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Original Research Article

Hair growth efficacy and safety assessment of an ayurvedic leave-on treatment: a clinical trial

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ABSTRACT

Background: This clinical study was undertaken to assess the safety, efficacy of an Ayurvedic leave-on treatment for hair loss in healthy Indian adults (both male and female). The study also aimed to elucidate the product's mechanism of action through in vitro analyses.
Methods: Healthy male and female volunteers experiencing hair loss and hair thinning were enrolled for clinical study. Subjects were screened based on predefined criteria, including specific hair density and thinning parameters, and the absence of underlying medical conditions that could contribute to hair loss. The study design included a washout period prior to treatment initiation. The treatment phase lasted for three months, during which participants applied the product regularly and visited the study center at scheduled intervals for follow-up assessments and measurements. An *in vitro* study was conducted using human dermal papilla cells to investigate the mechanism of action (MoA) of the Ayurvedic leave-on treatment.
Results: The Ayurvedic leave-on treatment demonstrated significant efficacy in reducing hair fall and improving hair density in both male and female subjects compared to baseline measurements. Noticeable improvements observed in increase in hair density, reduction in hair fall and over all hair and scalp health from the second week of application and continued throughout the treatment period.
Conclusion: The Ayurvedic leave-on formulation demonstrated a clinically significant increase in hair density and improvement in the anagen: telogen ratio, indicating reduced hair fall and normalization of the hair cycle. This is mechanistically supported by the upregulation of vascular endothelial growth factor (VEGF) in dermal papilla cells, promoting follicular angiogenesis.

Keywords: Ayurvedic, Hair growth treatment, Clinical trial, Bhringaraj, New hair growth

INTRODUCTION

Hair is mini organ of human body, with highest density on scalp. Humans have up to 5 million hairs on their body which are either vellus or terminal hair.[1] Vellus hair are thin colourless, or pale seen across the skin with short hair cycle, while terminal hair is thick, dark and can grow longer up to 6 years. In human, density of terminal hair is highest on scalp followed by beard. On an average healthy human scalp can have one lakh of hair spanning over 600-650 cm^2 of scalp.[2,3] Human hair growth is cyclical in nature with three phases in the cycle, the anagen, catagen and telogen. Anagen is an actively growing phase, during which the hair matrix cells grow rapidly that allows the hair shaft to grow. Hair follicles remain in this growth phase usually between 2-6 years and in healthy scalps anagen hair grows at the rate of 1 cm per month.[4] Catagen is the transition phase with 1-3 weeks, during which hair cells cease to divide undergoes programmed cell death, accompanied by rapid regression of perifollicular blood

vessels with apoptosis in endothelial cells and pushes the hair into resting phase, the telogen in which hair remains for 2-3 months.[5] At any given time point healthy scalp can have up to 90-95% of hair in anagen and 5-10% in telogen.[6] Hair loss or hair thinning becomes visible with more hair entering into telogen and exiting the scalp, with no new hair replaced or delayed and replaced with weaker or thinner hair.

Hair loss is seen both in women and men, however, manifestation of hair loss (alopecia) is very different in men and women in terms of presentation of the condition. In women this hair fall is called as female patten hair loss (FPHL), usually starts as diffuse hair loss, with loss in density at frontal scalp and vertex region and generally does not progress into complete baldness, while it is different in men with receding hair line in the frontal portion or hair thinning in the vertex region leading to balding spots, that broadens, culminating into complete loss of hair in the crown, most of the times sparing the hair in occipital region.[7,8]

Hamilton estimated that 30% to 50% of men develop androgenic alopecia by the age of 50 and 40% women develop FPHL by the age of 50.[9] While the prevalence of FPHL is reported to be high in pre- to post-menopausal women, male pattern baldness is due to genetic predisposition of hair follicle sensitivity to testosterone. Apart from the aforementioned causative factors for hair fall, role of oxidative stress, recurring dandruff, unhealthy scalp/hair grooming practices have been implicated in hair loss problems. The market is saturated with various types of leave-on formulations with different technologies and mechanism of action. These include oils—often made with natural or herbal ingredients—that tend to leave the hair and scalp feeling oily or greasy, which is generally considered an undesirable sensory experience. There are also topical solutions containing pharmaceutical actives such as Minoxidil, which has been associated with side effects like skin irritation and dryness, and Finasteride, which is contraindicated for women in reproductive age.[10,11]

