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Hair growth activity of *Cicer arietinum* Linn. *Ocimum sanctum* Linn and *Cyperus rotundus* Linn in Albino Rats

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Abstract
The ethanolic extract of *Cicer arietinum* Linn, Fabaceae, *Ocimum sanctum* Linn. Lamiaceae and *Cyperus rotundus* Linn. Cyperaceae were evaluated for their effect on hair growth in albino rats. The extract incorporated into cream were applied topically on shaved skin of rats and primary skin irritation test, hair growth initiation time, completion time, hair length and diameter were recorded.

Keywords: Alopecia, hair growth

1. Introduction
Alopecia is a universal problem, having affected both sexes of all races to different extents for as long as mankind has existed. It has been suggested that alopecia could have an adverse effect on physiological life and self-esteem between both the genders [1]. Alopecia effects approximately 50% of men over 40 years of age and may also affect just as many as women. The majority of men and women (90%) or more want to reverse, halt hair loss. Alopecia is a synonym of baldness, involves absence or loss of hair, especially of the head. Androgens are well known to cause regression and balding on the scalp in genetically disposed individuals. Alopecia has also been observed as major side effect of anticancer drugs, immunosuppressant and many other drug treatments. Minoxidil, a drug of scientific origin was scientifically proved for the treatment of alopecia [2]. Though the side effect associated with this drug has limited its pharmacological benefits hence the drug of plant origin is necessary to replace the synthetic one. India is a repository of medicinal plants [3-4]. Besides healthcare, herbs are also used for beautification of the body and for preparation of various cosmetics [5]. In traditional system of medicine, many plants and herbal formulations are reported for hair growth promotion [6-10] but lack of sound scientific backing and information limits their use.
The present study is an effort to evaluate hair growth promoting activity of *Cicer arietinum* Linn., *Ocimum sanctum* Linn and *Cyperus rotundus* Linn. The herbs *Cicer arietinum* Linn and *Cyperus rotundus* Linn were selected on the basis of their traditional use [11-12] and *Ocimum sanctum* Linn was selected based on its anti-androgenic property [13].

2. Material and Method
2.1 Plant material
The leaves of *Cicer arietinum* Linn, whole plant of *Ocimum sanctum* Linn and roots of *Cyperus rotundus* Linn were collected in the month of October locally from Bhopal. The plants were authenticated at Department of Pharmacy, Barkatullah University, Bhopal. The plants were dried under shade.

2.2 Extraction
Dried powered drug were taken and maceration was done by keeping them in 95% alcohol for 7 days with occasional stirring. After filtration, double maceration was done for next three days with 95% alcohol. The solvent was removed under reduced pressure and the extract obtained was air dried.

2.3 Preparation of test sample
Herbal hair creams were prepared by fusion method using o/w type base [14]. The formula of base contains glyceryl mono stearate 9% w/w, liquid paraffin (light) 20% w/w, cetyl alcohol 15% w/w, beeswax 15% w/w, propyl and methyl paraben 0.15% w/w, glycerol 4.5% w/w and water 59% w/w. The 5% extract mixture of *Cicer arietinum* Linn., *Ocimum sanctum* Linn and *Cyperus rotundus* Linn were incorporated in the base and named E_1, E_2 and E_3 respectively.

2.4 Animals
Albino rats, weighing 120- 150 g were used for hair growth activity. The study was approved by Institutional Animal Ethical Committee, Barkatullaha University, Bhopal. Animals were placed in cages and kept in standard environmental conditions, fed with standard diet *ad libitum* and allowed free access to drinking water. The prepared extracts were assessed for hair growth studies.

2.5 Primary skin irritation test
The rats were divided into five groups of six rats each. A 4cm^2 area of dorsal portion of all the rats were shaved and wiped with surgical spirit. Measured quantity of extract incorporated in cream E_1, E_2, and E_3 were applied over the site. The test sites were observed for erythema and edema for 48 h after application [15].

