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Effects of *Withania somnifera* root extract serum application on hair health in healthy adults: A prospective, double-blind, randomized, parallel, placebo-controlled study

Chinmai Yerram, Aditya Jillella *, Venkateswar Reddy

Skintis Clinic Medical and Cosmetic Dermatology and Gynecology, Hyderabad, India

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ABSTRACT

Background: Alopecia is a dermatological condition affecting genders, negatively impacting their personality and quality of life (QoL). The current approved entities are limited, inconsistently effective, and associated with negative side effects. To alleviate this issue, ayurvedic herbs such as Ashwagandha have being explored. As a result, this study was designed to evaluate the efficacy and safety of Ashwagandha (root extract) topical formulation (serum) on hair health in healthy adults.

Methods: In this prospective, double-blind, randomized, placebo controlled, two arm, parallel, comparative study, the effects of topical Ashwagandha on the hair health was evaluated. Healthy adults between 18 and 45 years with mild to moderate hair loss were randomized to either Ashwagandha (topical) or Placebo (topical) treatment. The participants were assessed at Day-1 and Day-75 for change in efficacy parameters, which included 60 Seconds Hair Comb, Trichoscan analysis, Hair Pull test, Investigator's Global Assessment (IGA) and QoL using Hair-specific Skindex-29.

Results: In the per protocol analysis of 61 participants, Ashwagandha group demonstrated significant reduction in hair shedding in the 60 Seconds Hair Comb test compared to Placebo at day 75. Similarly, Ashwagandha substantially enhanced hair density, growth, and thickness compared to the Placebo group (density = 7.3 vs. 2.8, $P < 0.001$; growth = 21.7 vs. 4.2, $P < 0.001$; thickness = 1.8 vs. 0.9, $P < 0.001$). In addition, Ashwagandha significantly improved QOL compared to placebo (Score = −17.3 vs. −6.1, $P = 0.011$).

Conclusion: The study found that topical Ashwagandha (serum) improved hair growth and hair health indicators. Thus, it can be an effective and safer alternative for alopecia.

Study registration: CTRI, Number CTRI/2022/11/047539, Registered on: 23/11/2022.

1. Introduction

Alopecia, or hair loss, is a dermatological disorder that affects both gender and has a significant impact on self-esteem and personal appearance, potentially leading to depression and other negative consequences [1,2].

Individuals may have isolated regions of hair loss or more widespread hair loss, such as substantial hair thinning or increased hair shedding [3]. Alopecia is caused by a combination of nutritional, autoimmune, and environmental causes [2,4,5]. There are other types of alopecia, including androgenic alopecia, alopecia areata (the formation of circular bald patches on the scalp), alopecia total (complete hair loss of the scalp), and alopecia universal (entire body hair loss), but the most prevalent is androgenic alopecia (AGA) [6–8].

Humans are born with roughly 100,000 terminal hair follicles on the scalp that are genetically predisposed to generate long and thick hair [9]. The hair development cycle is divided into three stages: anagen, catagen, and telogen. Anagen lasts for three to five years, catagen for two to three weeks, and telogen for three to four months, all of which are followed by the hair-shedding phase. Hair is released and lost during the telogen phase, also known as the resting period, and the next cycle can start at any time [10–12]. Typically, 90 % of the hair on a healthy scalp is growing, 1 % is involution, and the remainder is resting (<10 %). In general, all kinds of alopecia decrease the hair development cycle and

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* Corresponding author. Skintis Clinic Medical and Cosmetic Dermatology and Gynecology, Hyderabad, Telangana, 500035, India.
E-mail addresses: chinmayi.yerram@gmail.com (C. Yerram), aditya.jillella@gmail.com (A. Jillella), jvrobgdr@gmail.com (V. Reddy).

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cause hair loss [10,11,13,14].

Hair fall is a common and stressful symptom, distressing the quality of life of an individual, thereby necessitates optimal therapy [15]. For the treatment of alopecia, allopathic medications, including minoxidil and finasteride, are available on the market, but many of them fall short of expectations and have undesirable adverse effects, including hypotension, recurrence of alopecia, loss of libido, impotence, decreased ejaculate volume, swelling of the lips, skin rashes, and others [6,17–19].

There is an unmet need for therapies providing satisfying, long-term results for alopecia. Herbal therapies have been used to treat alopecia in traditional medical systems since ancient times. More than a thousand plant species, including the Ashwagandha plant, have been explored for their potential benefits in hair care [20,21]. Ashwagandha [*Withania somnifera*, (WS) fam. Solanaceae] is also known as "Indian Ginseng" or "Indian Winter Cherry". It is a vital herb in Ayurveda (India's traditional system of medicine), and has been used for millennia as a Rasayana for its numerous health benefits [20–24].

Ashwagandha is a versatile plant with antioxidant, anti-inflammatory, neuroprotective, adaptogenic, memory-enhancing, hematopoietic, sleep-inducing, and anxiolytic activities, according to pharmacological investigations [22,24,25]. Adaptogens, such as Ashwagandha, have long been used to lower stress and cortisol levels in the body [26]. Stress is a major cause of hair loss and shedding. Ashwagandha could potentially alleviate some of the symptoms associated with stress and anxiety, including minimizing hair loss. Ashwagandha can contribute to healthy hair as it exerts antioxidants and anti-stress effects [22,24,26], which can help to strengthen the hair and minimize breakage. Ashwagandha has been used for centuries to treat a variety of ailments, including hair loss. There is a scarcity of scientific data to back up the traditional facts.

Although there is scientific evidence supporting the use of Ashwagandha in hair health, it is limited in number and rigorous study methods. As a result, further research through rigorous randomized clinical trials is required.

The purpose of this clinical study was to assess and compare the efficacy and safety of an Ashwagandha root extract topical formulation (serum) on hair growth and hair health metrics in healthy adults to an identical placebo. The primary objectives of this study were to evaluate the changes in the hair shedding test and hair analysis parameters (hair density, hair diameter, hair growth, anagen/telogen ratio) between the two groups from baseline (Day 1) to the end of the study (Day 75).

2. Methods

2.1. Study design

The present study was a 75-day prospective, double-blind, randomized, placebo controlled, two arm, parallel, comparative clinical study. Its primary purpose was to evaluate the efficacy and safety of Ashwagandha root extract topical formulation (serum) on the hair health in healthy adult humans compared to placebo. It was conducted in a single centre cosmetology clinic in Hyderabad, Telangana (India).

