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Research Article

HAIR GROWTH ACTIVITY OF AQUEOUS EXTRACT OF *HIBISCUS ROSA-SINENSIS* L. FLOWERS

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ABSTRACT

The study was aimed to investigate the *in-vivo* and *in-vitro* efficacy of aqueous extract of *Hibiscus rosa-sinensis* L. flowers. *In vivo* 2.0 % w/v aqueous extract of flowers in absolute ethanol was applied topically over the shaved skin of albino rats and monitored for 30 days. The length of hair and the different phases of hair follicles, like anagen and telogen phases were determined at different time periods of study. *In vitro*, the hair follicles from neonates of albino rat were isolated and cultured in DMEM supplemented with 1.0 % w/v aqueous extract of flowers. From the study it is concluded that the flower extract has significant hair growth potential when compared to control and standard (Minoxidil 2.0 % w/v).

KEYWORDS

Hibiscus rosa-sinensis; hair follicle; in vitro; in vivo; anagen; telogen.

INTRODUCTION

It is an evergreen woody, glabrous, showy shrub of 5-8 ft in height. Leaves are bright green, ovate, coarsely toothed above, flower are solitary, axillary, bell shaped, large 4-6 inch in diameter with pistil and stamens projecting from the centre (Sastri B N., 2001, Asolkar *et al.,* 2005). The leaves and flowers are observed to be promoters of hair growth and aid in healing of ulcers (Adhirajan et al., 2003; Nadkarni, 1954; Ali and Ansari, 1997; Kurup *et al.,* 1979). According to traditional literature (Nadkarni, 1954; Kumar *et al.,* 1994), it is well accepted that the leaves and flowers of Hibiscus rosa sinensis have hair growth promoting and anti-greying properties. Folklorically the flowers are used as demulcent, emollient, refrigerant, aphrodiasic emmenagogue and hair growth potential. A decoction of flower is used in bronchial catarrh. The dark red petals in the form of mucilaginous infusion are used in ardor-urinae, strangury, cystitis and other irritable conditions of the genito-urinary tract. The flowers of *Hibiscus rosa-sinensis* have reported to possess various activities such as analgesic (Sawarkar et al., 2009), anti-convulsant (Birari et al., 2010), anti-diabetic (Venkatesh et al., 2008), wound healing (Nayak et al., 2007), antibacterial (Hena, 2010), immunomodulatory (Gaur et al., 2009), and hair growth (Adhirajan et al., 2003). Hence, the present study is focused on the scientific investigation of hair growth activity of *Hibiscus rosa-sinensis* flowers.

MATERIALS AND METHODS

Plant material: The flower of *Hibiscus rosa-sinensis* Linn were collected in the month of August from the gardens of Mathura district, Uttar Pradesh and aunthentified by Birbal Sahni Institute of Palaeobotany, Lucknow, Uttar Pradesh, India Ref. No. 13374

Preparation of extracts: The flower of *Hibiscus rosa-sinensis* were collected from gardens of Mathura (Uttar Pradesh) and dried in shade and coarsely powdered. It was then passed through the sieve no. 20. A weighted quantity (360g) of the powder drug was extracted with petroleum ether (60-80^{0}C) using soxhlet extractor. Defatted drug was subjected to ethanolic extraction and extract was dried by distilling off the solvent and then dried in desiccator. The marc collected after ethanolic extraction was subjected to aqueous extraction by maceration process for seven days consecutively and then extract was dried by evaporating the water and stored for further activity.

Animals: The experimental protocol described in the present study was approved by the Institutional animal ethical committee (IAEC) of Institute of Pharmaceutical Research, GLA University, Uttar Pradesh, India, with the permission from committee for the purpose of control and supervision of experiments on animals (CPCSEA). Healthy Wistar rats (200-300g) were used for the study. Rats were housed in small cages in environmentally controlled (25 ± 2^{0}C, 12h light and dark cycle, with free access to food and water *ad libitum*). Rats were fed with the standard laboratory chow diet during the period of study.