2.6 Hair growth activity
The rats were divided into five groups of six rats each. A 4 cm^2 area of dorsal portion of all the rats were shaved and wiped with surgical spirit. Hair remover was also applied over the shaved area to assure the removal of trace of hairs from denuded area. Group1 was kept as control, where there was no drug treatment. Group 2 was treated as standard, where 2% minoxidil lotion (Mintop) was applied over the shaved area, once a day. The animals of remaining groups were given application of 5% cream of extract, E_1, E_2 and E_3 respectively. The treatment was continued for 30 days during which time, hair growth initiation (minimum time to initiate hair growth on denuded skin region) and completion time (time taken to completely cover the denuded skin region with new hair) were recorded for each group of animals [16]. Hair was plucked randomly from the shaved area of selected rats, from each group on 10th, 20th and 30th day of the treatment and length and diameter of 24 hairs was measured [17]. The average length and diameter were determined. The determination and evaluation of these parameters have been considered as vital for accomplishment of hair growth. It is considered that these parameters are accomplishing the concept of hair growth.

3. Results and Discussion
3.1 Primary skin irritation test
This test was conducted to evaluate the irritancy of the extracts on intact skin of rats. None of the prepared extract showed any erythema or edema, indicating that the prepared extracts were non- irritant on the skin of rats.

3.2 Hair growth activity
In control group animals, initiation of hair growth in denuded area was observed in second week. Hair growth initiation was noted in the first week in rats of minoxidil treated standard group. The extract E_2 exhibited hair growth initiation on 10th day and E_1 on 6th day whereas with extract E_3, hair growth initiation time was reduced to 5th day. Similarly the time taken for complete hair growth on shaved area was affected with minoxidil treatment as well as treatment with extracts. Complete hair growth with minoxidil and control group was observed in 20 and 24 days respectively. In extract E_2 complete hair occurred after 23 days, in E_1 after 20th days, and in E_3 it was reduced to 19 days (Table 1). In comparison to control for extract E_3 the whole denuded area was covered with hair during the 4th week (Fig. 1). The experiment thus clearly demonstrates hair growth promoting activity in the extracts. The length of the hair began to increase until the end of the treatment course (Table 2). The extract E_3 produced a greater effect on the length of hair when compare to other group being 8.76 mm at the end of the course, compare to 8.38 mm in the E_1, 7.79 mm in E_2 and 8.5 mm in standard. This may be due to the premature switching of follicles from the telogen to anagen phase of hair growth cycle (18). On 30th day, in control animal diameter of hair was found 0.0279 mm, in standard it was 0.0458 mm, in extract E_1 and E_2 it was 0.0404 mm and 0.0279 mm respectively, but in extract E_3, it was highest around 0.0467 mm (Table 3).

Table 1: Effect of Various Formulations on Hair Growth Initiation and Completion Time of Albino Rats

S. No.	Compound Name	Initiation Time (in days)	Completion Time (in days)
1	Control	10.67±1.37	24.83±2.14
2	Standard	6.17±1.47**	20.00±2.61**
3	*Cicer arietinum* Linn. extract (E_1)	6.00±1.41**	20.17±1.94**
4	*Ocimum sanctum* Linn. extract (E_2)	10.00±2.28	23.50±2.74
5	*Cyperus rotundus* Linn. extract (E_3)	5.57±1.37**	19.43±1.58**

Value are mean ± S.D.
* P<0.01, ** P<0.001, When compare to control value by student's t-test (n = 6 animals)

Table 2: Effect of Various Formulations on Hair Length of Albino Rats

S. No.	Compound Name	Length of hair in mm		
		10 Days	20 Days	30 Days
1	Control	3.00±0.72	6.17±0.76	7.54±0.83
2	Standard	3.46±0.59 *	6.79±0.98*	8.5±1.10 **
3	*Cicer arietinum* Linn extract (E_1)	3.42±0.58 *	6.67±0.96*	8.38±0.88 **
4	*Ocimum sanctum* Linn. extract (E_2)	3.21±0.66	6.29±0.86	7.79±1.02
5	*Cyperus rotundus* Linn. extract (E_3)	3.87±0.59 ***	6.97±1.5 **	8.76±1.74 ***

