ORIGINAL RESEARCH PAPER

Phytochemical Investigation and Hair Growth Promoting Activity of *Hibiscus rosa-sinensis* Leaf Extract

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Abstract
The plant *Hibiscus Rosa-sinensis* Linn reportedly has a wide range of therapeutic effects on different ailments. Many traditional approaches have been employed to evaluate these therapeutic values. In present study the petroleum ether extract of *Hibiscus Rosa-sinensis* Linn leaf was evaluated for its hair growth promoting property in male albino rats. The hair growth promoting activity of test sample was compared with marketed formulation Mintop® (2% ethanolic solution of Minoxidil) which was taken as a standard. Both qualitative and quantitative analyses have been performed to evaluate the pharmacological activity of the prepared sample. The petroleum ether extract of *Hibiscus Rosa-sinensis* Linn shows the hair promoting activity significantly when compared to standard.

INTRODUCTION

Hair disorders include hair loss, increased hair growth, and hair structure defects with increased breakage, as well as unacceptable cosmetic appearance, such as reduced shine, strength, curliness, and elasticity.[1] Hair loss is a very common problem in human beings.[2] It is a symptom that is exceptionally difficult to quantify and to evaluate.[2-3] The most common form of alopecia is androgenic alopecia which affects equally both male & female & is characterized by a progressive loss of hair diameter, length, and pigmentation[2]. Androgenetic alopecia is hereditary thinning of the hair induced by androgens in genetically susceptible men and women[6]. Androgens are essential for the development of androgenetic alopecia.[4] Alopecia areata is a disorder in which there is loss of hair causing patches of baldness but with no scarring of the affected area. It can affect the entire scalp (alopecia totalis) or cause loss of all body hair (alopecia universalis). It is a relatively common condition affecting 0.15% of the population.[5,6] Individual's predisposition and different types of drugs administrations determine the degree and severity of alopecia.[7] Although in many cases it can be a self-limiting condition, nevertheless hair loss can often have a severe social and emotional impact.[5] *Hibiscus Rosa-sinensis* Linn is a versatile plant and its various species are cultivated throughout the world. It has reportedly shown analgesic property,[8] antioxidant and antibacterial activities,[9] hypoglycemic and hypolipidemic activities,[10] antidiabetic effect.[11] The present study focuses on the effect of petroleum ether extract of leaf of the *Hibiscus Rosa-sinensis* Linn. on hair growth in male albino rats.

MATERIAL AND METHODS

Procurement and Identification of Plant Material

Fresh leaves of *Hibiscus rosa-sinensis* were collected from Jhansi district in the month of September and the authenticity of plant was confirmed by Dr. R.K. Agrawal, Department of Botany, Bundelkhand University, Jhansi, India. Specimen voucher is deposited in Institute of Pharmacy, Bundelkhand University, Jhansi, India.

Chemicals

Minoxidil was obtained from Dr. Reddy's Laboratories, Hyderabad, India. All other chemicals and solvents used were of analytical grade and purchased from SD Fine Chemicals, Mumbai, India.

Study of Foreign Matter in Crude Drug

Five grams of sample was accurately weighed and spread in the thin layer. Sample was inspected with unaided eye or with the use of a 6x lens and separated the foreign organic matter manually as complete as possible and weighed, percentage of foreign matter was determined from the weighed of the drug taken.

Fluorescence Analysis of Crude Drug

A small quantity of dried and finely powdered leaves sample was placed on a grease-free microscopic slide and 1-2 drops of freshly prepared solutions of different chemicals were added separately as shown in Table 2, mixed by gentle tilting the slide and waited for 1-2 minutes. Then the slide was placed inside the UV viewer chamber and viewed in day light, short (254 nm) and long (366 nm) ultraviolet radiation. The colors observed by application of different reagents in various radiations were recorded.[12,13]

Study of Ash Values

The herbal drugs when incinerated, they leave an inorganic ash which fairly varies in wide limits and is therefore of considerable importance for the purposes of evaluation. In the present study, total ash, acid insoluble ash, water soluble ash, and sulphated ash values were determined as per the procedures described in Indian Pharmacopoeia 1996.[14]

Preparation of Leaf Extract

The collected leaves were dried in the shade, powdered and packed in soxhlet apparatus and extracted with different solvents such as petroleum ether, ethanol (95%), and water, successively at 60-80°C by continuous hot percolation. The extracts were dried under reduced pressure using rotary evaporator. The extracts were stored in desiccator until further use.