Primary skin irritation test: The skin irritation test was done by the method described by Suraj *et al.,* 2009 with some minor modification. The hairs from rats dorsal part were removed by commercial available hair removing cream (Veet). A 2-cm^2 dorsal area was shaved and cleaned with surgical spirit. The animal did not show any toxic effect when 10% w/v of extract was applied to shaved area of rats. Hence the prepared extract was considered safe for topical application.

***In vivo* hair growth activity:** The rats were divided in to three groups of six rats in each group. A 2-cm^2 area of the hair from the dorsal portion of the rats were shaved off and wiped with surgical spirit. One milliliter of prepared lotion and the standard (Minoxidil 2%v/v) were applied to the denuded area of the respective groups two times in a day and control group received no treatment. This treatment was continued for 30 days during which qualitative and quantitative parameter of hair growth was observed and recorded. Skin biopsies were taken on the 10^{th,} 20th and 30th day of extract and standard application of follicular analysis.

Hair length determination: Hairs were plucked randomly using sterile forceps from the shaved area of selected rats, from each group on 15th, 20th, 25th and 30th day of the treatment. The average length of 25 hairs was measured in millimeter and the results were expressed in mean ± SEM.

Histological studies: On the 10th, 20th and 30th day of treatment one rat from each group was euthanicated and skin biopsies were taken from the shaved area and fixed in 10% formalin buffer. Sections of tissue were embedded in paraffin wax and sectioned in to uniform thickness of 10µm. The sectioned tissues were stained with haematoxylin and eosin. From the stained tissue the number of hair follicles per millimeter of the skin and the percentage ratio of different cyclic phases were examined using microscope fitted with an ocular micrometer facility.

***In vitro* hair growth activity:** *In vitro* hair growth activity of *Hibiscus rosa-sinensis* was performed by the method described by the Adhirajan et al 2003[12] with some modification. Hair follicles were isolated from the neonates of Wistar rat. The neonate was killed and the dorsal skin was dissected out and washed thoroughly in phosphate buffered saline (PBS). The skin was cut in 0.5 cm^2 in size and placed in petri dish containing PBS. The individual skin pieces were chopped thoroughly until the intact hair follicle came out from the skin piece. The hair follicle was isolated using a fine Pasteur pipette under binocular dissecting microscope. Individually, freshly isolated hair follicles were placed in 96-well plate containing Dulbecco's Modified Eagles Medium (DMEM), extract in dimethyl sulfoxide (DMSO) and the standard. The plates were maintained at 37°C±1°C and length of hair follicles were measured after 24, 48 and 72h by using binocular inverted microscope equipped with micrometer facility.

RESULTS

Hair growth of *Hibiscus rosa-sinensis* was observed from the 2nd week of the treatment (Table 1) and the increase in the length of hair was compared with control and standard. With comparison to the control and standard the time taken to complete the hair growth was found to be 24 days for aqueous extract of flower. From the observation it was found that the extract treated group produced a significant effect with respect to control. However, in the extract treated group the length of hair was significantly higher than control group but not from the standard group (Table 2).

Histological studies: The histological observation was showed that there were 5-7 hair follicles per millimeter of skin of all the treatment groups. However, a marked difference in the different cyclic phases (Anagen/Telogen) were observed in treated and control group. On the 10th day (Table 4), nearly 40% of the hair follicles were in the anagen phase in all the groups but at the end of the course of treatment extract treated group showed 71% of anagen follicle, which is higher than control (53%) but to a lesser extent than standard (74%).

***In vitro* hair follicle culture:** The flower extract treated group showed a significant increase in the length over 72h in 96-well plate culture and the rate of growth was 1.73±0.18 mm per 72h (mean ±SEM). This value was found to be significantly higher than control (0.19±.0.03) but not than the standard (1.95± 0.14). However, after the 72h the rate of increase in hair follicle length was reduced and gets ceased subsequently (Table 3).

