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Pharmacological and Histochemical Screening for Hair Growth-Promoting Activity of *Daucus carota* Herbal Gel.

Namrta Shukla, Sridevi G* and Gopkumar P.

G.R.Y. Institute of Pharmacy, Borawan, Khargone – 451228, India.

Research Article

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***For Correspondence**

G.R.Y. Institute of Pharmacy, Borawan, Khargone – 451228, India

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ABSTRACT

Daucus *carota* L. (Carrot) (Apiaceae) is used in the traditional medicine for the treatment of variety of ailments. It has been used in salads, vegetable. In the titled research pet.ether and aqueous extract of Daucus *carota* was initially tested for the type of phytoconstitutents and then the extract was standardized for the marker compound. Animal studies were carried out by application of standardized extract in gel formulation to the shaved dorsal skin of albino rats. Histomorphometric analysis was employed to study induction of the hair follicle cycle. To determine the effect of extract on the telogen to anagen transition, the protein expression levels of β-catenin in hair follicles were determined by immunohistochemistry. We observed that pet ether Daucus *carota* extract promoted hair growth by inducing the anagen phase. Specifically the histomorphometric analysis data indicates that topical application of the extract in gel form induced an earlier anagen phase and prolonged the mature anagen phase, in contrast to control and 1% minoxidil treated group. Results also exposed the increase in both the numbers and size of hair follicles of the extract treated group. Moreover, the immunehistochemical analysis reveals earlier induction of β-catenin in hair follicles of the extract-treated group, compared to the control group.

INTRODUCTION

Alopecia is a disease of hair loss in allied patterns. It is mainly categorised by bald patches on the scalp or other parts of the body, and can ultimately cause baldness across the entire body. Hair, a complex mini-organ composed of terminally differentiated and dead keratinocytes, plays several roles in physical protection, sensory, thermoregulation, and sexual attractiveness. The cyclical process of hair growth is divided into 3 following phases: anagen (growth phase), catagen (regression phase), and telogen (resting phase) [1]. Dysregulation of the hair growth cycle has been shown to be associated with the pathogenesis of certain conditions, for example, androgenetic alopecia. Two key regulators of hair follicle growth, Sonic hedgehog (Shh) and β-catenin, are known to be involved in the induction of the transition from telogen to anagen, and when the level of either protein is low, hair growth is severely damaged[10-13]. The number of patients suffering from hair loss or alopecia has increased dramatically]. The drugs, finasteride and minoxidil, have been approved by the Food and Drug Administration for the treatment of alopecia, their efficacies are limited and transient, due to unpredictable efficacies and side effects. Therefore, it is urgent to develop more and better treatment options[2,3].

Carrot is a vegetable known scientifically as Daucus *carota* L. which belongs to family Umbelliferae *(Apiaceae)*. Regarding its nutritive value, the carrot has abundant amount of beta carotenes (provitamin A) which give the vegetable its characteristic color. Moreover, carrots are rich in dietary fibers, antioxidants and minerals, especially potassium. The nutrient contents of each 100 g raw carrot were estimated to be

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as follow: water (89 g), carbohydrates (8.71 g), proteins (0.98 g), lipids (0.24 g), dietary fibers (2.24 g), vitamin A (12 mg), vitamin C (7.1 mg), calcium (33 mg), magnesium (18 mg) and potassium (240 mg), as recorded by Ensminger Experimental and clinical studies on carrots (powder or extract) and its active constituents (mainly carotenoids) revealed that they have hyperglycemic effect, anticancer activity due to the presence of alpha carotene and falcarinol, protective effect against coronary heart disease, hypocholesterolemic and hypolipidemic activities [10,11,12,13] .Increased levels of DHT and 5α-reductase cause the balding scalp skin. The present study clued up to investigate the hair growth-promoting activity of pet.ether and aqueous extract and the underlying mechanism of action[5-9].

MATERIALS AND METHODS [2,3,11,12,13]

Preparation of Daucus *carota* solvent and aqueous extract

An authenticated voucher specimen of Daucus *carota* (DcHG-495) was deposited in the Herbarium of the College. Shade dried rhizomes were ground to a fine powder with a dry grinder and extracted 4 times with hot water for 4 h. Hot water extract was then chilled, filtered through the filter paper, and allowed to concentrate to dryness. Residues were extracted with hot water again at room temperature and filtered. Pet.ether extract was prepared in similar way using soxhlet extraction method. Extract was dried in a rotary evaporator under vacuum at 40°C and subsequently stored at -4°C until use. Extract was dissolved in water for animal experiments.

Preparation of Hair gel

The petroleum ether and aqueous extracts were incorporated into carbopol 934 gel base in concentration of 4% (w/w). Hair gel was prepared using 1.2% of polymer, 5% humectant, 2% of combination surfactants and 1% benzyl alcohol preservative.