Phytochemical Analysis

The preliminary phytochemical tests for dried leaves extracts were also carried out according to the standard procedures.[15,16]

Hair Growth Promotion Activity

Animals

Healthy male wistar albino rats, weighing 120-150 g, age 3-4 months were used according to the guidelines of the Institutional animal ethics committee of the animal house of institute of pharmacy, Bundelkhand University, India. The rats were placed in cages and kept in standard environmental conditions, fed with standard diet ad libitum and free access to drinking water.

Standard Drug

2% of Ethanolic solution of minoxidil (Mintop®) was used as standard drug.

Test Sample

1 g of petroleum ether extract was dissolved in 100 mL of liquid paraffin to produce the 1% active compound and it was further used for the evaluation of potential hair growth effects *in vivo*.

Method

24 male albino rats were selected and divided into 4 groups of six rats each. The dorsal of all the rats were trimmed and shaved using razor and finally marketed hair remover Anne French® was applied over the shaved area to remove all the hair.

The first group was treated as control while the second group was kept as standard where the ethanolic solution of 2% minoxidil (Mintop®) was applied over the shaved area once a day. Third group was treated with sample and fourth group with placebo. This treatment was continued for 30 days. During the course, hair growth pattern was observed visually and the differences were recorded qualitatively between control group, minoxidil ethanolic solution, placebo and sample treated groups.

Quantitative Model for Study of Hair Growth

Folliculogram

The morphometric method developed for evaluating the drugs effect on hair growth and for approaching the action of the drugs on follicular growth, is essentially a three dimensional reconstructions of all of the hair follicles in a given skin region. The data represented as a histogram called a folliculogram exhibits a proportional population of hair follicles in different cyclic phases and shows the size of each follicle. Thus a sequential analysis of folliculogram provides data showing dynamic changes in the follicular cycles at the growth of follicular size.[16]

Preparation of Folliculogram

After 30 days of drug application a rat from each group were selected randomly and shaved the drug treated area. The selected rats were sacrificed and skin biopsy was taken and fixed. After fixation the edge of the specimen was cut vertical to the skin surface parallel to the hair line using rotary microtome. The obtained serial sections of 10 μm thickness were fixed on the slide and stained with haemotoin and eosin dyes. With the aid of compound microscope growth cycle of hundred hair follicles were observed and the length of the hair follicle present in different growth phases were recorded using optical micrometer.

RESULTS AND DISCUSSION

The results of physicochemical evaluation of the leaves of *Hibiscus rosa-sinensis* are given in Tables 1 and 2. The total foreign matter in the crude drug was found to be 1.52%, which included 0.88% of dust and 0.64% of other plant material.

Different ash values may be considered as standard for further evaluation. The sulfated ash was high in the crude drug, which might be due to the presence of dust and other foreign matter. Table 2 shows the

fluorescence behavior of the crude drug in presence of different chemicals. The fluorescence analysis of the crude drug helps to identify it in powder form.