Additionally, cosmetic leave-on products like serums are available, featuring a range of natural or synthetic active ingredients. There is compelling clinical research evidence supporting the need for long term treatment to restore hair density in moderate to severe hair loss conditions. As a result, there is an ever-growing demand to develop safe and side effect free technologies and products that achieve a consumer perceivable improvement in hair growth and hair density. Ayurveda, Indian classical system of medicine, provides a time-tested holistic approach with multiple plant-based ingredients to treat hair loss and hair thinning problems.

As per Ayurveda, hair loss/hair fall is referred as Khalitya, and is classified under Kshudra Roga (minor diseases/ailments) according to classical texts like Ashtang Samgraha, Yoga Ratnakar, Madhav Nidana, and Sushrut Samhita.[12] Ayurveda considers this condition as non-life-threatening and is characterized by the gradual loss of hair, due to the vitiation of Pitta dosha, which, when combined with either Kapha or Vata dosha, leading to destruction of hair, culminating into hair loss. Bringha (*Eclipta alba*), commonly referred as Bringharaj or Keshraj is used in various classical ayurvedic formulations like Bringhadi taila or Nilibringadhi tail as main ingredient for hair loss treatment.[13] Along with bringha, other ingredients like amla, hibiscus, brahmi, currey leaf, neem, rosemary, and nilini. were also recommended to treat hair loss issues.

The present *in vitro* studies examine the mechanism of action of the Ayurvedic bringha+complex on the dermal papillary cells, which are essential for the survival and growth cycle of hair follicle and demonstrates the clinical efficacy of leave-on treatment to improve hair density and reduce hair fall in Indian volunteers suffering from hair loss.

METHODS

In vitro method

Cell culture

The human dermal papillary cells (DPC) procured from Inovaugmet Research were sub cultured in Dulbecco's Modified Eagle's medium (DMEM from GIBCOTM, USA) with high glucose supplemented with 10% heat-inactivated foetal bovine serum (FBS; MP Biomedicals, USA), 1X penicillin-streptomycin-amphotericin B (MP Biomedicals, USA) on 0.1% bovine gelatin (Sigma-Aldrich) coated surface. The DPC were maintained at 37°C in a humidified atmosphere at 5% CO_2 in the PhCbi incubator and 4th and 5th passage cells were used for the experiments.

Determination of non-toxic concentrations

The MTT assay was carried out to identify the noncytotoxic concentrations of Ayurvedic bringha+ complex which is made with extracts of bringh, amla, kumari, brahmi, pudina rosemary curry leaf. This assay is based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by metabolically active cells, resulting in purple colored formazan. The amount of formazan is quantitated using a microplate reader.

Briefly, DPC were seeded into 96-well plates (4×103 cells/well) and were exposed to different concentrations (5 ppm, 10 ppm, 25 ppm) of ayurvedic concoction. After a 48-hour incubation period, cells were treated with 150 µl MTT (0.5 mg/ml) at 37°C for 3 hours.

Following, the resulting formazan was solubilized using 150 µl of DMSO and the absorbance was read in Multiskan SkyHigh Microplate reader (Thermo scientific, USA) at 570 nm.

Vascular endothelia growth factor (VEGF) quantification

9×105 DPC were seeded in 6 well plates and cultured overnight. Following day, DPC were treated with different concentrations of ayurvedic Bringha+ as indicated in the figure legends. Medium was collected following 48 h treatment. VEGF was analysed from cell culture supernatants using a VEGF enzyme linked immunosorbent assay (ELISA) kit (Immunotag™, Geno Technology Inc., USA). An experiment for each condition was carried out in triplicate. 100 µl of the supernatant was subjected to ELISA.