The study participants were randomly allocated into two interventional treatment groups in a ratio of 1:1 to prevent treatment allocation bias. The patients and study investigators were blinded to the treatment allocation. Out of 92 subjects screened, 68 subjects were enrolled in the study. All the enrolled subjects were randomized to either of the two interventional treatments; Ashwagandha (topical formulation) or Placebo (topical formulation).

2.2. Study protocol and ethical aspect

The study protocol was approved by the Deccan Independent Ethics Committee (Study code: IB−HH−CT08−22, Approval date: 31−10−2022).

The study was conducted in accordance with the Declaration of Helsinki (2016 amendment) and Good Clinical Practice (GCP) guidelines. In addition, Ethical Guidelines for Biomedical Research on Human Subjects, were also followed. The study was registered with Clinical Study Registry of India (Registration number CTRI/2022/11/047539, Registered on: 23/11/2022) and is completed. Written Informed Consent was taken from all the subjects before enrolling in the study. A Consolidated Standard of Reporting Trials (CONSORT 2010) flow chart (Fig. 1) and checklist has been completed.

2.3. Participants

Healthy adults of either gender between 18 and 45 years of age were screened for study eligibility based on the inclusion and exclusion criteria. The study was conducted in a single clinic setup and the study participants were selected from the clinic and from the vicinity of the clinic.

All the participants were explained in detail regarding the purpose, procedure, and potential risks and benefits of the study and signed/written informed consent was obtained from them before the commencement of the study.

2.3.1. Inclusion criteria

The present study included healthy adults of either gender, aged between 18 and 45 years with mild to moderate hair loss including androgenic alopecia. Subjects having ability to comply with the study protocol, willing to give signed/written informed consent, willing to undergo Trichoscan hair test, and likely to be compliant with the prescribed investigational products, were included in the study.

2.3.2. Exclusion criteria

The exclusion criteria included a) Participants with severe seborrheic dermatitis, alopecic disease (except for androgenic alopecia) and scalp disorders, such as scalp psoriasis and infection, b) Participants with history of clinically significant medical conditions or psychiatric conditions, c) Pregnant and lactating women and those with known hypersensitivity or hypersensitivity to Ashwagandha, d) Participants who had undergone or planned for surgical correction of hair loss or hair transplantation and d) Participants on any drugs or supplements for hair loss, including finasteride, any other 5 α-reductase inhibitors, minoxidil, steroids, or hormonal products, during the last 3 months were also excluded.

2.4. Investigational products

Product details: KSM-66 Ashwagandha is a root extract of ashwagandha manufactured using an aqueous based extraction process. It is slightly hygroscopic and yellowish brown in color. It is standardized to >5 % of total withanolide content, consisting mainly of Withastramonolide A, Withanoside IV, Withanolide A, Withanone. It also consists of <0.1 % of withaferin A.

The investigational products used in this study were topical formulations of Ashwagandha root extract (5 %) serum and placebo. Topical hair serum formulations allow provide better absorption of the active ingredients and ease of application compared to other dosage forms and hence being selected. The investigational products were manufactured in a Good Manufacturing Practice (GMP)-certified facility (Shri Kartikeya Pharma, Telangana State, India) and were identical in appearance, color and packaging. The investigational product KSM66 Ashwagandha root extract was received as a gift sample from its manufacturer, Ixoreal Biomed Inc., Los Angeles, California, United States of America.

Quality control: The investigational product/subject material was tested for identification using HPTLC and for assay using HPLC (Available at https://doi.org/10.6084/m9.figshare.23912394.v2). The extract was subjected to various tests including microbial testing, pesticides, heavy metals, and aflatoxins. Extensive testing is done at all stages right from the receipt of raw materials, throughout the manufacturing

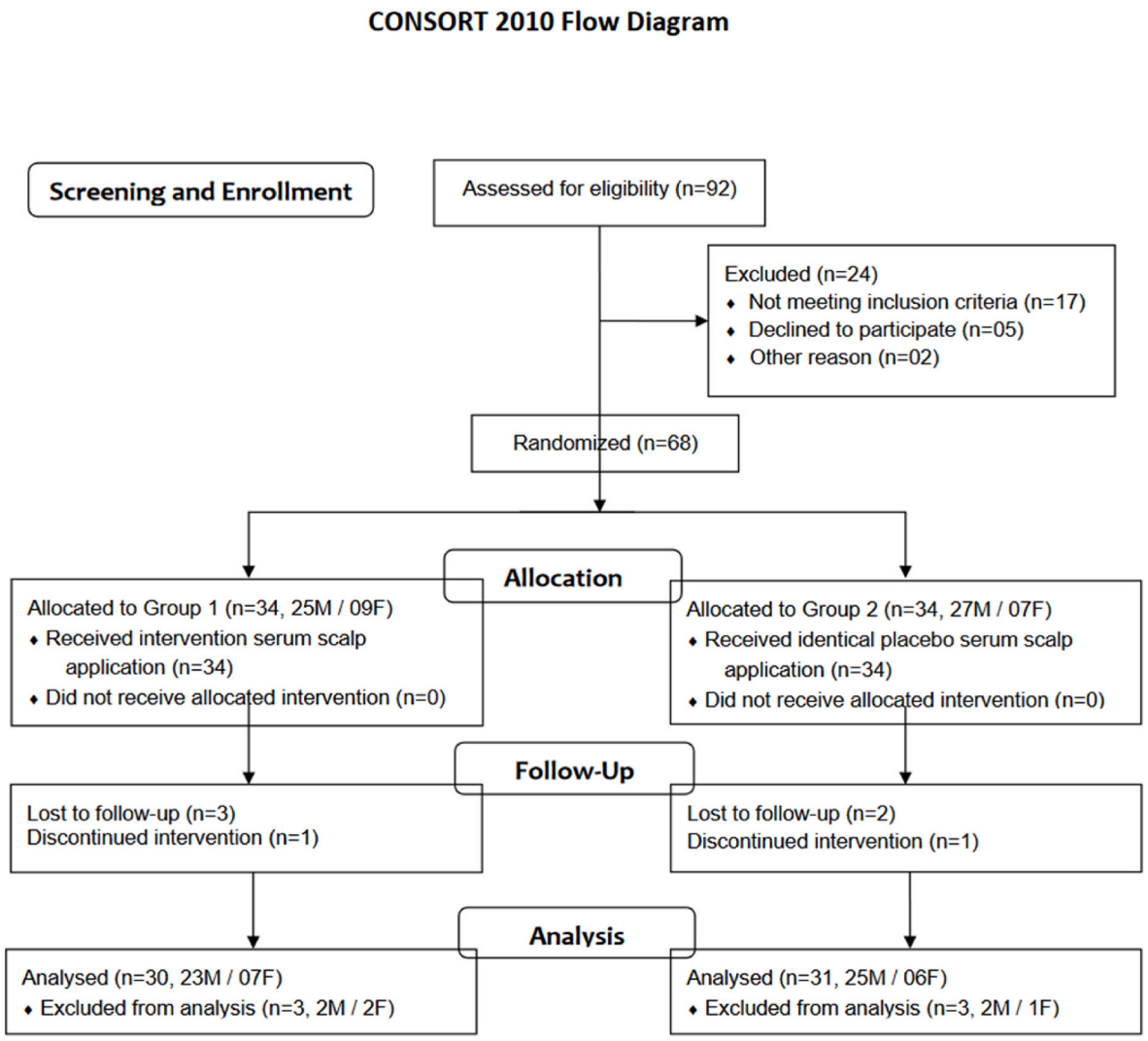

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flow chart- The enrollment, allocation, follow-up and analysis scenario of the study is depicted through the CONSORT flow diagram.

