



ISSN: 0975-833X

Available online at <http://www.journalcra.com>

International Journal of Current Research
Vol. 10, Issue, 03, pp.67151-67155, March, 2018

INTERNATIONAL JOURNAL
OF CURRENT RESEARCH

RESEARCH ARTICLE

EVALUATION OF HAIR GROWTH POTENTIATION ACTIVITY OF HIBISCUS ROSA SINENSIS IN DISTURBED CIRCADIAN RHYTHM

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ARTICLE INFO

Article History:

Received 17th December, 2017
Received in revised form
22nd January, 2018
Accepted 04th February, 2018
Published online 30th March, 2018

Key words:

Hibiscus rosa sinensis,
Minoxidil, wistar albino rats,
Hair growth, circadian rhythm.

ABSTRACT

Rupture Background: Alopecia is an age old problem affecting humans since dawn of civilization. While there are commercially available treatment, it's not for the mass population. The current millennials follow an unhealthy lifestyle and increasingly rely on synthetic hair products which only aggravates the hair growth and hair fall disorders. This study was conducted to observe the impact of one such factor and determine whether extracts of Hibiscus rosa sinensis can be used to treat such problems.

Objective:

- To evaluate the hair growth potentiation activity of Hibiscus rosa sinensis flower on wistar albino rats
- To evaluate the hair growth potentiation activity of Hibiscus rosa sinensis leaves on wistar albino rats
- To evaluate the hair growth potentiation activity of minoxidil
- To evaluate the effects of altering the circadian rhythm of wistar albino rats on hair growth
- To compare the efficacy of Hibiscus rosa sinensis leaves, Hibiscus rosa sinensis flowers and minoxidil in normal acclimatized rats and rats with disturbed circadian rhythm.

Method: After dividing the animals to two groups, they are acclimatized to different environmental conditions. After 7 days, the dorsal skin of the rats is shaved and divided into 4 regions. In both the groups of animals one region kept as control and remaining 3 regions are treated with minoxidil and Hibiscus rosa sinensis extracts for 30 days. Hair samples were taken at regular intervals and recorded.

Results: Hibiscus rosa sinensis leaves extract was more effective in promoting hair growth than the flower extract while minoxidil treated region recorded maximum hair growth. Rats with disturbed circadian rhythm had delayed hair growth initiation as compared to normal rats however the difference was statistically insignificant. After hair growth initiation, the hair growth trend observed in rats with disturbed circadian rhythm was identical to that of normal rats.

Conclusion: The active principle of Hibiscus rosa sinensis extract is not clear but with positive results in hair growth, it has a great potential in the drug discovery process with formulae's derived from its active principle can be used to treat the hair disorder that has plagued mankind.

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Citation: Dr. Swanand S Pathak and Dr. Monil Yogesh Neena Gala, 2018. "Evaluation of hair growth potentiation activity of hibiscus rosa sinensis in disturbed circadian rhythm", *International Journal of Current Research*, 10, (03), 67151-67155.

INTRODUCTION

Since the ancient times, hair loss is one of the commonest dermatological disorder to affect mankind. Viewed as a status symbol and flaunted like jewelry, solving the age old problem and discovering products with growth promoting properties is of utmost importance. (<http://www alopecia world.com/profiles/blogs/men-s-baldness-in-history>)

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In modern times factors such as disturbed sleep cycle, unhealthy diet and lifestyle, stress and synthetic chemical hair products have aggravated the hair fall problem. The loss of hair adds on to the stress of the man leading to a dangerous never ending cycle (Adhirajan et al., 2003). Although there are number of synthetic, herbal, ayurvedic and natural products available commercially, search for an economical natural alternative is a challenge for the mass population who although are affected by the hair loss disorder but are unable to afford any treatment for it (https://www.researchgate.net/publication/40773282_Traditional_medicinal_uses_of_Hibiscus_rosa-sinensis_Schweiger_et_al.,_2010). There are many medicinal plants

