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Original Research Article

Effect of citrullus colocynthis on hair growth in albino rats

Sandeep Kumar*
Department of Zoology, Government College, Matanhail, Jhajjar – 124106, Haryana, India
*Corresponding author, E-mail: sandeepsheoran0140@gmail.com

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ABSTRACT
Ayurveda, the conventional Indian medical system, usually praises Citrullus colocynthis Schrad (Cucurbitaceae) as a hair tonic. Therefore, research was done to determine how C. colocynthis extracts in petroleum ether and ethanol affected albino rat hair development. On the shaved, denuded skin of albino rats, the extracts mixed with the oleaginous ointment base were applied topically. The length of time needed for the beginning and end of the hair growth cycle was noted. The standard was a topically administered 2% minoxidil solution. When petroleum ether extracts were used as a treatment, the time it took for hair to start growing was dramatically shortened—by half—when compared to untreated control animals. Additionally, the amount of time needed for full hair growth was greatly decreased. More hair follicles (>70%) were successfully brought into the anagenic phase by the treatment than by regular minoxidil (67%). Treatment with petroleum ether extracts at 2 and 5% produced results that were comparable to those of regular minoxidil.

1. Introduction

For more than 2000 years, dermatologists have recognised hair loss as a condition. It is widespread throughout the world and is thought to afflict close to 2% of the population [1, 2]. Alopecia has been seen as a significant adverse effect of anticancer, immunosuppressant, and many other pharmacological therapies, in addition to metabolic and genetic reasons. Currently, minoxidil [3], which is helpful for both male and female pattern baldness, and finasteride [4], which is helpful for treating male pattern baldness, are two synthetic medications approved by the U.S. FDA that are used concurrently to treat androgenic alopecia. However, their adverse effects have decreased their use.

In the cosmetic and hair care industries, natural goods are categorically promoted, and around 1000 plant extracts have been tested for use in hair care. The grape seed proanthocynidine and the saw palmetto b-sitosterol have both demonstrated exceptional hair growth-promoting effects [5]. There are numerous products on the market that are made by combining one or more herbal medications and are acceptable as hair tonics, hair growth promoters, hair conditioners, hair cleaning agents, antidandruff agents, and for the treatment of alopecia and lice infection [6, 7].

Numerous herbal medications are advised by India's traditional medical systems (Ayurveda and Unani) to encourage hair growth. However, their use is constrained by a lack of reliable scientific data. Traditional literature has suggested the herb Citrullus colocynthis Schrad (Cucurbitaceae) as a hair growth promoter [8]. The medication colocynth of commerce is made from the dried pulp of the unripe but fully developed fruit that has been liberated from the rind and is used to cure hair loss [9]. According to reports, ethnic tribes utilise the plant's seed oil to prevent hair from thinning too quickly and becoming grey [10, 11]. Ayurvedic literature Bhavprakash discusses the usage of the medication for the treatment of "Indralupta," or the medication used to treat hair loss. The Sanskrit term "Indrayan" most likely refers to the drug's purported ability to rejuvenate hair growth [12]. The medication was listed in Martindales Extra Pharmacopoeia and the Indian Pharmaceutical Codex.

In addition to being used to stimulate hair growth, C. colocynthis is also utilised as a hypoglycemic [13], antifertility agent [14], and anti-inflammatory agent [15].

In the present work, we investigated the commencement and encouragement of hair development in response to ethanol and petroleum ether extracts of C. colocynthis.

2. Materials and methods

Plant material and extract preparation

The fruits of C. colocynthis were purchased in November at a local market in Charkhi Dadri (Haryana, India), and their authenticity was confirmed by comparing them to those listed in the Indian Pharmaceutical Codex [8]. In the herbarium at the Department of Pharmaceutical Sciences, G.J.U.S.&T., Hisar, a voucher specimen for the plant has been placed. The plant material was dried in the sun, and the medicine was extracted using a moderately coarse powder (all particles passed through a sieve of 710 µm, and not more than 40% passed through a sieve of 250 µm). Analytical grade materials were utilised throughout.

500 g of dried, coarse C. colocynthis powder were placed into a Soxhlet extractor, and petroleum ether (60–80 ^{o}C) was used to extract the material initially until it was all used up. The marc was further extracted with ethanol (95%) in a Soxhlet extractor until it was fully depleted. Petroleum ether and ethanol extracts produced yields of 4.7%w/w and 4.1%w/w, respectively, after solvent removal at reduced pressure.

Characterization of extracts

To characterise the extracts, precoated silica gel-G plates (10×10) (E. Merck, Darmstadt, Germany) were subjected to thin-layer chromatography. For the ethanol extract, chloroform: methanol: water (7:2.1:0.9 v/v) provided the best resolution. The optimum mobile phase for resolving petroleum ether extract was toluene:ethyl acetate (84:16 v/v) [16]. Using 50% ethanol H_2SO_4 as a derivatizing agent, the spots were seen.