process, and the finished extract. The extract is manufactured using purely a water-based extraction method which is devoid of any chemical solvents or alcohol.

2.5. Intervention

All the enrolled subjects fulfilling the inclusion and exclusion were randomized to either of the two interventional treatment arms. Treatment group 1: Ashwagandha topical formulation (1–2 drops once a day for 75 days) or Treatment group 2: Placebo topical formulation (1–2 drops once a day for 75 days).

All the subjects were evaluated at baseline (day 1), day 37 and day 75. The outcomes measures mentioned below were assessed at baseline-Day 1 and Day 75 post-treatment. The subjects in the respective treatment arms were given required quantity of the study topical formulation for self-application, sufficient till the Day 75 $\pm$ 4 (End of study visit). Each subject was instructed to apply the study topical formulation 1 to 2 drops once a day for the study duration. They were instructed to take the formulation drops on the palm of hand and then to apply to the hair, working from the ends up to the middle of hair strands. They were given subject diaries to document the dosing details along with adverse events (if any).

2.6. Study procedure

Signed/written Informed consent was obtained from the participants during screening and enrolment. The participants were screened for brief medical history, general physical examination, vital parameters and hair assessment by a qualified dermatologist. Once enrolled, the participants were assessed for the efficacy parameters for hair health and quality of life (QoL) assessments which included: 60 Seconds Hair Comb test, Trichoscan hair parameters (density, growth, thickness, anagen phase, telogen phase and anagen/telogen ratio), Hair Pull test, Investigator's Global Assessment (IGA) and Hair specific Skindex-29. The safety parameters were assessed based on the reported adverse events.

2.7. Sample size

Sample size calculations was performed to determine the number of participants needed to detect effect sizes similar to those that have been reported in recent skin health trial [27]. The study was powered to detect a difference of 10.16 in hair density between the two treatment arms, with 80 % power and a significance level alpha of 5 % [27.] A sample of 31 subjects in each group with a total sample of 62 completed cases was determined. Furthermore, considering the drop-out rate of 10 %, the sample size was set to 68 (34 in each arm) to achieve 62

completed cases for the present study.

2.8. Randomization and blinding

Randomization schedule was generated by certified statistician by using a validated software (Rando version 1.2 for Windows). After signing the informed consent, the subjects were assigned a unique screening number. After eligibility of subjects was confirmed for the study, each subject was assigned a unique randomization number according to randomization schedule and accordingly, treatments were assigned by the clinical research coordinator. It was double-blind study; subjects as well as the investigator were blinded to the treatment allocation. Subjects were assigned randomly as per the randomization schedule, in a 1:1 ratio to receive either treatment of Ashwagandha topical formulation or Identical Placebo topical formulation by clinical research coordinator.

Tamper-proof and similar in look and color, the Ashwagandha and placebo topical formulation packets were created. All of the packets were coded to obscure their contents, as well as properly labelled with the subject serial number (ID of the study). Upon enrolment, participants were given a topical formulation pack with exactly the matching serial number. Unblinding was allowed only after completion of the data collection or in case of any serious adverse event. The randomization codes were and placed in a separate sealed envelope for each patient. Neither any member of study site nor the study monitor had access to the randomization code until the end of the study and data base was locked. The date and the reason for unblinding was recorded. In the event the Investigator required to break the blind for an individual subject during the study, the investigator would unseal the envelope and break the blind for that subject only.

2.9. Outcome measures

2.9.1. Primary outcome

2.9.1.1. Second hair comb test. Shedding of hair was assessment using 60 Second Hair Comb test. The test is simple, practical and objective tool for assessment of hair shedding [28]. The 60 s hair count test involves the following steps: using the same comb/brush for combing the hair for 60 s (over a sheet/towel of contrasting color for easier collection of hairs) from the (Vertex) back top moving to the front of the scalp, counting of hairs from comb/brush plus sheet and recording hair fall with bulb and without bulb values [28,29].

Primary efficacy assessment was change in the shedding range of the hair in the "60 Second Hair Comb test" from baseline (Day 1) to End of study (Day 75) in the two groups.

2.9.1.2. Trichoscan hair analysis. Trichoscan is a validated and precise tool for measurement of hair growth parameters It is a method that combines epiluminescence microscopy with automatic digital image analysis for the measurement of human, and potentially animal hair, in situ [30–32].

Procedure: The measurement site was chosen. The covered surface was dyed and then cleaned with an alcohol-based solution. Images were captured with a camera, with the lens pressed into the wet assessment area so that no bubbles were entrapped. The captured images were loaded into Trichoscan software, which performed the analysis automatically. In the subsequent visit, the same region was identified and evaluated [30–32].

Primary efficacy assessment was change in hair analysis parameters, hair density (n/cm2), hair diameter (μm), hair growth rate (mm/day), or anagen/telogen ratio from baseline (Day 1) to End of study (Day 75) in the two groups.

2.9.2. Secondary outcomes

2.9.2.1. Investigator's Global Assessment (IGA) scale. Investigator's Global Assessment (IGA) was used to measure distinctly and clinically relevant gradations of scalp-hair loss. It consists of a five-category ordinal response scale (*'None', 'Limited', 'Moderate', 'Severe' and 'Complete'*). It's corresponding percentage ranges with hair loss descriptors are as follows: *('None', 0 %; 'Limited', 1–20 %; 'Moderate', 21–49 %; 'Severe', 50–99 %; and 'Complete', 100 %)* [33].

Secondary efficacy assessment was change in Investigator's Global Assessment (IGA) from baseline (Day 1) to End of study (Day 75) in the two groups.