that promote hair growth or prevent hair fall, are safer than the commercially available synthetic alternatives but fail to a mark (Falto-Aizpurua et al., 2014). According to ancient literature and texts, in medicine, red colored *Hibiscus Rosa Sinensis* (HRS) flowers and leaves have shown to have great potential for promoting hair growth as well as anti-greying property. Apart from this property, this widely cultivated evergreen shrub has other several medicinal properties such as aid in healing ulcers, hypertension, antifertility, demulcent etc. (Adhirajan et al., 2003; https://www.researchgate.net/publication/40773282_Traditional_medicinal_uses_of_Hibiscus_rosa-sinensis; Paus, 2006; https://www.researchgate.net/publication/288970136_Review_on_Hibiscus_rosa_sinensis) The present study was thus conducted to determine the possible effects of disturbed circadian cycle on hair growth and hair growth potentiation activity of *Hibiscus rosa sinensis* (HRS).

MATERIALS AND METHODS

Plant Material

Fresh HRS red flowers and leaves were collected from area around JNMC, Sawangi. They were then authenticated by a taxonomist from MGAC, Sawangi.

Extract preparation

Leaves and flowers were shade dried and powdered in a blender. They were stored in air tight containers till further use. The powder was extracted in soxhlet apparatus using 95% of ethanol at 60-80 degree Celsius for 12 hours. The extracts were filtered and the filtrate was then concentrated with a rotary evaporator under reduced pressure to obtain crude extract. 7 grams from reddish brown semisolid extract was obtained from 50 grams of dried powder of flowers while Dark greenish brown semi solid extract was obtained when the 60 grams of dried powder of the HRS leaves was treated the same way yielding 8 grams.

Chemicals

Minoxidil and ethanol was purchased from Maharashtra Scientific, Wardha.

Animals

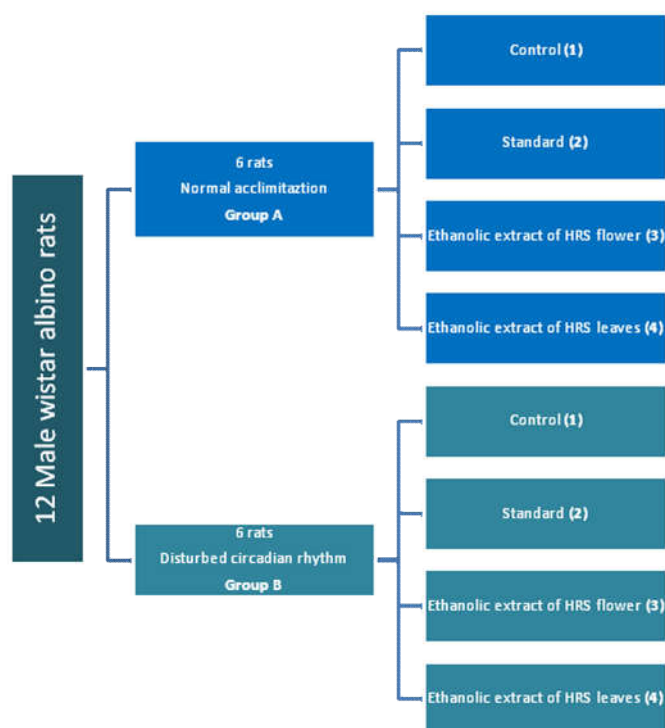
12 Male wistar albino rats, 150-250gms body weight were procured from the Animal House, DMIMS (Reg.No. 571/02/a/CPCSEA) after due approval from the Institutional Ethics Committee, DMIMS. Animals were divided into 2 groups equally, with one group (Group A) exposed to normal 7-day acclimatization period (12:12 hour light dark cycle) to the laboratory environment. The second group (Group B) were acclimatized with continuous light for 7 days to disturb the circadian rhythm. Based on the guidelines of the ethics committee, the rats were kept in standard cages, fed on standard diet ad libitum with free access to drinking water. The conditions were kept same throughout the duration of experiment.

Primary skin irritation test

The skin irritation test was done by a method loosely based on the test described by Suraj et al (Adhirajan et al., 2003). A 2cm² dorsal area was trimmed and then shaved. After cleaning the area with surgical spirit, 10% w/v of extract and 2% v/v standard drug was applied topically to gauge any adverse reaction. Animals did not show any adverse effects and thus the prepared extract was considered safe for topical application.