Animals

Wistar strain albino rats of either sex, weighing 120–150 g, were given access to a regular diet and unlimited amounts of water. The animals were kept in a standard day/night cycle (0600 h to 1800 h) at room temperature (24±2^{o}C). The Institutional Ethical Committee of G.J.U.S.&T, Hisar approved the protocol before any animal testing was done. The CPCSEA, India, regulations were strictly adhered to.

Preparation of samples

2% and 5% (w/w) of the petroleum ether and ethanol extracts, respectively, were added to the ointment base. Ointments were created in accordance with the Pharmacopoeia of India's instructions [17]. The created oleaginous base was then given the extracts.

Treatment

Each group of animals contained six rats. The following treatment was administered to various groups of animals:

Group I: Used as the control and merely a vehicle.

Group II: Topically applying an ointment base containing 2% petroleum ether extract.

Group III: Topically applying an ointment base containing 5% petroleum ether extract.

Applying 2% ethanol extract topically in an ointment base is Group IV.

Group V: Topically applying an extract of 5% ethanol based ointment.

Application of a 2% alcohol solution of minoxidil topically is considered Group VI (standard).

Hair clippers and electric shavers were used to trim the hair on the rats' dorsal sides. To accomplish total denual of a 6 cm^2 area, a commercial hair removal product (Anne French) was also utilised [18].

Toxicity studies

In order to check for skin rashes, irritation, or allergic reactions, the petroleum ether and ethanol extracts were applied topically to denuded skin for seven days at a concentration of five percent [19]. The produced extracts were therefore suitable for topical application. The institutional ethical review board gave its approval for using animals in research.

Statistical analysis

Data is presented as the mean SEM. Using the Instat v 2.1 statistical analysis programme, one-way ANOVA was used to compare all test groups to the control group, followed by Dunnett's test.

Qualitative hair growth study

The two metrics of (a) hair growth initiation time, which is the shortest amount of time required to begin noticeable hair growth, and (b) hair growth completion time, which is the shortest amount of time required to completely cover the denuded skin region with new hair, were used to evaluate the quality of hair growth [20]. For each group of animals, the time between the start and finish of hair development was noted.

Quantitative hair growth study

For the quantitative evaluation of C. colocynthis extract, the Uno technique [21] was used. After 10, 20, and 30 days of therapy, one rat from each group was slaughtered. Skin biopsies were obtained from the shaved area, and specimens were stored in 10% formalin. Blocks were made for microtomy after the specimens were set on paraffin wax. After fixation, a semiautomatic rotary microtome was used to cut vertical pieces of the skin. Hematoxylin and eosin was used to stain the sections. A microscope was used to calculate the ratio of hair follicles in the anagen (active growth phase) and telogen (resting phase) cyclic phases as well as the number of hair follicles per millimetre area of skin. By analysing the growth cycle of 100 hairs and the length of the hair follicle, a hair folliculogram was created [22].

3. Results and discussion

Qualitative studies on hair growth

The petroleum ether extract of C. colocynthis significantly shortened the time it took for hair growth to begin and finish. Hair growth in the denuded area started in the control group animals in the second week, whereas it was noticed in the petroleum ether extract-treated groups and the minoxidil-treated groups in the first week. On the fourth day, petroleum ether extract of C. colocynthis was applied to start hair growth. In the standard group that received minoxidil treatment, hair growth didn't start until the sixth day, whereas it did on the fifth day with the 2% C. colocynthis ointment. Even though the 2% and 5% ethanol extract-treated groups showed a marginally shorter hair growth initiation time than the petroleum ether extract-treated group did, the difference was not as noticeable (Table 1). The petroleum ether extract of C. colocynthis also had a substantial impact on how long it took for all of the hair to grow back on the shaved area. Complete hair growth was seen on days 18 and 16 of petroleum ether extract treatments, respectively. On day 19, full hair growth was seen with regular minoxidil. After 24 days, full hair growth was observed in the animals in the ethanol extract and vehicle control groups. Further research revealed that the hair in the petroleum ether extract-treated group was coarse, hard, and rough in comparison to the short, silky hair in the minoxidil (2%)-

treated group, and the coarse, hard hair in the ethanol extract-treated animals (Figure 1).

Table 1: Effect of Citrullus colocynthis Schard extracts on qualitative hair growth.