2.9.2.2. Hair Pull Test. Hair Pull test is also known as the 'traction test' or 'Sabouraud's sign'. It is used to determine how tightly hair is anchored to the hair papilla. This test is based on the concept of 'gentle' pulling of the hair to bring about shedding of telogen hairs. It aids in determining the extent and area of hair loss [29,34,35]. If more than 10 % of the hairs are pulled out of the scalp, this is regarded as a positive pull test and indicates active hair shedding [29,34,35].

Secondary efficacy assessment was change in Hair Pull Test from baseline (Day 1) to End of study (Day 75) in the two groups.

2.9.2.3. Hair-specific Skindex-29. Assessment of the quality of life (QoL) was conducted using the Hair-Specific Skindex-29. The Hair-Specific Skindex-29 scale comprises of three kinds of domains: a symptom scale (7 items), a function scale (12 items), and an emotion scale (10 items). Each statement in the Skindex-29 questionnaire will be scored on a 5-point Likert scale (*points; never = 1, rarely = 2, sometimes = 3, often = 4, all-the-time = 5*). Answers to each item is transformed to a linear scale, ranging from 0 (never bothered) to 100 (always bothered). A scale score is the average score from the responded items and a global score is the mean of the sums of each scale. A high score denotes impaired QoL, and a low score denotes mild damage in the QoL [36,37].

Secondary efficacy assessment was change in Hair-specific Skindex-29 scores from baseline (Day-1) to End of study (Day-75) in the two groups.

2.10. Safety assessment and statistical analysis

Assessment of the adverse events reports and serious adverse events was considered as part of the safety evaluation.

All the participants enrolled in the present study were analyzed according to their randomized group in the per-protocol dataset and intent to treat dataset for baseline, regardless of compliance with the treatment or any other deviation from protocol. MedCalc® Statistical Software version 20.217 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2023) was used to perform all relevant statistical computations. A descriptive analysis of demographic factors, primary and secondary efficacy measures was conducted out. For numerical and ordinal data, mean and standard deviation were calculated. Percent change from baseline were computed and reported as a percentage. The data normality was determined using Shapiro Wilk test. Independent sample *t*-test was performed to compare the differences in efficacy metrics between the two treatment groups. The study used 95 % confidence intervals (CI) to ensure best statistical standards. Cohen's d effect size was used to compute the mean difference to variability. All tests were performed using two-sided tests with statistical significance level of 5 %.

3. Results

3.1. Participants

A total of 68 healthy participants were enrolled in the study, with 34

participants randomized to each treatment group. The intent to treat (ITT) data represented from these 68 randomized patients and were used for analysis of baseline characteristics, efficacy and safety parameters. A total of 7 participants (4 in Ashwagandha group and 3 in Placebo group) were lost to follow-up or discontinued after treatment intervention. In summary, data from 30 participants receiving Ashwagandha and 31 participants receiving placebo were analyzed as Per-protocol (PP) for primary and secondary parameters (Fig. 1).

3.2. Baseline characteristics

The baseline characteristics of the participants in the two groups are depicted Table 1. The baseline demographic and baseline vital parameters were similar between the Ashwagandha group and Placebo group. There were 25 males and 9 females in the Ashwagandha group, and 27 males and 7 females in the Placebo group. No significant differences between the two groups were observed with respect to age, gender and anthropometric measurements (Height, Weight and BMI) (Table 1).

3.3. Baseline hair-related efficacy parameters

Table 2 summarizes the baseline hair-related efficacy parameters in the two groups (ITT and PP). No significant differences between the two groups were found at baseline with reference to the following efficacy parameters: 60 Seconds Hair Comb test, Trichoscan hair parameters (density, growth, thickness, anagen and telogen and anagen/telogen ratio), Hair Pull test, Investigator's Global Assessment (IGA) and Hair specific Skindex-29 QOL scores (Table 2).

3.4. Primary outcome

Post intervention day 75.
Per protocol analysis of Primary efficacy outcomes.
Mean change in hair related parameters at day 75 from baseline.

3.4.1. Seconds Hair Comb test

At day 75, the mean change in 60 Seconds Hair Comb test from baseline was significantly reduced in Ashwagandha group than with Placebo group (with bulb = -6.90 vs. −3.13, P < 0.001; without bulb = −7.07 vs. −3.13, P < 0.001) (Table 3).

Ashwagandha group exhibited significantly lower percent change from baseline in 60 s hair comb test compared to Placebo group (Supplementary file S1).

3.4.2. Trichoscan hair parameter analysis

At Day-75, the mean change from baseline in hair density, hair growth and hair thickness were significantly greater (increased) in Ashwagandha group than with Placebo group (hair density = 7.3 vs. 2.81, P < 0.001; hair growth = 21.76 vs. 4.25, P < 0.001; hair thickness = 1.80 vs. 0.90, P < 0.001) (Table 3). The ashwagandha group showed a substantial improvement in hair density, growth, and thickness at day 75 compared to baseline (Table 3).

The anagen and telogen phases in the two groups also exhibited a significant shift. At day 75 from baseline, the mean change in telogen was significantly decreased (−3.04 vs. −0.79, P < 0.001) in the Ashwagandha group compared to the placebo group, whereas the mean change in anagen was significantly improved (3.04 vs. 0.90, P < 0.001) in the Ashwagandha group compared to Placebo (Table 3).

3.5. Secondary outcomes

Per protocol analysis of Secondary efficacy outcomes.

3.5.1. Hair pull test

At day 75, the mean change from baseline in Hair Pull test was significantly reduced in Ashwagandha group than with Placebo group (no hair pulled = −1.83 vs. −0.71, P < 0.001; percent hair pulled = −8.07.07 vs. −4.84, P = 0.003) (Table 3).

3.5.2. Investigator's Global Assessment (IGA) scale

Similarly, the mean change in IGA score for hair loss at day 75 from baseline in the Ashwagandha group was significantly lower than in the Placebo group (no hair pulled = −8.50 vs. −3.87, P < 0.001) (Table 3).

3.5.3. Hair specific Skindex-29

At day 75, the mean change from baseline in Hair specific Skindex-29 and its subdomain scores significantly reduced (improvement in QOL) in Ashwagandha group than with Placebo group, with the exception in symptom subdomain (Symptom = −4.07 vs. −1.81, P = 0.052; Function = −8.47 vs. −3.0, P = 0.008; Emotion = −4.80 vs. −1.39, P = 0.024; Total score = −17.33 vs. −6.19, P = 0.011) (Table 3).