Animals

Wistar albino rats of either sex, weighing 200–250 g, were used for animal studies. All the animals were housed in cages at a room temperature of 25 ± 3°C with 12h dark/12h light cycles. The rats were fed with rat pellets and water ad libitum. The experiments were approved by the Institutional Animal Ethical Committee.

Primary skin irritation test

Hairs from the dorsal side of the rats were removed using hair clippers and electrical shavers from 12 sq cm area. The shaved area was cleaned with surgical spirit and the animals did not show any toxic effects when petroleum ether and water extracts were applied in a concentration of up to 10% for 48 hours post application. Hence the prepared extracts were considered safe for topical application.

Experimental studies for hair growth activity in vivo

Twenty four rats were divided into four groups of six animals in each group. Hairs from a 3*4 cm2 area at the dorsal portion of all the rats were shaved using electric shavers and applied with marketed hair remover to completely remove hair. Group 1 served as a control was applied with simple gel base. Group 2 – 3 were topically applied with formulated hair gel of petroleum ether and water extract respectively. Group 4 was applied with 1ml of 2% minoxidil over the shaved area. All the gels and standard drug were applied twice in a day. The treatment was continued for 21 days and hair growth pattern was observed and tabulated (Mithal and Shah, 2000; Tortora, 1996). Skin biopsies were taken on the 10th, 20th, and 30th day for follicular observation. Visible hair growth was recorded at 0, 10, 15, 20, 25, and 30 days.

Hair length determination

Regrown hairs were plucked randomly using sterile forceps from the shaved dorsal area of rats on test days of treatment. Hair length was measured and the results were recorded as mean length ± SEM of 25 hairs.

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Quantitative histomorphometrical studies

One rat from each group was forfeited after 10, 20 and 30 days of treatment. Skin biopsies were obtained from the shaved portion and preserved in 10% formalin. Sections of tissues were implanted in paraffin wax and sectioned into a thickness of 10 µm. The sectioned tissues were stained with haematoxylin and eosin and the follicular phases of hairs were examined under microscope with an ocular micrometer (Sawada et al., 1987). Individual hair follicles were confined to specific hair cycle stages (telogen or anagen I–VI), based on the classification of Chase. The percentage of hair follicles in each defined cycle stage at 10, 20, and 30 days was calculated.

Hair follicle counting

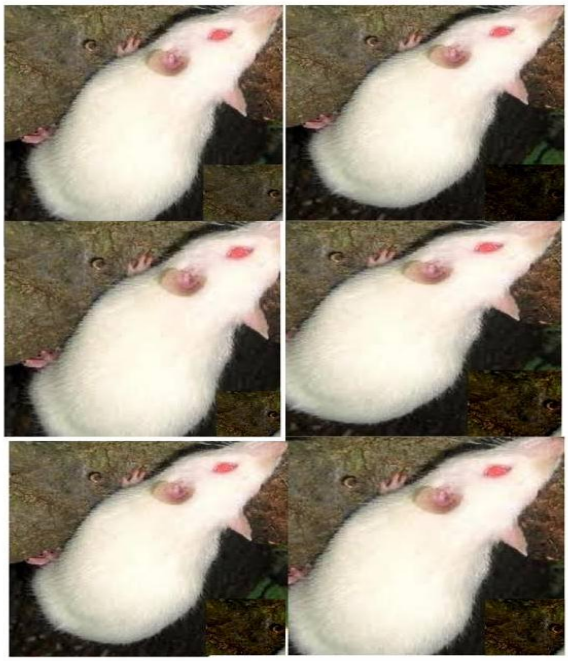
Digital photomicrographs were taken from representative areas of slides at a fixed magnification of 100 ×. All images were cropped in a fixed area with a width of 1500 um. We then manually counted hair follicles in deep subcutis (n = 25/rat).

Statistics

The data for hair growth length of albino rats were expressed as mean ± SEM. The values obtained for the above parameters were compared with control group using Student's t-test. The values of $p<0.01$, $p<0.05$ and $p<0.001$ were considered to indicate a significant difference between the groups.

RESULTS

Hair gel containing both pet.ether and aqueous extract of Daucus *carota* exhibited positive results during the in-vivo animal experimental studies for promoting hair growth, results of studies shown the better effect of hair gel with pet.ether extract than aqueous extract. Results of study are reported in table 1 for hair length determination on test days. The time taken to complete the hair growth cycle was also affected by the hair gel treatment and was found to be 20 days for petroleum ether gel followed by minoxidil (20days) and then aqueous (25days) and finally control (30 days). Though there was not much change in results in terms of hair length between minoxidil and pet.ether treated animal group but in terms of quantitative histo morphological studies pet.ether gel treated animal showed the better response than all the other groups both interms of number of follicle and conversion between growth phase rate. Results of the study are tabulated in three different part as time taken for initiation and complete growth of hair follicle in standard, control and hair gel treated group of animals (Table 1).