Statistical analysis

The results obtained from 3 separate experiments are expressed as mean ± standard error of the mean (SE). The results were analysed in the GraphPad Prism using unpaired t test where differences with $p<0.05$ were considered significant.

Clinical study method

Present clinical study was carried out as per good laboratory practice (GLP) and International Conference of Harmonization (ICH) guidelines in compliance to the local government regulations. The study was reviewed and approved by independent ethics committee in India and an informed consent was obtained from all study subjects. The study was registered on Clinical Trial Registry of India, under ayurvedic study with study number CTRI/2024/09/073415 prospectively and executed at Mascot SpinControl India Pvt Ltd, Mumbai, India with dermatologist as the principal investigator and Ayurvedic doctor as co-investigator between September 2024 to December in 2024.

Study design

The study was a controlled, full head, blinded and monocentric, with one test product which is a scalp leave-on treatment. Study participants were recruited as per inclusion and exclusion criteria: both male and female in the age group of 20-45 years suffering from hair fall and hair loss, participants with mild to moderate hair fall and self-perceived hair fall, volunteers presenting hair thinning basis dermatological visual assessment (for males: Hamilton scale class II to III (including III, IIIA and III vertex); for females: Ludwig scale 1-2 to 1-4). Pregnant and lactating women were excluded from the study. Volunteers with severe hair fall or any scalp related problems or allergies that can potentially interfere in the study results or on food supplements were excluded from the study.

Volunteers undergone hair transplantation and have participated in similar studies in last 6 months were also excluded. Subjects with hair density 100-150 per cm^2 were recruited and underwent a wash-off phase for 1 week with neutral shampoo. Study volunteers were restricted from using any hair care product or treatments except the products provided to use during the study period. Test products were used by volunteers at home as per the instructions provided and applied on the scalp once a day. Subjects were instructed not to wash their hair 48 hours before assessment visit.

Methodology

Hair density and A:T ratio was assessed using phototrichogram imaging analysis. Hair fall was evaluated by counting hair fallen from roots by combing test. Hair anchorage was assessed by performing hair pull test (dermatologist). For hair pull test, approximately, 50-60 hair were gently grasped between the thumb, index, and middle fingers from the base of the hair near the scalp, with a firm but not forceful tug, the hairs were pulled away from the scalp.[14] Number of pulled away from the scalp with roots were counted and documented.

Dermatologist visual assessment was done to grade hair density by Hamilton/Ludwig scales and denolite microscopic imaging to assess the improvement of hair fibre thickness. There was a total of 9 visits (including shaving visits) for trichogram imaging. During the visit to the site the participants were acclimatized at a temperature of 22+5°C and relative humidity of 50-60% for 15 minutes prior to scalp imaging and assessments.

Study was executed at Mascot Spincontrol India Pvt Ltd, Mumbai, India.

Statistical analysis

All statistical tests were performed at two-sided 5% level of significance and 95% confidence interval and statistical software PAST 4.03 and SigmaStat 3.5 were used for the analysis of the data. Paired t-test was used to evaluate the efficacy of the product.

Demographics of the study volunteers

48 participants were recruited in the study, and 4 participants were lost to follow up, with 44 subjects completing the study. Demographic characteristics of the participants are depicted in Table 1.

Table 1: Demographics and volunteers' characteristics at baseline.

Characteristics	N
Age range (years)	20-45
Gender category - N (male: female)	44 (1: 3)
Hair density at baseline (no. of hair/cm^2)	100-150
Race category	Asians with Indian origin

RESULTS

In vitro study: determination of viability of DPC

To determine the effect of Ayurvedic bringha+complex on the viability of DPC, MTT assay was performed. There is a dose dependent increase in cell viability between (11 to 15%) in the tested range of concentrations, 5 ppm, 10 ppm and 25 ppm as shown in Figure 1, indicating its protective benefit of bringha+ complex on DPC.