Table 1. Foreign matter and ash values of crude drug (*Hibiscus rosa-sinensis* leaves)

S No	Parameter	Result
1	Foreign matter (dust + other plants/parts) (%)	1.52 ± 0.09
2	Total ash (%)	13.75 ± 0.35
3	Acid insoluble ash (%)	6.25 ± 0.08
4	Water soluble ash (%)	7.48 ± 0.46
5	Sulfated ash (%)	20.47 ± 0.59

Table 2. Fluorescence behavior of powdered crude drug (*Hibiscus rosa-sinensis* leaves) when treated with different chemicals

S No	Chemical Treatment	Observation	
		At 254 nm	At 366 nm
1	Normal drug	No Change	No Change
2	1N NaOH in methanol	Yellowish Brown	Brown
3	50% KOH	Light Brown	Dark Brown
4	GAA	Brown	Light Brown
5	50% HCl	Light Brown	Brown
6	50% HNO$_3$	Dark Brown	Light Brown
7	50% H$_2$SO$_4$	Dark Brown	Light Brown

Table 3 shows the presence of different phytochemicals in different successive extracts. The presence of phytochemicals depends on the solvent used for extraction. In non-polar solvents, fat soluble chemicals get extracted whereas, polar components get extracted in the polar solvents. A similar pattern of extraction was observed in the present study also.

Table 3. Phytochemical composition of different extracts of *Hibiscus rosa-sinensis* leaves

S No	Phytochemical constituents	Pet. Ether extract	Ethanol extract	Aqueous extract
1	Alkaloids	+	+	+
2	Carbohydrates	+	+	+
3	Phenolic compounds and tannins	-	+	-
4	Resin	+	-	-
5	Phytosterols and triterpenoids	+	-	-
6	Flavonoids	-	-	+
7	Proteins and free amino acids	-	+	+
8	Glycosides	+	+	+

− indicates Absent; + indicates Present

Based on the phytochemical analysis, petroleum extract of leaves of *Hibiscus rosa-sinensis* was selected for the evaluation of hair-growth promoting activity. The petroleum ether extract contains phytosterols and triterpenoids, which are beneficial for hair growth. The ethanol extract was not considered for the activity since it contains tannins, which suppress hair growth. The results of hair growth analysis are given in Table 4 and Fig 1. The albino rats treated with the petroleum ether extract were compared with control group, standard drug and the placebo.

Table 4. Visual observation of hair growth in albino rats after treatment with *Hibiscus rosa-sinensis* leaf extract