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Initially the skin color of denuded area was pink and became dark along with anagen initiation. Since the active growth of hair follicles and black pigmentation during the anagen phase, the hair growth-promoting activity of extract based hair gel was evaluated by observing the skin color. More blacken skin areas were observed in pet ether extract-treated group at 10 days, compared to the control or 1% minoxidil group. There were 5 to 7 hair follicles counted per mm of the skin for both pet.ether, aqueous extract and control treated groups and on histological observation, there was no difference in the number of hair follicles post treatment in group 1, and 4 but group 2 and 3 exhibited increase in the number of follicle in the range of 30-40%. The difference in the various cyclic phases of the hair follicles such as anagen and telogen phases for the treated and control groups were examined and the findings were recorded in table 3. At 20 days, we observed that pet.ether extract promoted hair growth more prominently as comparable as 1% minoxidil treated group. At 20 days, dorsal skin hairs were fully recovered in extract-treated rats, whereas only 50% of the dorsal skin area in the control group was covered with hairs. These results suggest that extract induces early telogen-to-anagen conversion of hair follicles. Immuno histochemical analysis reveals earlier induction of β-catenin and Shh proteins in hair follicles of the extract-treated group. Quantitative analysis of effect of herbal gel on hair length or growth of the hair in albino rats reported in table 2. Percentage of hair growth follicle on anagen/telogen phases during the treatment time in table 3. Results tabulated are self-explanatory indicating pet. ether extract based herbal gel as effective hair growth promotion activity, and was able to sustain the hair follicle between anlagenic to telogenic conversion.

Table 1: Time taken for initiation and complete growth of hair follicle in standard, control and hair gel treated group of animals.

Treatment Groups	Dose (%)	Number of days for hair growth initiation	Number of days for Complete hair growth
Control(G-1)	--	11	30
Pet. Ether hair Gel (G-2)	5%	6	20
Aqueous extract Hair Gel (G-3)	5%	7	25
Standard Drug (minoxidil) Treated Group (G-4)	1%	5	20

Table 2: Effect of herbal gel on hair length or growth of the hair in albino rats

Treatment Groups	Dose (%)	Length of the Hair(mm)			
		Day15	Day 20	Day 25	Day 30
Control(G-1)	--	7.1±1.4	10.5±0.6	12.5±0.4	13.5±0.6
Pet. Ether hair Gel (G-2)	4%	8.2±0.4***	15.6±0.7**	17.1±0.6***	18.1±1.2*
Aqueous extract Hair Gel (G-3)	4%	7.9±0.2	13.5±0.3**	15.5±1.2*	16.5±0.3***
Standard Drug (minoxidil) Treated Group (G-4)	1%	8.4±0.6**	15.4±0.6***	17.3 ±0.5*	17.9±0.6*
*p < 0.01, **p < 0.05, ***p <0.001, Compared to control group by students t-test (n=25hairs).					

Table 3: Percentage of hair growth follicle on anagen/telogen phases during the gel treatment time

Treatment Groups	Dose (%)	Percentage of hair folicles					
		Day 10		Day 20		Day 30	
		Anagen	Telogen	Anagen	Telogen	Anagen	Telogen
Control(G-1)	--	34	61	40	60	56	44
Pet. Ether hair Gel (G-2)	5%	43	67	62	48	70	30
Aqueous extract Hair Gel (G-3)	5%	42	60	48	52	67	33
Standard Drug (minoxidil) Treated Group (G-4)	1%	41	58	57	43	69	31

CONCLUSION

In this study the denuded dorsal area of albino rats when treated with herbal hair gels prepared with both pet.ether and aqueous extracts of Daucus *carota* revealed both the extract possess hair growth promotion activity, that petroleum ether extract captivated greater potential in promoting the hair growth and induced the anagen phase in telogen (resting) phase of hair follicles. This pattern of growth may be due to the premature shifting of hair follicles from the telogen to anagen phase (Philpot *et al*., 1992). The growth of hair was dense in petroleum ether extract treated group which explains the increased induction of follicles in anagen phase. The transformation of hair follicles from telogen phase to anagen phase in treated groups may be due to the epithelial cell proliferation near the base of the follicles that induce vasodilatation of blood vessels in the scalp as reported by Savin and Atton (1993). However, the exact

mechanism of the Daucus *carota* has been unknown and hence identification and isolation of active constituents from the extracts may laminate new directions for treatment of alopecia. Further research is needed for structural elucidation and identifying the mechanism of action responsible for using cabbage as an apparent hair growth promoter.

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