.3±0.12*
Group III	300 mg/kg	3±0	2.8±0.16*	2.5±0.09*	2.2±0.09*	1.9±0.07*	1.5±0.11*	0.9±0.12*	0.4±0.13*	0±0
Group IV	2%	3±0	2.8±0.12*	2.7±0.16*	2.5±0.15*	1.9±0.11*	1.3±0.13*	1.0±0.11*	0.3±0.11*	0±0

Group I-Control(water);Group II-aqueous extract of *Abrus precatorius* 150mg/kg; Group III- aqueous extract of *Abrus precatorius* 300mg/kg; Group IV-Standard(2% Minoxidil). Results are expressed as mean ± SD, n=6. *P<0.05, considered significant by Dunnett Multiple comparison Test(Test Vs control).

Table 2: Qualitative observation of hair growth

Treatment group	Dose	Number of days taken to initiate hair growth	Number of days taken to complete hair growth
Group I	2ml	3	23
Group II	150 mg/kg	2	21
Group III	300 mg/kg	2	17
Group IV	2%	2	16

Group I-Control(water);Group II-aqueous extract of *Abrus precatorius* 150mg/kg; Group III- aqueous extract of *Abrus precatorius* 300mg/kg; Group IV-Standard(2% Minoxidil).

Table 3: Quantitative observation of hair growth

Treatment group	Dose	Percentage of hair follicles					
		Day 10		Day 20		Day 30	
		Anagen	Telogen	Anagen	Telogen	Anagen	Telogen
Group I	2 ml	30	70	51	49	55	45
Group II	150 mg/kg	33	66	59	40	60	38
Group III	300 mg/kg	36	64	67	33	68	32
Group IV	2%	48	52	68	32	69	31

Group I - Control(water); Group II - aqueous extract of *Abrus precatorius* 150 mg/kg; Group III - aqueous extract of *Abrus precatorius* 300 mg/kg; Group IV - Standard (2% Minoxidil).

Table 4: Effect of aqueous extracts on hair length of albino rats

Treatment group	Dose	Length of hair (mm)avg. of 25 hairs		
		Day 10	Day 20	Day 30
Group I	2 ml	7.83±0.052	18.68± 0.055	20.51±0.061
Group II	150 mg/kg	9.23±0.81*	19.2±0.053*	21.70± 0.046*
Group III	300 mg/kg	9.70±0.06*	19.94± 0.050*	21.53±0.043*
Group IV	2%	9.48±0.057*	18.97± 0.038*	22.45±0.024*

Group I-Control(water); Group II-aqueous extract of *Abrus precatorius* 150 mg/kg; Group III- aqueous extract of *Abrus precatorius* 300 mg/kg; Group IV-Standard(2% Minoxidil). Results are expressed as mean ± SD, n=6.*P<0.05,considered significant by Dunnett Multiple comparison Test (Test Vs control)

Table 5 : Change in concentration of minerals

Treatment group	Dose	Minerals	Mineral Conc. in blood		
			Day 0	Day 15	Day 30
Group I	2ml	Iron µg/dL	63.0	63.5	63.9
		Zinc µmol/L	84.4	84.9	85.3
		Protein total g/dL	6.2	6.43	6.72
Group II	150 mg/kg	Iron µg/dL	64.0	65.5	66.8
		Zinc µmol/L	84.6	85.3	85.7
		Protein total g/dL	6.3	6.58	6.85
Group III	300 mg/kg	Iron µg/dL	65.7	66.7	67.4
		Zinc µmol/L	85.6	85.9	86.1
		Protein total g/dL	6.58	6.89	7.01
Group IV	2%	Iron µg/dL	58.1	58.9	59.1
		Zinc µmol/L	84.3	84.6	84.8
		Protein total g/dL	6.0	6.6	6.9

Group I-Control(water); Group II-aqueous extract of *Abrus precatorius* 150 mg/kg; Group III- aqueous extract of *Abrus precatorius* 300 mg/kg; Group IV-Standard (2% Minoxidil).

4. Discussion

Preclinical screening of aqueous extract of *Abrus precatorius* suggest that the plant is a potent hair growth promoter as compared to 2% minoxidil. The effect of aqueous extract on hair growth promotion exhibited a promising activity in covering bald patch of treated animals.The activity was found similar to that of standard treated group. Since androgens (metabolite product of testosterone by 5-alpha reductase enzyme) have been implicated for the development of common baldness and alopecia, research also, has been conducted in search of natural compound having antiandrogenic activity or testosterone 5-alpha reductase inhibition. Hence it can be assumed that the phytoconstituents in *Abrus precatorius* also may posses the similar mechanism. The bald patch disappeared in 8 days and the area was totally covered with hair for standard 2% minoxidil and *Abrus precatorius* 300 mg/kg treated group of animals.

Treatment given to the depelited skin of Wistar albino rats with crude extracts and standard minoxidil had exhibited a pronounced effect on hair growth initiation and completion.The result obtained showed that aqueous extract of*Abrus precatorius* at a dose of 300 mg/kg reduced the time taken for hair growth initiation and completion.

The aqueous extract at 300 mg/kg converted hair follicles in telogen phase hair to anagen phase which indicated follicle stimulatng property and can be used for non-androgenic alopecia. It has been reported that some of the phytoconstituents aid in the promotion of hair growth. Few examples are proanthocyanidins, 3, 4 dimethyl 3-hydroxy flavanone, ginsenoside from red ginseng, senegin II, III and senega saponin B[6]. Among these, proanthrocyanidins have been investigated to larger extent for hair growth property. As per the literature review it was found that *Abrus precatorius* is rich in anthocyanins, gallic acid,saponins and flavonols. The mechanism of action reported for the proanthocyanins is that it converts the telogen (non growing phase of hair growth) into anagen (growing phase of hair growth) [6]. The mechanism of flavonoids for increased hair growth may involve enhanced microcirculation around hair follicles or direct stimulation of resting hair follicles. These phytochemicals selectively inhibit protein kinase C intensively and promote hair epithelial cell proliferation *in vitro* and increase anagen production *in vivo* [6]. As per the chemical test and TLC profiles presence of flavonoids and saponins were determined it may be suggested that these constituents may be responsible for the follicle stimulating property.