S No	Group	No. of rats	Growth initiation time (Days)	Growth completion time (Days)
1	Control	6	10	24
2	Sample	6	5	18
3	Standard (Minoxidil)	6	6	20
4	Placebo	6	10	23
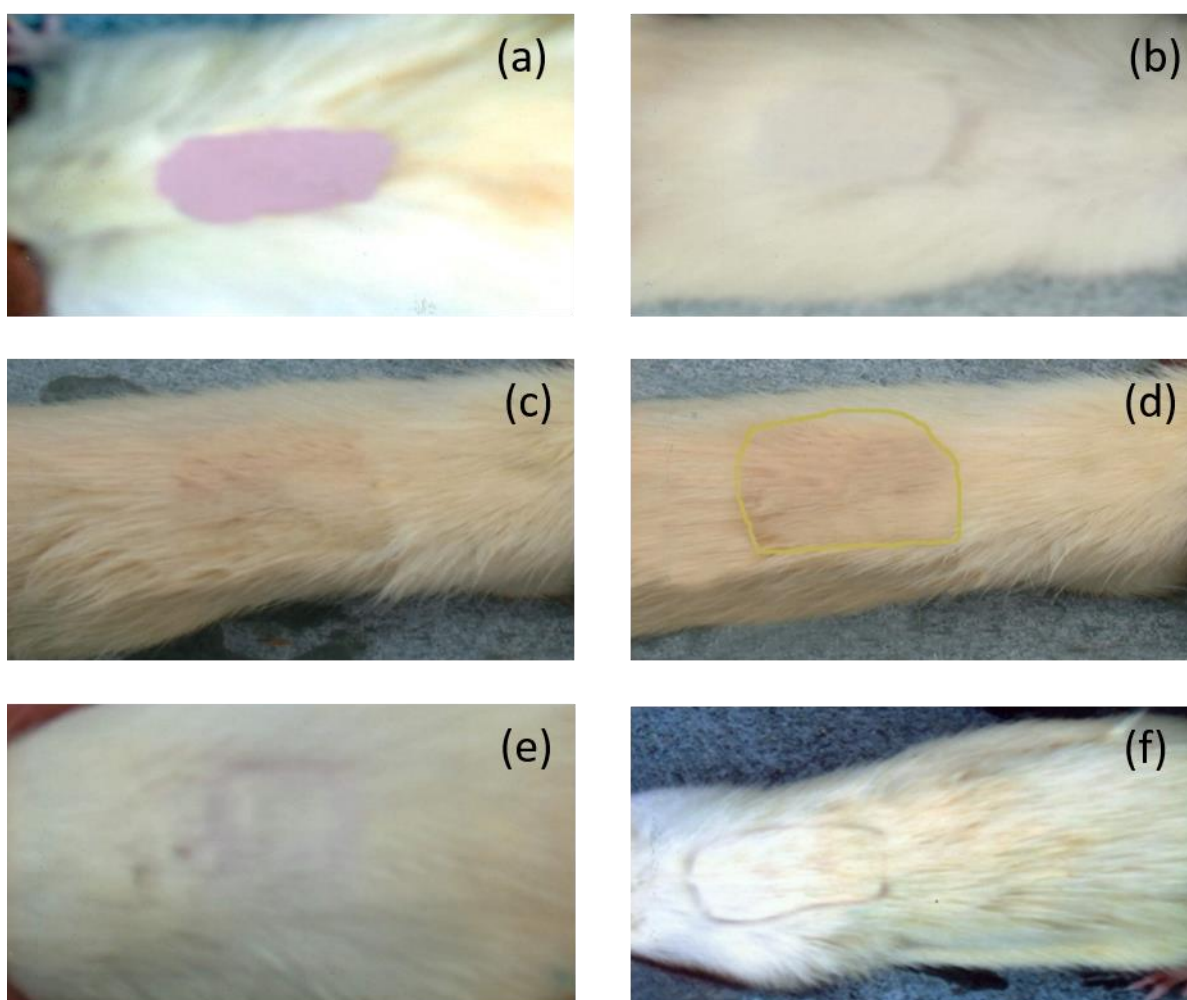
Fig 1. Visual observation of hair growth in various groups of Albino rats
(a) Initially shaven albino rat
(b) Hair growth in control group after 15 days
(c) Hair growth in standard (Minoxidil treated) group after 15 days
(d) Hair growth in sample (Hibiscus treated) group after 15 days
(e) Hair growth in placebo treated group after 15 days
(f) Hair growth in albino rat after 30 days

The *in vivo* study was performed and test samples were evaluated for their hair growth activity using the model developed by Uno et al (1991).[17] In quantitative analyses, the time taken to restart the growth of hair in shaved area observed visually and the complete hair growth was recorded. Almost all the developed diluted test sample found to be better hair growth activity, and the time period taken for sampling was 5 days to restart hair growth which was completed in 18 days. In case of placebo treatment using liquid paraffin, the time taken to restart hair growth was 10 days, which was completed in 23 days. In control group neither drug nor paraffin applied on to the shaved area and was observed visually and found no initiation of growth up to 10th day and took 24 days for completing hair growth. The experiment thus clearly demonstrates hair growth promoting activity of test sample. In standard formulation (Minoxidil), the time period of initiation of hair growth was 6 days, which was completed in 20 days.