3.5.4. Percent change from baseline

The % change from baseline in hair-related efficacy parameters in the two groups according to per protocol analysis is available in **Supplementary file S1**. There were significant differences in the % change in the two groups for the 60 Seconds Hair Comb test, Trichoscan hair parameters (density, growth, thickness, anagen, telogen and anagen/telogen ratio) and, and Hair Pull test. Overall, compared to the Placebo group, the Ashwagandha group showed a significant % improvement in hair-related efficacy parameters (**Supplementary file S1)**.

3.6. Safety assessment

Over the course of the study, neither group experienced any adverse events or major adverse events. Topical application of Ashwagandha

Table 1
Demography and baseline vital parameters in patients randomized.

	Treatment	ITT dataset			PP dataset		
		N	*Mean (SD)*	*t (p)**	*N*	*Mean (SD)*	*t (p)**
Age (yrs.)	Ashwagandha	34	32.35 (9.53)	0.026 (0.980)	30	32.40 (8.02)	−0.258 (0.798)
	Placebo	34	32.29 (9.36)		31	32.97 (9.13)	
Height (cm.)	Ashwagandha	34	164.96 (8.95)	0.265 (0.792)	30	165.25 (8.70)	−0.004 (0.997)
	Placebo	34	164.41 (7.95)		31	165.26 (7.44)	
Weight (kg.)	Ashwagandha	34	62.63 (15.95)	−0.084 (0.934)	30	63.03 (15.01)	0.003 (0.997)
	Placebo	34	62.90 (10.11)		31	63.02 (10.43)	
BMI (Kg/sq.m)	Ashwagandha	34	22.78 (4.52)	−0.557 (0.580)	30	22.91 (4.42)	−0.215 (0.831)
	Placebo	34	23.34 (3.78)		31	23.13 (3.83)	
		N	*Count*	χ^2 *(p)*ˆ	*N*	*Count*	χ^2 *(p)*ˆ
Gender (M/F)	Ashwagandha	34	25/09	0.327 (0.567)	30	23/07	0.144 (0.704)
	Placebo	34	27/07		31	25/06	

ˆChi-square test; * Independent sample *t*-test (between group comparisons).
ITT, Intent-to-treat; M, Male; F, Female; PP, Per-protocol; SD, Standard deviation.

Table 2
Baseline scores in two groups for the randomized patients.

		ITT dataset			PP dataset		
	Treatment	*N*	*Mean (SD)*	*t (p)**	*N*	*Mean (SD)*	*t (p)**
60 Seconds Hair Comb test							
Hair with bulb (count)	Ashwagandha	34	15.94 (2.96)	0.045 (0.964)	30	15.47 (2.49)	−0.954 (0.344)
	Placebo	34	15.91 (2.37)		31	16.06 (2.41)	
Hair without bulb (count)	Ashwagandha	34	11.09 (3.01)	0.045 (0.964)	30	10.63 (2.58)	−0.670 (0.506)
	Placebo	34	11.06 (2.37)		31	11.06 (2.45)	
Trichoscan							
Hair density (/cm^2)	Ashwagandha	34	160.76 (21.10)	−0.012 (0.990)	30	160.53 (22.47)	0.027 (0.979)
	Placebo	34	160.82 (18.96)		31	160.39 (19.83)	
Hair Growth (μm/d)	Ashwagandha	34	300.35 (45.62)	−0.088 (0.930)	30	302.88 (47.99)	−0.034 (0.973)
	Placebo	34	301.35 (47.55)		31	303.31 (49.41)	
Hair Thickness (μm)	Ashwagandha	34	50.94 (2.96)	0.045 (0.964)	30	50.47 (2.49)	−0.954 (0.344)
	Placebo	34	50.91 (2.37)		31	51.06 (2.41)	
Anagen (%)	Ashwagandha	34	63.08 (3.16)	−0.327 (0.744)	30	63.04 (3.26)	−0.347 (0.730)
	Placebo	34	63.35 (3.77)		31	63.36 (3.94)	
Telogen (%)	Ashwagandha	34	36.92 (3.16)	0.327 (0.744)	30	36.96 (3.26)	0.347 (0.730)
	Placebo	34	36.65 (3.77)		31	36.64 (3.94)	
A:T Ratio	Ashwagandha	34	4.15 (0.89)	−0.570 (0.570)	30	4.15 (0.92)	−0.599 (0.551)
	Placebo	34	4.30 (1.18)		31	4.32 (1.24)	
Investigator's global assessment							
Percentage hair loss	Ashwagandha	34	41.03 (11.60)	−0.144 (0.886)	30	41.33 (12.03)	−0.182 (0.856)
	Placebo	34	41.44 (12.00)		31	41.90 (12.39)	
Hair Pull test							
Hair pulled (count)	Ashwagandha	34	3.38 (1.74)	0.226 (0.822)	30	3.23 (1.68)	−0.470 (0.640)
	Placebo	34	3.29 (1.47)		31	3.42 (1.41)	
Percentageof hair pulled	Ashwagandha	34	11.18 (3.70)	0.165 (0.869)	30	11.17 (3.87)	−0.129 (0.898)
	Placebo	34	11.03 (3.65)		31	11.29 (3.64)	
Hair specific Skindex-29							
Symptom score	Ashwagandha	34	18.71 (6.61)	0.148 (0.883)	30	18.57 (6.94)	−0.051 (0.959)
	Placebo	34	18.50 (4.73)		31	18.65 (4.85)	
Function score	Ashwagandha	34	31.29 (13.84)	0.055 (0.957)	30	31.03 (13.91)	−0.037 (0.971)
	Placebo	34	31.12 (12.79)		31	31.16 (13.11)	
Emotional score	Ashwagandha	34	27.06 (11.71)	−0.181 (0.857)	30	26.97 (11.57)	−0.280 (0.780)
	Placebo	34	27.53 (9.65)		31	27.74 (10.01)	
Total score	Ashwagandha	34	77.06 (30.38)	−0.013 (0.990)	30	76.57 (30.58)	−0.135 (0.893)
	Placebo	34	77.15 (25.17)		31	77.55 (26.11)	

* Independent sample *t*-test (Between group comparisons).
A:T, Anagen:Telogen; ITT, Intent-to-treat; PP, Per-protocol; SD, Standard deviation.

was well tolerated, with no reported local reactions or adverse reactions after application.

4. Discussion

The key results from this research indicate that using a topical formulation of Ashwagandha root extract (serum) for 75 days resulted in a significant improvement in hair growth and hair-related efficacy metrics when compared to a placebo.