DISCUSSION AND CONCLUSION

There is only two FDA approved drugs i.e. Minoxidil (topical) and Finasteride (oral and topical) that are used as hair growth promoter but these drugs have serious side effect such as pruritis, dryness, scaling, impotence and loss of libido. Minoxidil act as a potassium channel opener and act through by converting telogen (resting phase) to anagen phase. Finasteride is a 5-α-reductase enzyme inhibitor which inhibits the conversion of testosterone to dihydrotestosterone (DHT) and act through the same manner as minoxidil i.e. stimulate anagen phase and depress the telogen or increase rate of cell proliferation. However, the rate of minoxidil benefit is 60% and 48% in case of finasteride but the baldness recurs when the treatment is discontinued. The effect of these drug varies from individual to individual and types of baldness, as we know there are different type of baldness and more common is androgenic or male pattern baldness (Tripathi, 2003). So the need of the present study was to identify the novel hair growth promoter with minimum side effect. The result of the study showed that flower extract has turned telogen to more anagen at the end of 30 days treatment and

confirmed the folkloric use of the plant as hair growth promoter. The transformation of hair follicles from telogen phase to anagen phase in treated group may be due to vasodilating effect of alcohol present in extract lotion and in the same time extract showed increase in epithelial cell proliferation. The aqueous extract showed significant hair promoting activity which was well comparable with that of standard drug minoxidil. The in-vitro study of aqueous extract of *Hibiscus rosa-sinensis* revealed that extract has direct effect on hair follicles and thus may improve hair growth.

It is concluded from the study that flower extract of *Hibiscus rosa-sinensis* has a potential hair growth effect in in-vivo and in-vitro study and it may be due to presence of flavonoids in flower. From the study it is suggested that flower extract of *Hibiscus rosa-sinensis* have the constituent which may improve the hair growth and need to identify, isolate and structural elucidation of that constituent and exact mechanism of action of that constituent.

Table 1: Qualitative observation of aqueous extract of *Hibiscus rosa-sinensis* on hair growth

Groups	Treatment	Dose	Number of days taken to initiate hair growth	Number of days taken to complete hair growth
Control	No Treatment	Nil	9	30
Test	Aqueous extract	2%	7	24
Standard	Minoxidil	2%	6	23

Table 2: Effect of aqueous extract of *Hibiscus rosa-sinensis* on hair length of albino rats

Groups	Treatment	Length of hair (mm)±SEM			
		Day 15	Day 20	Day 25	Day 30
Control	No treatment	3.43±0.2	4.45±0.2	8.21±0.5	13.56±0.4
Test	Aqueous extract (2%)	6.16±0.3	11.76±0.3	14.00±0.3	18.68±0.3
Standard	Minoxidil (2%)	8.72±0.4	13.56±.5	15.08±.2	19.24±0.4

P<0.05, when compared to control values by student's t-test (n=25 hairs)

Table 3: *In vitro* hair growth activity of *Hibiscus rosa-sinensis*

Group	Treatment	Increase in hair follicle length (mm)±SEM		
		24h	48h	72h
Control	DMEM + DMSO	0.12±0.02	0.16±0.02	0.19±0.03
Test	DMEM + Aqueous extract	0.82±0.13	1.55±0.10	1.73±0.18
Standard	DMEM + Minoxidil	0.86±0.11	1.73±0.07	1.95±0.14

P<0.05, when compared to control values by student's t-test (n=10 hair follicle)

Table 4: Effect of aqueous extract of *Hibiscus rosa-sinensis* on cyclic phases (Anagen/Telogen ratio)

Groups	Treatment	Percentage of hair follicles					
		Day 10		Day 20		Day 30	
		Anagen	Telogen	Anagen	Telogen	Anagen	Telogen
Control	-	35	65	46	54	53	47
Test	Aqueous extract (2%)	42	58	63	37	71	29