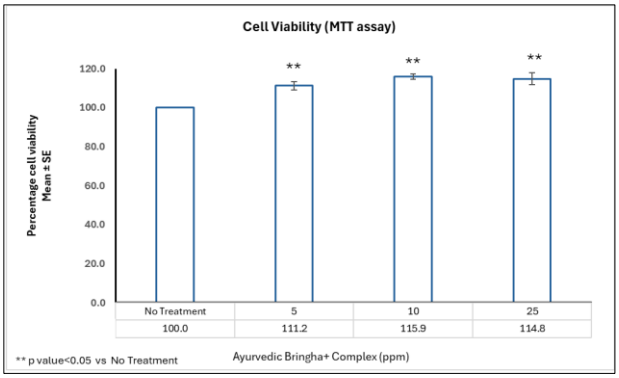

Figure 1: Determination of non-toxic concentrations of Ayurvedic bringha+complex.

**P value <0.05 versus baseline, values expressed as change from baseline

Quantification of VEGF secretion by DPC

One of the proven mechanisms to improve hair growth is upregulating the growth factors like VEGF that aids in improved vasculature to hair follicle and supply of nutrients. To determine the effect of Ayurvedic bringha+ complex on secretion of VEGF by DPC, above mentioned concentrations were used to treat the same. There is a dose dependent increase VEGF secretion by DPC at 5 ppm, 10 ppm, 25 ppm by 525 pg/ml, 627 pg/ml and 660 pg/ml respectively as shown in Figure 2, which could be one of the mechanisms of action of this Ayurvedic bringha+ complex, that can potentially help in treating hair loss problems.

Clinical result

With demonstrated mechanism of action of bringha+ complex, the present leave on treatment (formula) was developed with the functional levels the same to establish the efficacy in treating hair loss issues in male and female participants suffering from hair fall and hair thinning problem.

Improvement in hair density

With regular use of scalp leave-on treatment there is a significant improvement in hair density from week 2 till end of treatment phase i.e., 3 months (Figure 3) with 12% improvement in hair density from baseline. By the end of 3 months of treatment there was an increase of 17 hair strands per cm^2 based on triochogram analyzed scalp area. Extrapolating this improvement in hair density to average Asian scalp area (~650 cm^2) it is estimated to have improved by 11,082 new hairs on the scalp at the end of 3 months.

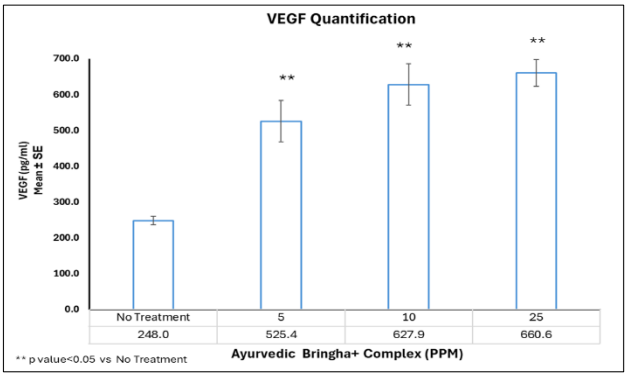

Figure 2: Quantification of VEGF.

**P value <0.05 versus baseline, values expressed as change from baseline

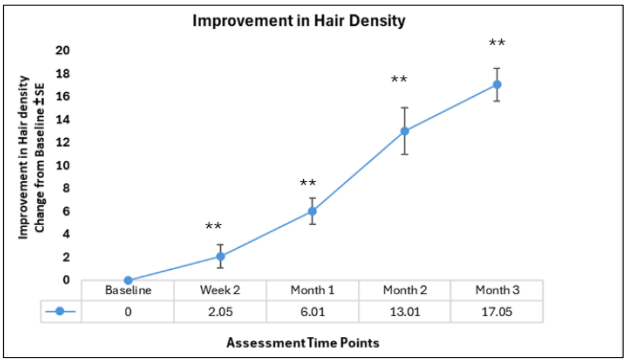

Figure 3: Phototrichogram assessment for hair density.