Alopecia is a disorder that affects both men and women [1,2]. Although it is not incapacitating, it can cause considerable psychological and social discomfort [1,2,15]. Currently, alopecia treatment standards include surgical procedures and pharmacological treatments that include topical and oral pharmaceutical drugs [16,17]. Finasteride and minoxidil are the two most regularly used pharmaceutical drugs. Finasteride is a type 2 5-alpha reductase inhibitor that inhibits testosterone conversion to dihydrotestosterone (DHT) and enhances scalp hair growth in males by lowering DHT levels in the scalp [16,18]. Minoxidil, a vasodilator is hypothesized to promote hair growth by activating potassium channels and/or increasing prostaglandins [16,19]. Surgical procedures are costly and intrusive, while the pharmaceutical drugs now in use have an inconsistent effectiveness with a considerable risk of local and systemic side effects [16–19].

Traditional medicinal systems are being investigated in attempt to enhance hair health outcomes. Ashwagandha (*Withania somnifera*) is one such medicinal plant that has been explored. It is traditionally known that Ashwagandha has multiple benefits, including control of hair fall [21,24]. There is limited scientific evidence available to establish the traditional information available especially robust clinical study to support the use in hair health. In the present clinical study, an attempted was made to assess and compare the efficacy and safety of an Ashwagandha (root extract) 5 % topical formulation (serum) on hair growth and hair health metrics in healthy adults compared to identical placebo.

In the present study, we found a that Ashwagandha root extract topical formulation (serum) daily for 75 days resulted in improvement in the hair growth and hair health metrics in healthy adults in comparison to placebo. The subjects receiving Ashwagandha topical formulation showed a statistical reduction in hair shedding as reflected in reduction in hair fall in the 60 s comb test and Hair Pull test, respectively. Similarly, Ashwagandha topical formulation substantially enhanced hair density, hair growth, and hair thickness compared to the Placebo group as reflected in the Trichoscan hair analysis.

The anagen and telogen hair phases shifted significantly in individuals receiving Ashwagandha. By day 75, telogen hairs was considerably lower (−3.04 vs. −0.79, $P < 0.001$), but anagen hairs was significantly higher (3.04 vs. 0.79, $P < 0.001$) in the Ashwagandha group compared to the Placebo group. There was significant increase in the AT ratio in the Ashwagandha groups denoting increase in the number of growing hairs.

Studies have associated alopecia negatively impacting the personality and quality of life (QoL) of an individual [15,37]. In this study, we observed that participants receiving Ashwagandha topical formulation (serum) had a substantial improvement in quality of life, as seen by a lower score in Hair specific Skindex-29 scale as compared to the Placebo group ($p < 0.05$).

This study provides valuable insight on the usefulness of topical formulation of Ashwagandha root extract (serum) on the hair growth and hair health.

Table 3
Change form baseline in hair parameters in PP dataset.

		N	*Change from baseline*	*Difference between treatments*	*t (p)**	Effect size
			Mean (SD)	*Mean (95 % CI)*		*Cohens 'd' (95 % CI)*
60 Seconds Hair Comb test						
Hair with bulb (count)	Ashwagandha	30	−6.90 (1.58)	−3.77 (−4.75 to −2.79)	−7.692 (<0.0001)	−1.97 (−2.58 to −1.35)
	Placebo	31	−3.13 (2.19)			
Hair without bulb (count)	Ashwagandha	30	−7.07 (1.68)	−3.94 (−4.92 to −2.95)	−8.010 (<0.0001)	−2.05 (−2.67 to −1.42)
	Placebo	31	−3.13 (2.13)			
Trichoscan						
Hair density (/cm^2)	Ashwagandha	30	7.30 (1.70)	4.49 (3.74–5.25)	11.952 (<0.0001)	3.06 (2.31–3.80)
	Placebo	31	2.81 (1.19)			
Hair Growth (μm/d)	Ashwagandha	30	21.76 (5.30)	17.51 (15.40–19.61)	16.648 (<0.0001)	4.26 (3.34–5.17)
	Placebo	31	4.25 (2.44)			
Hair Thickness (μm)	Ashwagandha	30	1.80 (0.85)	0.90 (0.46–1.34)	4.077 (<0.0001)	1.04 (0.50–1.58)
	Placebo	31	0.90 (0.87)			
Anagen (%)	Ashwagandha	30	3.04 (0.62)	2.25 (1.96–2.54)	15.483 (<0.0001)	3.97 (3.09–4.83)
	Placebo	31	0.79 (0.51)			
Telogen (%)	Ashwagandha	30	−3.04 (0.62)	−2.25 (−2.54 to −1.96)	−15.483 (<0.0001)	−3.97 (−4.83 to −3.09)
	Placebo	31	−0.79 (0.51)			
A:T Ratio	Ashwagandha	30	−2.17 (0.61)	0.32 (−0.08 to 0.73)	1.610 (0.113)	0.41 (−0.10 to 0.92)
	Placebo	31	−2.50 (0.93)			
Investigator's global assessment						
Percent loss (%)	Ashwagandha	30	−8.50 (4.18)	−4.63 (−6.32 to −2.94)	−5.475 (<0.0001)	−1.40 (−1.96 to −0.84)
	Placebo	31	−3.87 (2.13)			
Hair Pull test						
No Hair Pulled (count)	Ashwagandha	30	−1.83 (0.46)	−1.12 (−1.52 to −0.73)	−5.733 (<0.0001)	−1.47 (−2.03 to −0.90)
	Placebo	31	−0.71 (0.97)			
Percent Hair Pulled (%)	Ashwagandha	30	−8.00 (4.84)	−3.16 (−5.17 to −1.16)	−3.153 (0.003)	−0.81 (−1.33 to −0.28)
	Placebo	31	−4.84 (2.73)			
Hair specific Skindex-29						
Symptom score	Ashwagandha	30	−4.07 (5.83)	−2.26 (−4.54 to 0.02)	−1.980 (0.052)	−0.51 (−1.02 to 0.01)
	Placebo	31	−1.81 (2.50)			
Function score	Ashwagandha	30	−8.47 (10.02)	−5.47 (−9.42 to −1.51)	−2.764 (0.008)	−0.71 (−1.22 to −0.19)
	Placebo	31	−3.00 (4.50)			
Emotional score	Ashwagandha	30	−4.80 (7.86)	−3.41 (−6.36 to −0.47)	−2.317 (0.024)	−0.59 (−1.10 to −0.08)
	Placebo	31	−1.39 (2.32)			
Total score	Ashwagandha	30	−17.33 (22.44)	−11.14 (−19.61 to −2.67)	−2.632 (0.011)	−0.67 (−1.19 to −0.15)
	Placebo	31	−6.19 (7.10)			

A:T, Anagen:Telogen; CI, Confidence intervals; ITT, Intent-to-treat; PP, Per-protocol; SD, Standard deviation.