Table 5. Percentage population of hair follicles

S No	Group	Population of hair follicles in phase (%)		
		Anagen	Catagen	Telogen
1	Control	49	5	46
2	Standard (Minoxidil)	64	1	35
3	Sample	65	2	33
4	Placebo	47	4	49

Table 6. Length V/S percentage population of hair follicles

S No	Length of hair follicles (mm)	Population (%)			
		Control	Standard	Sample	Placebo
1	0.1	6	4	1	7
2	0.15	9	7	6	13
3	0.2	14	10	11	9
4	0.25	12	6	7	10
5	0.3	7	4	4	7
6	0.35	7	5	12	9
7	0.4	4	4	5	3
8	0.45	8	7	5	5
9	0.5	4	11	4	6
10	0.55	5	7	6	7
11	0.6	8	5	7	5
12	0.65	7	4	6	4
13	0.7	4	7	9	4
14	0.75	2	6	5	3
15	0.8	2	3	3	4
16	0.85	1	4	4	2
17	0.9	-	3	3	1
18	0.95	-	2	1	1
19	1.0	-	1	1	-

In quantitative analysis, the per cent population of hair follicles in different cyclic phases has been determined and the length of each hair follicle was measured using ocular micrometer. Table 5 and 6 show the quantitative estimation of hair follicles in different phases of development, such as anagen, catagen, and telogen phases.
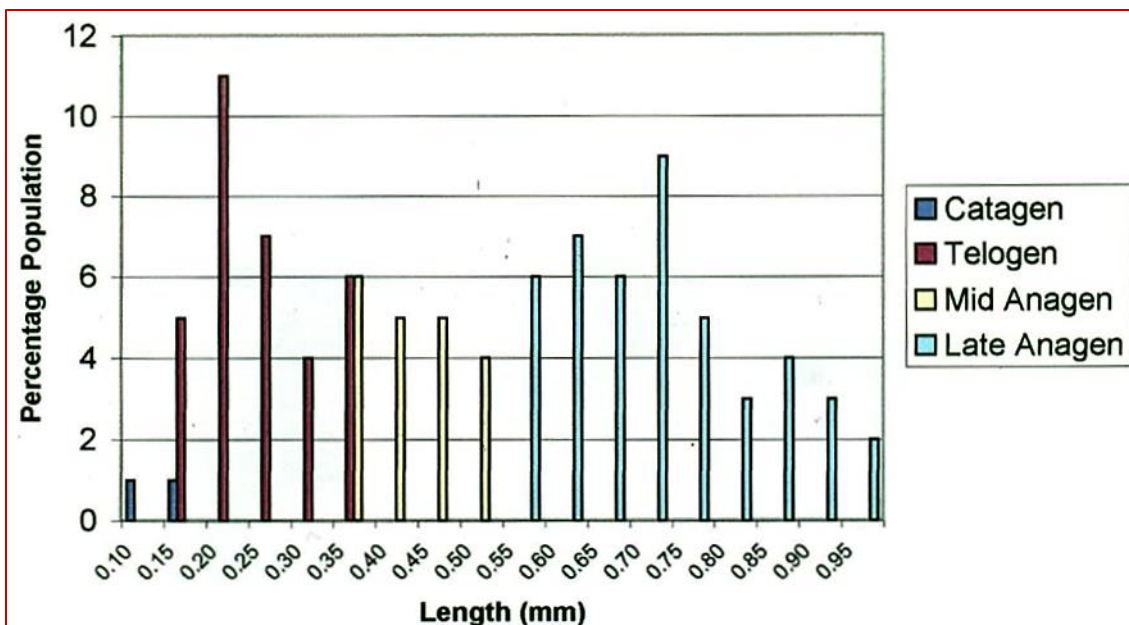
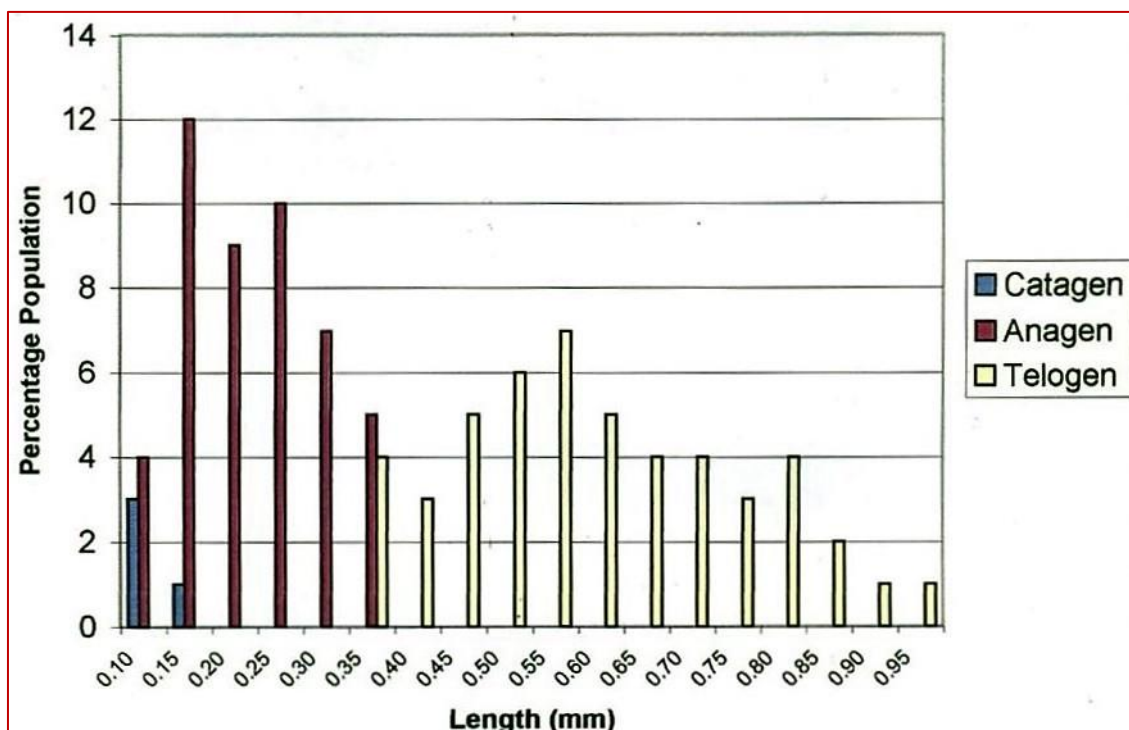Fig 2. Folliculogram of albino rats treated with *Hibiscus* leaf extract