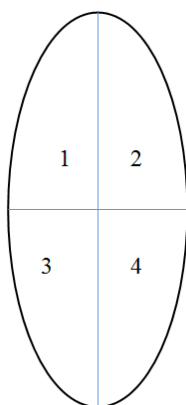
Treatment of animals

After dividing the animals to 2 main groups, the back (dorsal) of the rats was divided and marked into 4 regions. The dorsal skin of all the rats was first trimmed and then shaved off completely using trimmer and shaving blade accordingly. The denuded area was cleaned with surgical spirit.



Grouping of animals

Grouping of animals



- Control (1)
- Standard (2)
- Ethanolic extract of HRS flower (3)
- Ethanolic extract of HRS leaves (4)

RESULTS

Table 1. Group A

Groups	Treatment	Dose	No. of days taken to initiate hair growth	No. of days taken to complete hair growth
Control (1)	No treatment	Nil	9	28
Standard (2)	Minoxidil	2%	5	23
Test (3)	Ethanolic extract of HRS flower	2%	8	26
Test (4)	Ethanolic extract of HRS leaves	2%	7	25

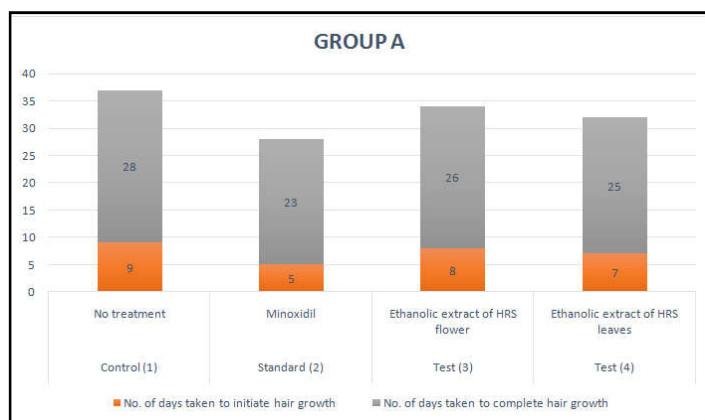


Chart. 1. Group A

Table 2. Group B

Groups	Treatment	Dose	No. of days taken to initiate hair growth	No. of days taken to complete hair growth
Control (1)	No treatment	Nil	11	30
Standard (2)	Minoxidil	2%	6	25
Test (3)	Ethanolic extract of HRS flower	2%	10	28
Test (4)	Ethanolic extract of HRS leaves	2%	9	27

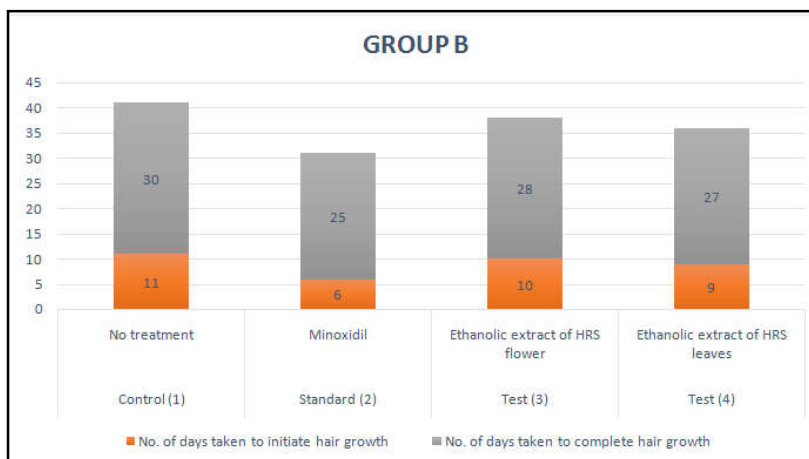


Chart. 2. Group B

Table 3. Group A Hair growth

Groups	Treatment	Length of hair (mm)± SEM				
		Day 15	Day 20	Day 25	Day 30	
Control (1)	No treatment	3.5±0.5	6.6±1.0	10.4±1.2	13.6±1.5	
Standard (2)	Minoxidil	8.7±0.4*	13.56±0.5*	15.02±0.2*	19.36±0.4*	
Test (3)	Ethanollic extract of HRS flower	4.5±1.0	8.2±1.0	12.4±1.2*	15.8±1.2*	
Test (4)	Ethanollic extract of HRS leaves	6.0±1.08*	9.8±1.0*	14.6±1.2*	17±1.2*	