The aqueous leaf extract of *Abrus precatorius* produced a greater effect on the length of hair which was comparable to the standard minoxdil. This may be due to the premature switching of follicles from telogen to anagen phase [22].

In terms of healthy hair growth, the most important minerals and vitamins are Iron (and the blood iron carrier ferritin), Zinc, Copper, Selenium, Biotin (vitamin H), Vitamin B6 (pyridoxine), Vitamin B12 (Cobalamin) [23]. Hair follicles are metabolically active tissues that require nutrients to support both structural and functional activities [24]. They comprise dietary deficiencies of protein, phosphorus, iodine, zinc, and vitamins A and E, as well as dietary excesses of selenium, iodine, and vitamin A. Other possible nutritional imbalances that can affect hair growth include B-vitamin and vitamin C deficiencies, copper and cobalt deficiencies, and molybdenum toxicosis [25]. The results obtained for the changes in the mineral concentrations in the blood for the test treated group of animals revealed no significant changes as compared to control and standard treated group of animals.

The concentration of iron, zinc and protein levels were almost similar for the test and control treated animals.

The overall results obtained for the current research investigation shows that *Abrus precatorius* is a potent hair growth promoter. The qualitative and quantitative parameters evaluated enlighten the potency of the plant as a promising solution for the treatment of hair fall. As no marked changes in the mineral concentrations were observed it shows that the plant does not alter the mineral concentrations in the blood. Hence it can be concluded that as the synthetic hair growth promoters available in the market are reported to have side effects, it can be strongly suggested that *Abrus precatorius* which has the potential components to stimulate hair growth can be an effective alternative. The plant could also be screened for alopecia, male pattern baldness as it showed follicle stimulating action.

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Reference

1. Devi VK, Jain N, Valli KS. (2010) *Phcog Rev*. 4: 27-31.

2. Paus, Ralf, Cotsarelis, George. (1999) *The New England Journal of Medicine*. 341(7): 491-497.

3. http://brucebud1.over-blog.com/article-causes-and-home-remedies-for-hair-problems-68792604. html.

4. Mark Dunnett, the diagnostic potential of equine Hair: a comparative review of hair Analysis for assessing nutritional Status, environmental poisoning, and Drug use and abuse. www.ker.com/library/advances/309.pdf 85-106

5. Awe EO, Makinde JM. (2009) *Journal of Natural Products*.2:70-73.

6. Rudrappa Nandeesh, Bagepalli Srinivasa Ashok Kumar, Kuruba Lakshman, Saleemulla Khan, *et al.* (2008) *Iran J Basic Med Sci*.11(4): 236-241 .

7. Nadkani KM.(2002) *Indian Material Medica*, Popular Prakashan, Mumbai; 1235-1236.

8. Kritikar KR, Basu BD. (2006) *Indian medicinal plants*, International book distributors, New Delhi; 1112-1114.

9. Deepak Acharya, Anshu Shrivastava, Sanjay Pawar and Garima Sancheti, The medicinal value of Indian herb *Abrus precatorius L.* used in herbal medicines inIndia, http://www.disabledworld.com/medical/alternativeherbal/*abrusprecatorius*.php #ixzz0vbbnCgCI. Retrieved on 18/3/2010.

10. *Indian medicinal plants compendium of 500 species*. (2002) Orient Longman Pvt. Ltd. Chennai ;320-322.

11. Madhava KC, Sivaji K, Tulasi KR. (2008) *Flowering Plants of Chitoor Dist A.P. India*, Students Offset Printers, Tirupati; 141.

12. Khare CP. (2007) *Indian medicinal plants and illustrated Dictionary*, Springer Science publishers: New Delhi; 671-672.

13. Kokate CK. (2008) *Practical Pharmacognosy*, Vallabh Vrakashan: Delhi; 149-156.

14. Kokate CK, Purohit AP, Gokhale SB. (2006) *Pharmacognosy,* Nirali Prakashan: Pune; 593-597.

16. Roy RK, Thakur M, Dixit VK. (2007) *J Cosmet Dermatol*. 6(2):108-12.

17. Thorat RM, Jadhav VM, Kadam VJ. (2009) *International journal of pharmtech research*. 1(4): 1251-1254.

18. Vaishali R, Jagdish Chandra R, Sengodan T. (2009) *Pharmacognosy Research*.1(4): 234-237.

19. Suraj R, Rejithaa G, Anbu Jeba Sunilsona B, K Anandarajagopala,P Promwichita. (2009) *Biology and Medicine*. 1 (4): 34-38.

20. Awe EO, Makinde JM. (2009) *Journal of Natural Products*. l: 270-73.

21. Semalty M, Semalty A, Geeta P. Joshi, Rawat MSM. (2010) Int. J. *Pharmacol*. 6: 53-57.

22. Adhirajan N, Ravi Kumar T, Shanmugasundaram N, Mary Babu. (2003) *J. Ethnopharmacology*. 88: 235-239

23. Ma PB. (2002) *Di Yi Jun Yi Da Xue Xue Bao*.22(5):421-2.

24. Galbraith, H. (1998) Proc. Nutr. Soc. 57: 195-205.

25. Scott, D.W. 1988. Large Animal Dermatology. W B Saunders, Philadelphia; 306-311.