The exact mechanism of action of Ashwagandha is unknown, but the following are plausible mechanisms/reasons via which it might influence hair formation and hair health [21–28]:

1) Plant composition (alkaloids, flavonoids, ergostane steroids, amino acids, withanolides and withanoferins)
2) A wide range of pharmacological action (antioxidant, anti-inflammatory and immunomodulatory)
3) Lowering cortisol levels, hence affecting hair follicle function and cyclic control
4) Anti-stress/adaptogen activity

In this study, it was also observed that Ashwagandha was well tolerated, with no adverse events or serious adverse events reported by the subjects.

Overall, Ashwagandha (serum) appears to be a feasible alternative for meeting the unmet demand for an effective, safe, and well-tolerated therapy for all adults suffering from hair loss or alopecia in the community.

4.1. Limitation of the study

First, the study was conducted in a single centre. A larger population research with a more diverse cross-section of participants as well as multicentric locations might yield more conclusive results. Second, the research only looked at short-term impacts and did not look at long-term effects. Future research should extend the study period to assess the long-term effects of Ashwagandha on hair formation and hair health. Third, no biological markers or hormones were assessed in this study because it was a topical formulation, and we may not expect any changes in the hormone status of biomarkers. Future research that involves oral or a combination of oral and topical formulations may include biological markers and hormones to determine the exact effect of Ashwagandha on biological indicators and hormones.

5. Conclusion

Topical administration of Ashwagandha root extract (serum) daily for 75 days improved hair growth and hair health metrics. No adverse drug events were encountered. Thereby, it can be concluded that Ashwagandha can be an effective and safer alternative for individuals suffering from alopecia. However, further research with a large cohort study, in diverse strata, and with biochemical evaluations is needed to substantiate the current findings.

Author contributions

Chinmai Yerram: Conceptualization, Methodology, Investigation, Resources, Formal.

Analysis, Writing – Original Draft, Writing – Review & Editing.

Aditya Jillella: Investigation, Formal analysis, Writing – Original Draft, Supervision.

Venkateswar Reddy J: Investigation, Formal analysis, Writing – Review & Editing.

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Ethical statement

This research was reviewed and approved by the Deccan Independent Ethics Committee (Study code: IB−HH−CT08−22, Approval date: 31−10−2022). Informed consent was obtained from all participants.

Data availability

The data that support the findings of this study are available within the article and its supplementary material, or Protocol are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Author reports drugs, or supplies was provided by Ixoreal Biomed Inc, Los Angeles, California, USA (for supplying the KSM-66 Ashwagandha root extract used in the study).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaim.2023.100817.