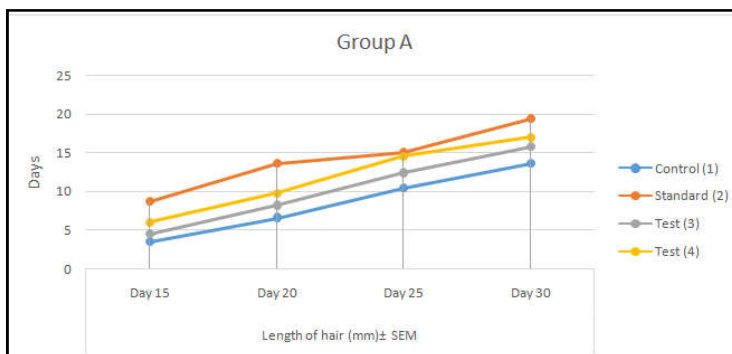


Chart 3. Hair growth progress

Table 4. Group B Hair growth

Groups	Treatment	Length of hair (mm)± SEM			
		Day 15	Day 20	Day 25	Day 30
Control (1)	No treatment	4.8±0.6	7.4±1.0	11.9±0.5	12.6±1.0
Standard (2)	Minoxidil	9.3±0.5*	14.2±1.0*	16.5±1.2*	21.1±1.5*
Test (3)	Ethanollic extract of HRS flower	5.7±0.5	9.4±1.0	13.3±1.2*	16.5±1.2*
Test (4)	Ethanollic extract of HRS leaves	7.3±0.8*	10.5±1.0*	15.9±1.28	18.6±1.2*