**P value <0.05 versus baseline

Anagen: telogen ratio

Most of the hair remains in anagen phase in healthy scalps, with 10% in telogen /catagen. Hence the ratio of anagen: telogen is a good indicator for healthier hair cycle. Significant improvement in A: T ratio is noted at all assessment time points from month 1 till month 3 compared to baseline (Figure 4) indicating the efficacy of leave-on treatment in restoring the hair cycle balance.

Hair thinning: dermatologist's visual assessment

Effect of the leave-on treatment on visible improvement of hair density was monitored by dermatological visual assessment using Hamilton and Ludwig scales in men and women respectively (lower the score, lesser is the hair thinning). Significant reduction in hair thinning (improvement in hair density) compared to baseline (Figure 5), was observed from month 1 till the end of the treatment phase (month 3).

Hair anchorage/hair loss from roots - hair pull test

Hair pull test determines the health of hair cycle balance and hair anchorage. Scalps with alopecia or hair loss problems show more hair being pulled off from scalps with pull test. There is a significant reduction in the number of hairs pulled off (with roots) compared to baseline from month 1 onward till the 3 months assessment (Figure 6), indicating the improvement in hair cycle balance and hair anchorage upon using the leave-on treatment with more hair being anchored strongly to dermal papilla.

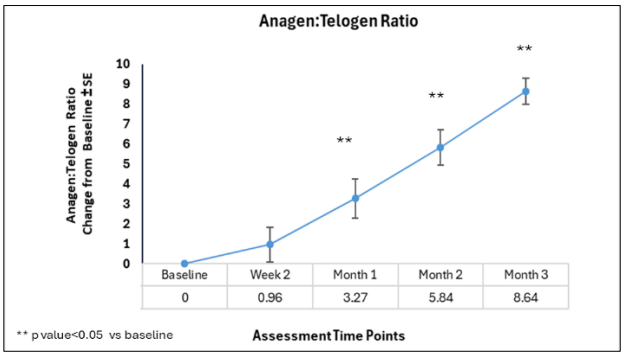

Figure 4: Phototrichogram assessment for anagen: telogen ratio.

**P value <0.05 versus baseline

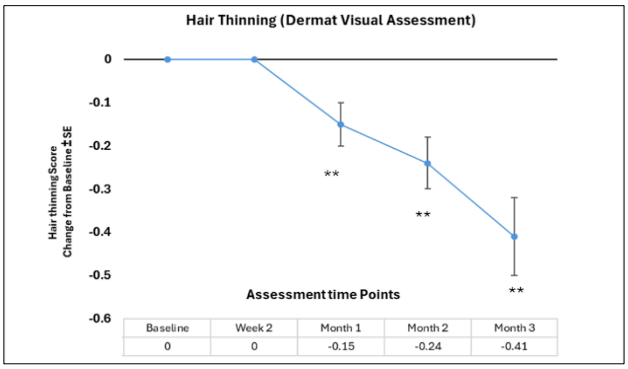

Figure 5: Dermatological assessment for hair thinning.

**P value <0.05 versus baseline

Hair fall from roots

Hair fall from roots (with hair bulb) was determined by combing test results indicated a significant reduction in hair fall as early as 2 weeks, with significant and sustained reduction till 3 months assessment compared to baseline (Figure 7). At the end of the study there is a mean reduction in hair fall from roots by 50% post-treatment compared to baseline.

Measurement of hair thickness by deno-lite microscope imaging

Hair fiber thickness was assessed by denolite microscopic imaging. There is a significant increase in the diameter of hair stands compared to baseline at 3rd month of treatment phase (Figure 8) indicating improvement in the hair thickness post treatment.