Fig 3. Folliculogram of albino rats treated with placebo

Table 6 gives data of the population of hair follicles with different lengths. The Folliculograms (histogram) depicting percentage population of hair follicles and their length are shown in Figures 2 to 5. The per cent population of hair follicles for control group was found to be 49% anagen, 5% catagen and 46% telogen; whereas, in the standard treatment (2% ethanolic minoxidil), it was found to be 64% anagen, 1% catagen and 35% telogen. The sample (*Hibiscus rosa-sinensis*) treated group showed the predominant activity of 65% anagen, 2% catagen and 33% telogen. The placebo showed less activity then standard and test (Table 5).
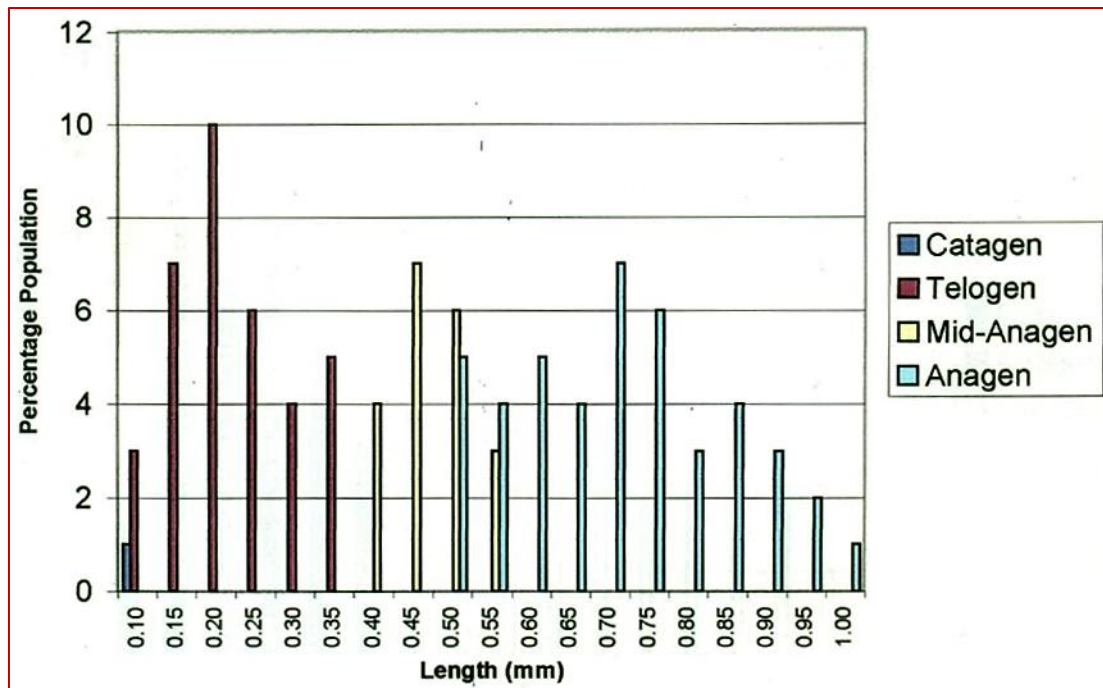
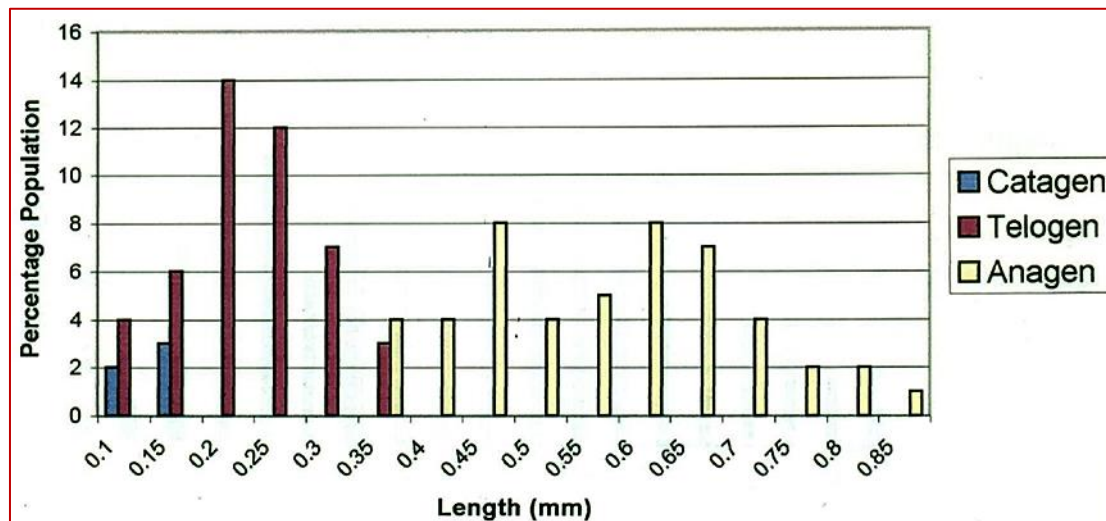Fig 4. Folliculogram of albino rats treated with standard (Monoxidil)

Fig 5. Folliculogram of control group of albino rats

CONCLUSION

Hibiscus Rosa-sinensis Linn has shown a significant hair growth promoting activity through its petroleum ether extract of leaves. Therefore, the present study supports the use of *Hibiscus Rosa-sinensis Linn.* in human beings for hair growth in future, for the treatment of alopecia. This study also supports the traditional use of this plant for hair growth promotion.

DECLARATION OF INTEREST

It is hereby declared that this paper does not have any conflict of interest.

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