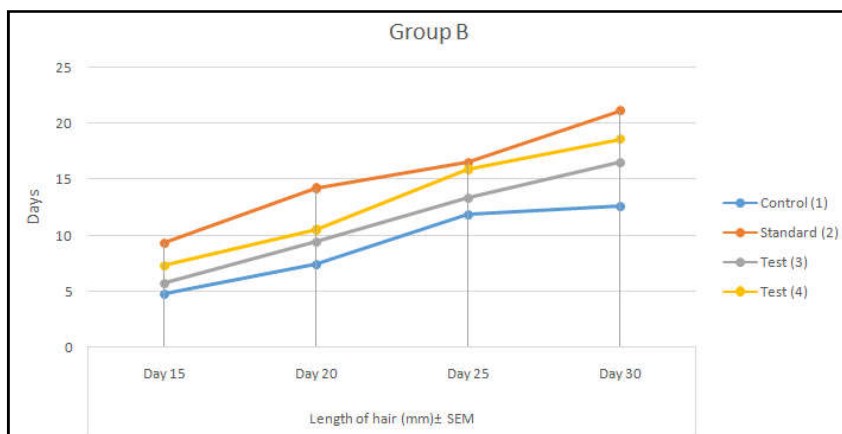


Chart 4. Hair growth progress

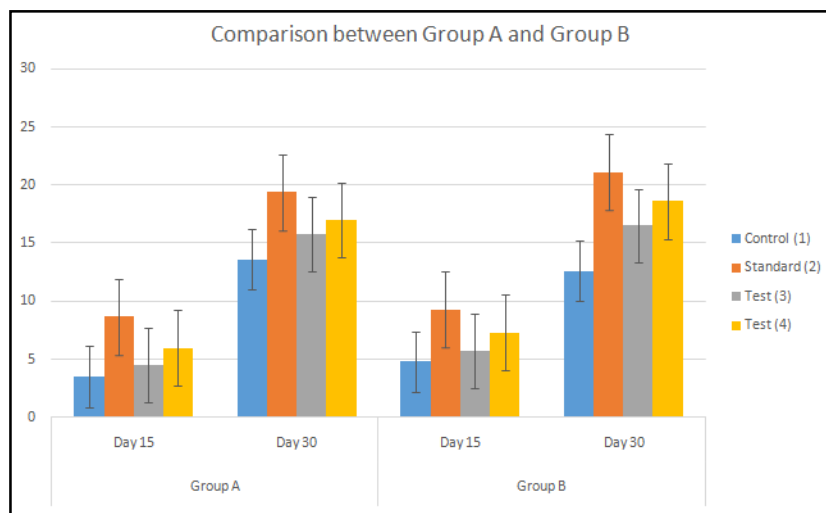


Chart 5.

The respective areas were treated accordingly daily with topical applications for 30 days with recording made on 15th, 20th, 25th and 30th day of treatment.

Hair length determination

Small tufts of hair were plucked randomly using sterile forceps from the designated area from the selected rats for each group on 15th, 20th, 25th and 30th day of treatment and placed in separate test tubes. A pair of sharp-ended tweezers was used to handle the hair, which was placed on a 10 cm ruler, illuminated with the lamp. The measurement was taken under the mounted magnifying glass. For each tube, lengths of ten hairs was recorded and the average length was estimated. A graph was plotted for the average hair growth recorded every week for three weeks. The average length was measured in millimetres and results were expressed in mean \pm SEM

Parameters for measuring hair growth

Hair growth initiation and completion time, length of hair.

Statistical analysis

Statistical analysis of data was carried out by one-way ANOVA and Dunnett's test. Data was reported as mean \pm SEM. $p < 0.05$ is considered as level of significance.

DISCUSSION

The shaved dorsal skin of the wistar albino rats was treated topically with Minoxidil, HRS flower and leaves extract over a period of 30 days in 2 groups of rats acclimatized to different conditions. It was observed that hair growth initiated on the dorsal skin of the rats mostly in the 2nd week of the treatment. The denuded area was covered with hair by the end of the experiment. The minoxidil treated portion of the shaved region showed maximum hair growth in both the groups. In both the groups, HRS extracts gave significantly better hair growth than control. Although HRS leaves extract showed better results compared to HRS leaves extract, the difference was insignificant in most of the recordings. The difference between minoxidil and the HRS extracts was significant. The altered circadian rhythm disturbed the hair growth cycle, thus indirectly affecting hair growth initiation period. However, post hair growth initiation, the hair growth pattern of Group A and Group B were similar. The hair growth property of HRS may be due various flavonoids, alkaloids and phenolic compounds present in the extracts (https://www.researchgate.net/publication/288970136_Review_on_Hibiscus_rosa_sinensis). Minoxidil promotes conversion of telogen to anagen phase while Finasteride is another approved drug for hair growth that has similar mechanism to promote hair growth (Schweiger et al., 2010; Olsen et al., 1987; Boyera et al., 1997; Libecco and Bergfeld, 2004; Olsen et al., 2006). However, they have various side effects with some serious ones like loss of libido and impotence.

Conclusion

From the above findings, it can be concluded that HRS flower and leaves extracts have similar significant hair growth promoting characteristics with minimal chances of developing serious adverse effects as compared. Since the exact component and mechanism of HRS promoting hair growth is not clear, it is a prime candidate for further experiments involving isolation of the active components and synthesizing formulae based on them to treat hair growth disorders in

humans. Further studies involving multiple dose of the HRS extracts can be carried out to find out the most efficacious extract. Unhealthy life style adopted by majority of the millennials also has an indirect effect on hair growth. An awareness programme and promoting a healthy lifestyle will have a positive effect on hair growth in the long term.

Acknowledgement

The authors are thankful to the members of Department of Pharmacology, Animal House and the Institutional Ethics committee of Jawaharlal Nehru Medical College, Sawangi, Wardha, Maharashtra, India for providing the necessary facilities and permission to carry out the research work.

Conflict of interest

Declared none

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