Standard	Minoxidil (2%)	45	55	65	35	74	26

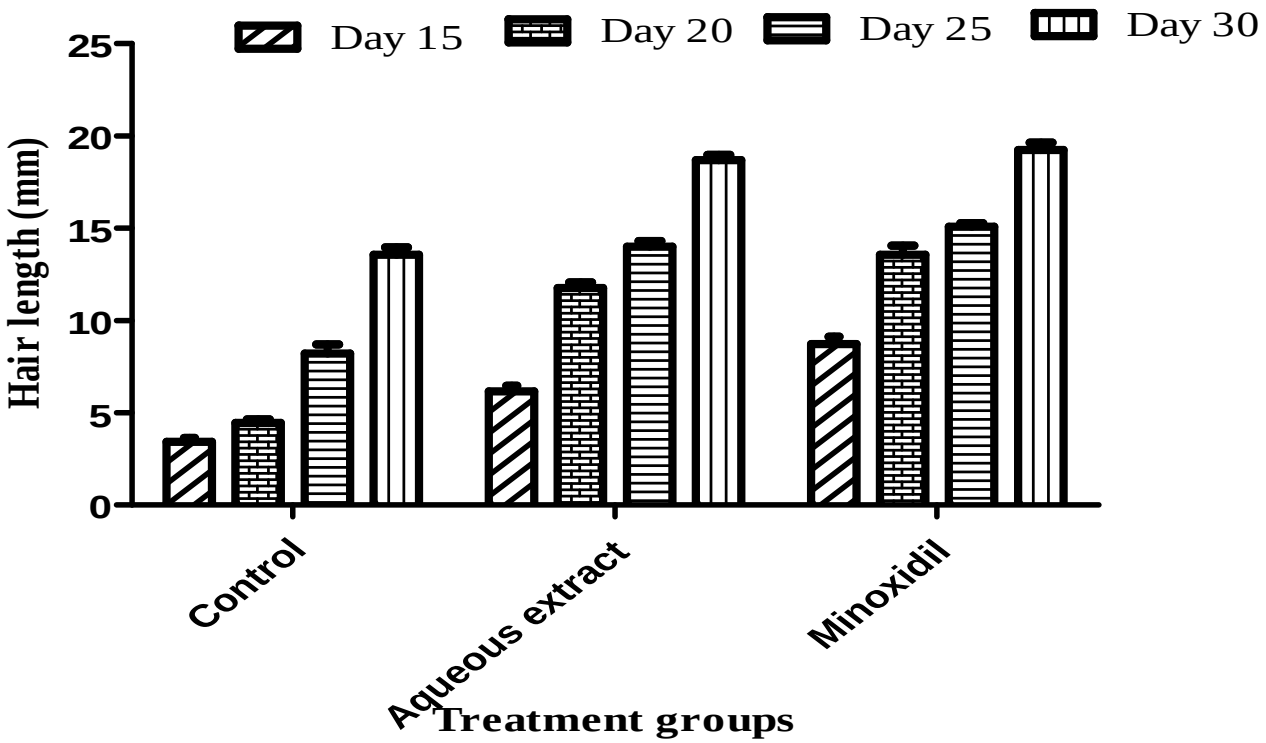


Fig 1: Evaluation of hair growth on different days of aqueous extract by *in-vivo* method