Value are mean ± S.D.
* P<0.05, ** P<0.01, and *** P<0.001
When compare to control value by student's t-test (n = 24 hairs)

Table 3: Effect of Various Formulations on Hair Diameter of Albino Rats

S. No.	Compound Name	Diameter of hair in mm		
		10 Days	20 Days	30 Days
1	Control	0.0192±0.0065	0.0233±0.007	0.0279±0.0066
2	Standard	0.0246±0.0083*	0.0358±0.0102 ***	0.0458±0.0102***
3	*Cicer arietinum* Linn extract (E_1)	0.0229±0.0062*	0.0333±0.007***	0.0404±0.010***
4	*Ocimum sanctum* Linn. extract (E_2)	0.0192±0.003	0.0238± 0.0071	0.0279±0.0078
5	*Cyperus rotundus* Linn. extract (E_3)	0.0252±0.0132**	0.0365±0.0084***	0.0467±0.0082***

Value are mean ± S.D.
* P<0.05, ** P<0.01, and *** P<0.001
When compare to control value by student's t-test (n = 24 hairs)

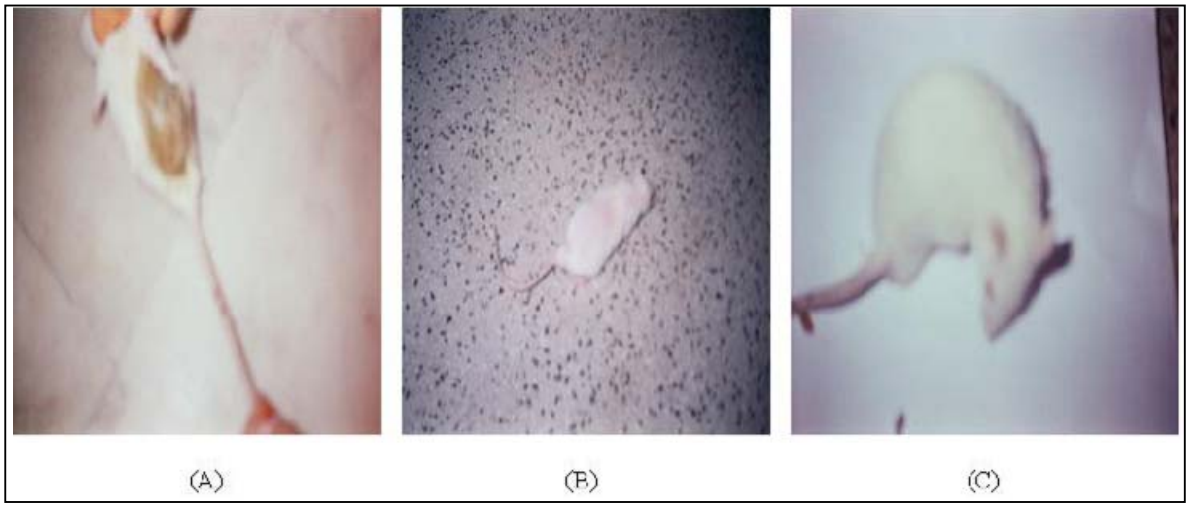

Fig 1: (A) Initially shaved albino rat (B) Control group after 30 days (C) Albino rat treated with formulation E_3 for 30 days showing complete hair growth.

4. Conclusion

Among the various extracts, the extract E_3, showed better growth initiation and hair growth completion time at the same time extract E_3, showed remarkable improvement in hair length and diameter compare to control, standard and other extract. Hence it can be concluded that the extract E_3 proved excellent growth activity and the extracts E_3, might be hold a promise of potential herbal alternative for synthetic drugs used for alopecia.

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