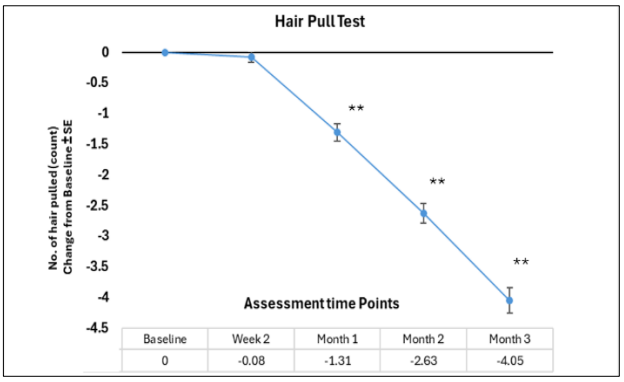

Figure 6: Dermatological assessment for hair anchorage.

**P value <0.05 versus baseline

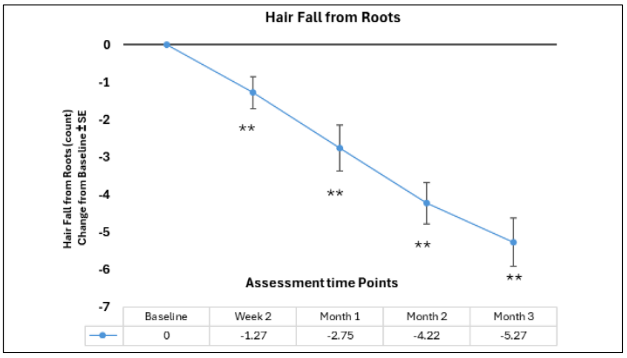

Figure 7: Hair fall assessment.

**P value <0.05 versus baseline

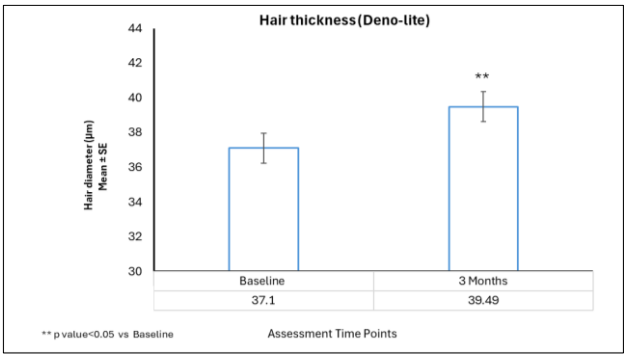

Figure 8: Instrumental assessment for hair thickness.

**P value <0.05 versus baseline

DISCUSSION

Role of vascular endothelial growth factor (VEGF) has been established in maintenance of anagen phase of hair.[15] Bringha+ complex used in development of present leave-on treatment has improved the viability of DPC up to 15% and upregulated the levels of VEGF by 2 times indicating its possible role in maintenance of hair anagen by

upregulating vascular supply to hair follicle. The current clinical study demonstrates efficacy of scalp leave-on treatment product through improved hair density by 12% and reduced hair fall by 50% in both male and female volunteers at the end of 3 months of treatment period.

Bringha in Ayurveda is called Keshraj, proven to impart hair benefits and used in classical ayurvedic formulations like Bringhamalkadi taila, Bringharaja taila and Nilibringhadi taila.[16] As per Ayurveda hair loss (known as Khalitya), is a consequence of destruction of hair follicles due to vitiated Pitta dosha, along with Kapha or Vata doshas. Siroabhyangana with tailam (oil) processed by ayurvedic vidihis/ingredients along with lifestyle changes is recommended to restore scalp health and lustrous hair.

Scientific research elucidates the role of multiple signalling events and complex network of chemical messengers in the cyclical process of hair growth, to maintain the hair density on the scalp. Human hair grows in cycles and an average human being can have 10-30 hair growth cycles.[17] Growth factors like insulin-like growth factor-1 (IGF-1), hepatic growth factor (HGF), and VEGF have been implicated in maintenance of anagen phase, whereas transforming growth factor β isoforms (TGF-β1 and TGF-β2) are known to drive the progression of hair from anagen to catagen characterized by apoptotic cells death in hair epithelial cells, thus pushing the hair into telogen phase.[18,19] TGF-β1 levels increase in late anagen and remain at highest at during catagen phase.[20] High levels of TGF β receptor with TUNEL positive cells (indicates the onset to apoptosis/cell death) were detected in catagen hair.[21] Lu et al demonstrated enhanced TGF β signalling in hair follicles isolated from balding region compared follicles in non-balding region concluding the role TGF β in development of androgenic alopecia, the male pattern baldness.[22]