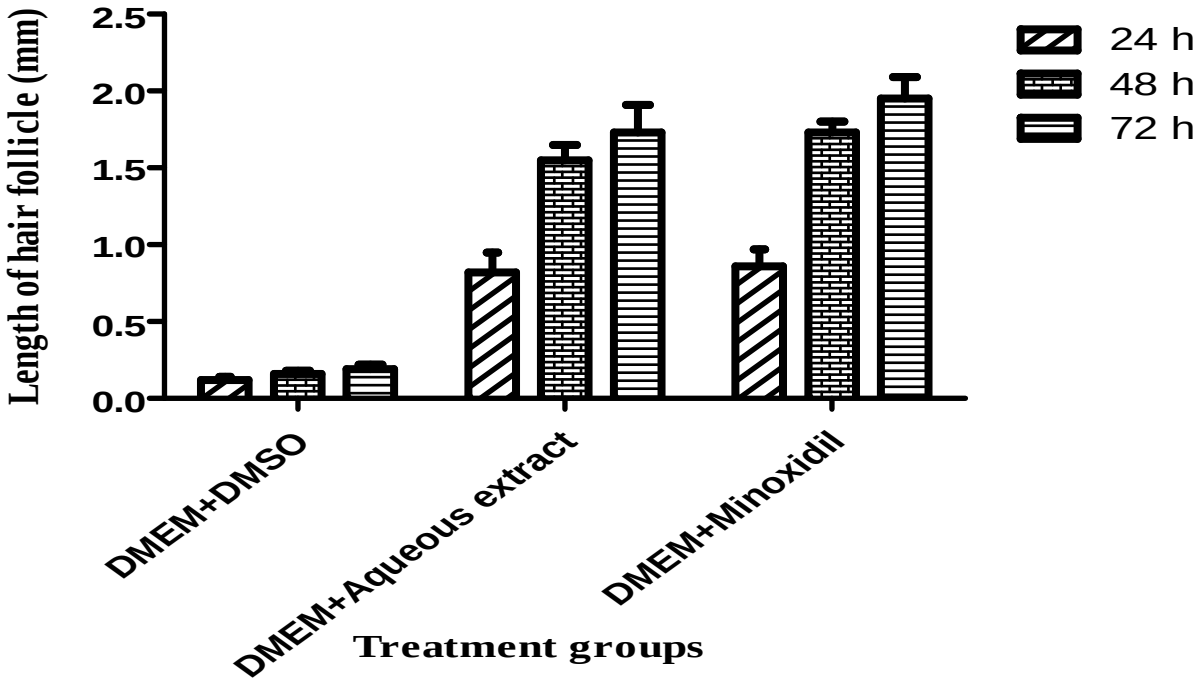


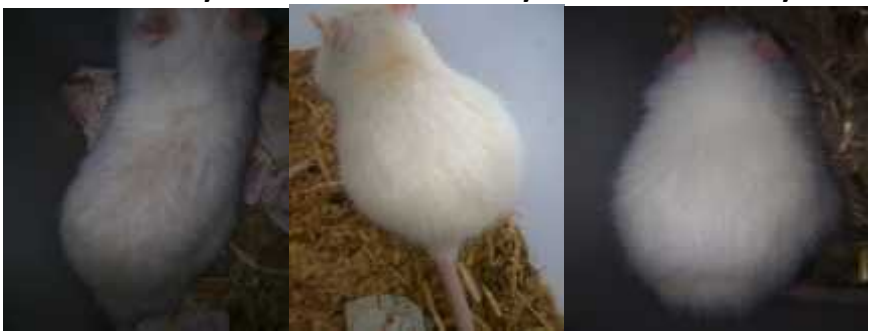
Fig 2: Evaluation of hair follicle growth of aqueous extract by *in-vitro* method

Control Day 1 **HAE Day 1** **Minoxidil Day 1**

Control Day 15 **HAE Day 15** **Minoxidil Day 15**

Fig 3: *In-vivo* hair growth activity on different days

Control Day 20 **HAE Day 20** **Minoxidil Day 20**



Control Day 28 **HAE Day 28** **Minoxidil Day 28**

Fig 3: *In-vivo* hair growth activity on different days


DMEM +Extract (96-well plate) **96-well plates in incubator**

Fig 4: *In-vitro* Hair follicle growth activity

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