Sample no.	Treatment (topical)	Hair growth (days)	
		Initiation time	Completion time
1.	Control (vehicle only)	11±0.78	23±1.01
2.	Standard (2% minoxidil solution)	5±0.39***	19±1.07***
3.	Petroleum ether extract (2% ointment)	4±0.31***	17±0.55***
4.	Petroleum ether extract (5% ointment)	3±0.99**	17±0.52**
5.	Ethanol extract (2% ointment)	10±0.56	23±1.55
6.	Ethanol extract (5% ointment)	9±0.97*	23±1.27

Values are mean ±SEM, $n = 6$.
*$p < 0.05$
**$p < 0.01$
***$p < 0.001$, significance versul control.

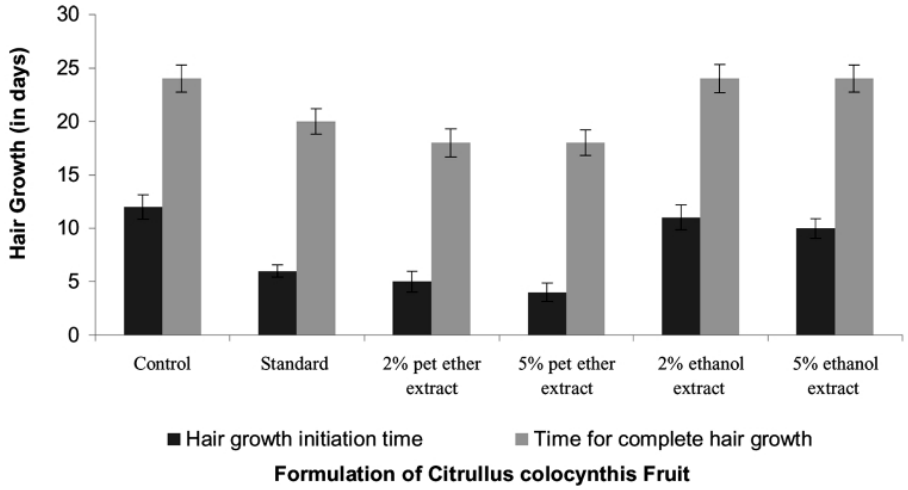

Figure 1: Qualitative effect of extracts of Citrullus colocynthis Schrad on hair growth.

The outcomes unmistakably demonstrate that the petroleum ether extract was effective in shortening the time required for the commencement and completion of hair growth. Further findings point to C. colocynthis petroleum ether extract having superior efficacy than minoxidil in terms of initiating and completing hair growth.

Quantitative studies on hair growth

The cyclic phase of hair development varied significantly between the groups that received minoxidil and the petroleum ether extract of C. colocynthis. Most of the hair follicles in the vehicle-treated control group animals are in the telogenic phase; only one or two are in the catagenic phase (PM I). Similar results are seen in the ethanol extract-treated group, where the majority of follicles are in the telogenic phase and just a small number are in the anagenic phase, with no anagenic hair follicles (PM II). In the petroleum ether extract-treated and minoxidil-treated groups, the situation is completely reversed, with the majority of hair follicles in the anagenic phase and very few in the catagenic phase. The number of telogenic follicles is extremely small (PM III-IV).

Table 2: Hair growth population (anagen/telogen ratio) after treatment with extracts of Citrullus colocynthis Schard.

Treatment[a]	Percentage of hair fossicles (%)						
	After 10 days		After 20 days		After 30 days		T/A ratio
	Telogen	Anagen	Telogen	Anagen	Telogen	Anagen	
Control (vehicle)	57±0.3	41±0.3	54±0.3	44±0.3	52±0.2	46±0.2	1.13
Standard (2% minoxidil)	53±0.2	45±0.2	36±0.8	61±0.8	32±0.4	66±0.4*	0.48
2% pet. ether extract	51±0.2	47±0.2	36±0.1	62±0.1	27±0.1	71±0.1*	0.38
5% pet. ether extract	46±0.3	53±0.3	33±0.1	65±0.1	24±0.1	74±0.1*	0.32
2% ethanol extract	54±0.4	44±0.4	49±0.5	49±0.5	45±0.7	53±0.7	0.85
5% ethanol extract	53±0.3	45±0.4	45±0.1	53±0.1	42±0.5	56±0.5	0.75

Values are % mean ± SEM.
T/A telogenic/anagenic ratio after 30 days of treatment.
[a]All extracts were applied topically; *$p < 0.05$ (considered siginifcant).

The percentage of the anagenic population increased noticeably (Table 2). The percentage of anagenic hair follicles was determined to be 47±0.3% in the vehicle-treated control group, 72±0.2% in the 2% petroleum ether extract treatment group, and 75±0.2% in the 5% petroleum ether extract treatment group. 67±0.5% of hair follicles were seen to be in the anagenic phase when treated with minoxidil. Treatment with ethanol extract also improved the population of anagenic hair follicles, although it was not as significant as treatment with petroleum ether extract (Table 2).