References

[1] Alfonso M, Richter-Appelt H, Tosti A, Viera MS, García M. The psychosocial impact of hair loss among men: a multinational European study. Curr Med Res Opin 2005;21(11):1829–36. https://doi.org/10.1185/030079905X61820. PMID: 16307704.
[2] Schmitt JV, Ribeiro CF, Souza FH, Siqueira EB, Bebber FR. Hair loss perception and symptoms of depression in female outpatients attending a general dermatology clinic. An Bras Dermatol 2012;87(3):412–7. https://doi.org/10.1590/s0365-0596201200030. 0010. PMID: 22714757.
[3] Mounsey AL, Reed SW. Diagnosing and treating hair loss. Am Fam Physician 2009;80(4):356–62. PMID: 19678603.
[4] Chartier MB, Hoss DM, Grant-Kels JM. Approach to the adult female patient with diffuse nonscarring alopecia. J Am Acad Dermatol 2002;47(6):809–18. quiz 818-20. doi: 10.1067/mjd.2002.128771. PMID: 12451364.
[5] Shrivastava SB. Diffuse hair loss in an adult female: approach to diagnosis and management. Indian J Dermatol Venereol Leprol 2009;75(1):20–7. quiz 27-8. doi: 10.4103/0378-6323.45215. PMID: 19172026.
[6] Santos Z, Avci P, Hamblin MR. Drug discovery for alopecia: gone today, hair tomorrow. Expet Opin Drug Discov 2015;10(3):269–92. https://doi.org/10.1517/17460441.2015.1009892. Epub 2015 Feb 9. PMID: 25662177; PMCID: PMC4339524.
[7] Motofei IG, Rowland DL, Baconi DL, et al. Androgenetic alopecia; drug safety and therapeutic strategies. Expet Opin Drug Saf 2018;17(4):407–12. https://doi.org/10.1080/14740338.2018.1430765. Epub 2018 Jan 24. PMID: 29363345.
[8] Strazzulla LC, Wang EHC, Avila L, et al. Alopecia areata: disease characteristics, clinical evaluation, and new perspectives on pathogenesis. J Am Acad Dermatol 2018;78(1):1–12. https://doi.org/10.1016/j.jaad.2017.04.1141. PMID: 29241771.
[9] Price VH. Treatment of hair loss. N Engl J Med 1999;341(13):964–73. https://doi.org/10.1056/NEJM199909233411307. PMID: 10498493.
[10] Blume U, Ferracin J, Verschoore M, Czernielewski JM, Schaefer H. Physiology of the vellus hair follicle: hair growth and sebum excretion. Br J Dermatol 1991;124(1):21–8. https://doi.org/10.1111/j.1365-2133.1991.tb03277.x. PMID: 1993141.
[11] Wolff H, Fischer TW, Blume-Peytavi U. The diagnosis and treatment of hair and scalp diseases. Dtsch Arztebl Int 2016;113(21):377–86. https://doi.org/10.3238/arztebl.2016.0377. PMID: 27504707; PMCID: PMC4908932.
[12] Hoover E, Alhajj M, Flores JL. Physiology, hair. 2022 Jul 25. In: StatPearls [internet]. Treasure Island (FL: StatPearls Publishing; 2022 Jan. PMID: 29763123.
[13] Rambwawasvika H, Dzomba P, Gwatidzo L. Alopecia types, current and future treatment. J Dermat Cosmetol 2021;5(4):93–9. https://doi.org/10.15406/jdc.2021.05.00190.
[14] Whiting DA. Chronic telogen effluvium. Dermatol Clin 1996;14(4):723–31. https://doi.org/10.1016/s0733-8635(05)70398-3. PMID: 9238330.
[15] Williamson D, Gonzalez M, Finlay AY. The effect of hair loss on quality of life. J Eur Acad Dermatol Venereol 2001;15(2):137–9. https://doi.org/10.1046/j.1468-3083.2001.00229.x. PMID: 11495520.
[16] Nestor MS, Ablon G, Gade A, Han H, Fischer DL. Treatment options for androgenetic alopecia: efficacy, side effects, compliance, financial considerations, and ethics. J Cosmet Dermatol 2021;20(12):3759–81. https://doi.org/10.1111/jocd.14537. Epub 2021 Nov 6. PMID: 34741573; PMCID: PMC9298335.
[17] Leyden J, Dunlap F, Miller B, et al. Finasteride in the treatment of men with frontal male pattern hair loss. J Am Acad Dermatol 1999;40(6 Pt 1):930–7. https://doi.org/10.1016/s0190-9622(99)70081-2. PMID: 10365924.
[18] Gupta AK, Venkataraman M, Talukder M, Bamimore MA. Finasteride for hair loss: a review. J Dermatol Treat 2022;33(4):1938–46. https://doi.org/10.1080/09546634.2021.1959506. Epub 2021 Aug 2. PMID: 34291720.
[19] Suchonwanit P, Thammarucha S, Leerunyakul K. Minoxidil and its use in hair disorders: a review. Drug Des Dev Ther 2019;13:2777–86. https://doi.org/10.2147/DDDT.S214907. Erratum in: Drug Des Devel Ther 2020;14:575. PMID: 31496654; PMCID: PMC6691938.
[20] Patel S, Sharma V, Chauhan NS, Thakur M, Dixit VK. Hair growth: focus on herbal therapeutic agent. Curr Drug Discov Technol 2015;12(1):21–42. https://doi.org/10.2174/1570163812666150610115055. PMID: 26058803.
[21] Sharma CG. Rastantra sar evam sidhyaprayog sangrah. Nagpur, Krishna– Gopal Ayurveda Bhawan. Dharmarth Trust); 1938. p. 743–4.
[22] Singh N, Bhalla M, de Jager P, Gilca M. An overview on ashwagandha: a Rasayana (rejuvenator) of Ayurveda. Afr J Tradit, Complementary Altern Med 2011;8(5 Suppl):208–13. https://doi.org/10.4314/ajtcam.v8i5S.9. Epub 2011 Jul 3. PMID: 22754076; PMCID: PMC3252722.
[23] Kaul KN. The origin, distribution and cultivation of ashwagandha the so called withania somnifera of Indian literature," symposium on the utilization of Indian medicinal plants. New Delhi: Council of Scientific & Industrial Research; 1957. p. 7–8.
[24] Umadevi M, Rajeswari R, Rahale SC, et al. Traditional and medicinal uses of withania somnifera. Pharma Innov 2012;1(9):102–10.
[25] Salve J, Pate S, Debnath K, Langade D. Adaptogenic and anxiolytic effects of ashwagandha root extract in healthy adults: a double-blind, randomized, placebo-controlled clinical study. Cureus 2019;11(12):e6466. https://doi.org/10.7759/cureus.6466. PMID: 32021735; PMCID: PMC6979308.
[26] Chandrasekhar K, Kapoor J, Anishetty S. A prospective, randomized double-blind, placebo-controlled study of safety and efficacy of a high-concentration full-spectrum extract of ashwagandha root in reducing stress and anxiety in adults. Indian J Psychol Med 2012;34(3):255–62. https://doi.org/10.4103/0253-7176.106022. PMID: 23439798; PMCID: PMC3573577.
[27] Majeed M, Majeed S, Nagabhushanam K, Mundkur L, Neupane P, Shah K. Clinical study to evaluate the efficacy and safety of a hair serum product in healthy adult male and female volunteers with hair fall. Clin Cosmet Invest Dermatol 2020 Sep 24;13:691–700. https://doi.org/10.2147/CCID.S271013. PMID: 33061509; PMCID: PMC7522433.
[28] Wasko CA, Mackley CL, Sperling LC, Mauger D, Miller JJ. Standardizing the 60-second hair count. Arch Dermatol 2008;144(6):759–62. https://doi.org/10.1001/archderm.144.6.759. PMID: 18559765.
[29] Dhurat R, Saraogi P. Hair evaluation methods: merits and demerits. Int J Trichol 2009;1(2):108–19. https://doi.org/10.4103/0974-7753.58553. PMID: 20927232; PMCID: PMC2938572.
[30] Hoffmann R. TrichoScan: a novel tool for the analysis of hair growth in vivo. J Invest Dermatol Symp Proc 2003;8(1):109–15. https://doi.org/10.1046/j.1523-1747.2003.12183.x. PMID: 12895006.
[31] Saraogi PP, Dhurat RS. Automated digital image analysis (TrichoScan®) for human hair growth analysis: ease versus errors. Int J Trichol 2010;2(1):5–13. https://doi.org/10.4103/0974-7753.66905. PMID: 21188016; PMCID: PMC3002414.
[32] Majeed M, Majeed S, Nagabhushanam K, Mundkur L, Neupane P, Shah K. Clinical study to evaluate the efficacy and safety of a hair serum product in healthy adult male and female volunteers with hair fall. Clin CosmetInvestig Dermatol 2020;13:691–700. https://doi.org/10.2147/CCID.S271013. PMID: 33061509; PMCID: PMC7522433.
[33] Wyrwich KW, Kitchen H, Knight S, et al. The Alopecia Areata Investigator Global Assessment scale: a measure for evaluating clinically meaningful success in clinical trials. Br J Dermatol 2020;183(4):702–9. https://doi.org/10.1111/bjd.18883. Epub 2020 Apr 3. PMID: 31970750; PMCID: PMC7586961.
[34] Camacho-Martínez FM. Hair loss in women. Semin Cutan Med Surg 2009;28(1):19–32. https://doi.org/10.1016/j.sder.2009.01.001. PMID: 19341939.
[35] Guarrera M, Semino MT, Rebora A. Quantitating hair loss in women: a critical approach. Dermatology 1997;194(1):12–6. 34. 10.1159/000246049. PMID: 9031784.
[36] Chren MM, Lasek RJ, Flocke SA, Zyzanski SJ. Improved discriminative and evaluative capability of a refined version of Skindex, a quality-of-life instrument for patients with skin diseases. Arch Dermatol 1997;133(11):1433–40. PMID: 9371029.
[37] Han SH, Byun JW, Lee WS, Kang H, Kye YC, Kim KH, Kim DW, Kim MB, Kim SJ, Kim HO, Sim WY, Yoon TY, Huh CH, Hwang SS, Ro BI, Choi GS. Quality of life assessment in male patients with androgenetic alopecia: result of a prospective, multicenter study. Ann Dermatol 2012;24(3):311–8. https://doi.org/10.5021/ad.2012.24.3.311. Epub 2012 Jul 25. PMID: 22879715; PMCID: PMC3412240.