Length of hair follicles

Treatment with C. colocynthis petroleum ether extract at 2% and 5% had a notable impact on hair follicle length. Only 34±0.4% of the control group's hair had an average length of 0.5 mm, but in the extract-treated groups, 46±0.3% and 48±0.1% of the hair population had an average length of more than 0.5 mm, respectively. The treatment's results were equivalent to those of the minoxidil group, where 49±0.1% of the population had hair that was at least 0.5 mm long (Figure 2).

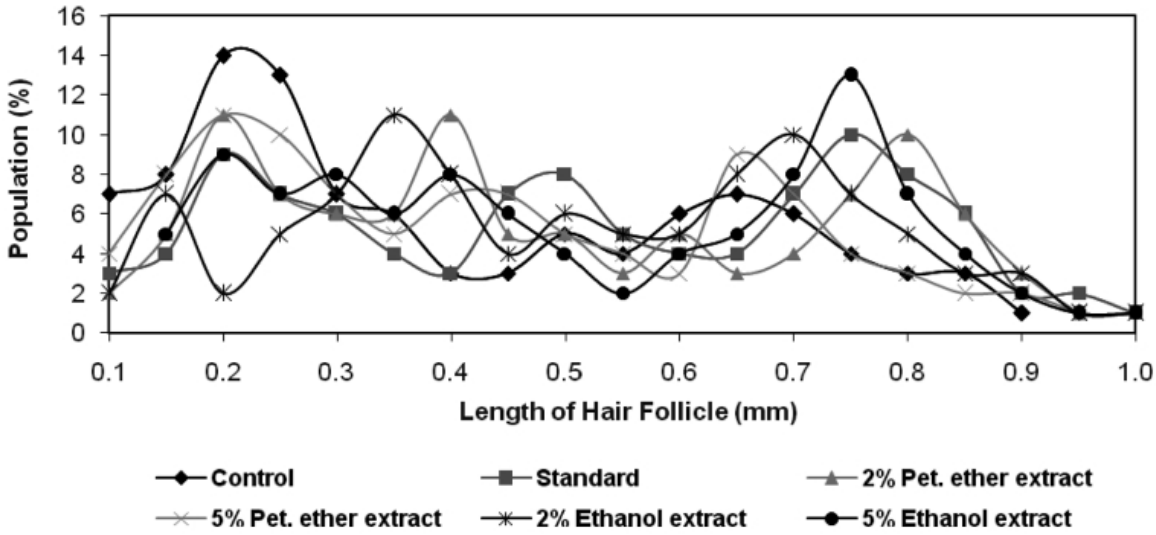

Figure 2: Percent frequency distribution of hair follicle population after 30 days of treatment with C. colocynthis.

4. Conclusions

The petroleum ether extract of the C. colocynthis fruit, when applied topically, was more effective than the conventional (minoxidil 2%) solution in shortening the time it took for hair to start growing. The minoxidil-treated group had fine, soft hair, but the petroleum ether extract-treated group's was coarse, harsh, and hard.

The treatment with petroleum ether extract resulted in an early transition of the hair follicle from the dormant telogenic phase to the active anagenic phase. It also aided in the retention of anagenic hair follicles in the treated mice. In comparison to the control group, the percentage of hair follicles in the anagenic phase showed a significant improvement.

The best extract for triggering both the beginning and the end of hair growth was petroleum ether. Treatment with minoxidil came next. Thus, the study demonstrates that in terms of hair development in rats, the 2% minoxidil therapy and the petroleum ether extract (5%) treatment are comparable. The herb's effects in promoting hair development are also supported by the remarkable increase in hair follicle length.

Dihydrotestosterone (DHT) is a key player in the process of androgenetic alopecia (AGA), which is characterised by the persistent miniaturisation of androgen-responsive hair follicles and the accompanying perifollicular fibrosis of follicular units in histologic analysis [23]. Therefore, 5a-reductase inhibitory activity may be responsible for the retention of late anagenic follicles as well as an increase in follicular length and inhibition of their miniaturisation. To investigate the potential of this mechanism, however, in-depth studies in this area are required.

The regular transition of hair follicles from the telogen to the anagen phase in the petroleum ether extract-treated group is a positive confirmation of C. colocynthis' ability to grow hair. The secondary germ associated with aggregated dermal papilla cells in telogen follicles increased as a result of the treatment, and the ongoing expansion and differentiation of these cells may have led to the development of new anagenic follicles [24].

The ethnomedical use of this herb for treating hair loss is supported by the current investigation. Commercial use of C. colocynthis calls for further application of the petroleum ether extract and its inclusion in a formulation.

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