Kesharaj, the Bringha (*Eclipta alba*) is highly recommended in ayurvedic classical formulations to treat alopecia and hair fall problems and is rejuvenating in nature. Extracts of *Eclipta alba* were proven to down regulate TGF β isoforms and enhances the proliferation of keratinocytes prolonging the anagen phase in experimental models justifying its potency as hair growth modulator. Expression of TGF β was also reported to be high in scalps suffering from telogen effluvium, an alopecia condition in which most of the hair follicles proceeds into telogen phase faster than usual hair cycle.[23,24] Telogen effluvium is known to occur as a result of stressful events, fevers, hormonal changes, and administration of drugs.[25] Proven the role of *Eclipta alba* to attenuate the chemical messengers that drive the hair follicle to resting phase, with potent antioxidant benefits, it is a promising ingredient to treat hair fall both in men and women which is used at functional levels in the present leave-on treatment and have shown significant improvement in hair density (by 12%) at the end of 3 months treatment phase.

Amla (*Phyllanthus emblica*) is highly recommended in Ayurvedic literature for promoting scalp and hair health.[26] It is widely used both oral and topical use, addressing a range of conditions from digestive problems to hair fall, greying to impart healthy and lustrous hair.[27] Amla fruit extract has been shown to enhance the proliferation of human dermal papilla cells which are essential for maintaining hair density, thickness, and prolonging the anagen phase.[28] Dermal papillary cells are the resident cells of hair follicles that stimulate the hair matrix cells to enter into hair growth phase, the anagen by releasing various growth factors/cytokines.[29] Proven the role of oxidative stress and inflammation in the aetiology of androgenic alopecia with hair thinning 24 amla with its rich antioxidant benefit support the use of same in hair loss treatment. One of the proven strategies to treat androgenic alopecia is inhibition of enzyme 5-alpha reductase, which is known to affect the survival of testosterone sensitive hair dermal papillary cells and amla extracts were proven to inhibit this enzyme. Aforementioned mechanism of action of Amla along with established antioxidant and anti-inflammatory benefits makes it a potent active for treating hair fall and hair thinning conditions which is used in the present leave-on treatment at optimised levels, showed hair fall reduction in 100% of the study participants in 3 months in the current study.

A randomized placebo-controlled blinded clinical study conducted on women with androgenic alopecia with Amla syrup showed a significant improvement in anagen-telogen ratio in 12 weeks compared to placebo with no remarkable side effects is a testament to use Amla in the treatment of hair loss.[30]

Charaka Samhita explains use of oils as Siroabhyanga (massaging on scalp and hair) to restore the vitality of scalp and hair and is effective of hair loss treatment.[31] Use of curry leaf (*Murraya koenigii*) processed in oil is one of the effective hair fall control treatments in Indian classical medicine.[32] Kaidarya siddha narikela taila (coconut oil processed with curry leaf) is single ingredient oil with proven hair fall control in subjects suffering from hair loss issues.[33] With proven antioxidant and anti-inflammatory properties and clinical efficacy of the single ingredient formula to treat hair loss, justifies the usage in the present leave-on treatment to reduce hair fall and hair loss and to improve the vitality of hair and scalp.[34]

Impact of emotional stress and anxiety on hair fall was reviewed in meta-analysis by Almudimeegh et al and conclusively suggest that people with stress issues suffer from some or other forms of alopecia. Hans Selye, the father of stress concept in biology , stated that “an intense psychic shock may also exert pronounced effects on the hair, e.g., graying and generalized loss of hair.[35,36] Activation of hypothalamic-pituitary-adrenal axis (HPA) is a classical stress response leading to release of corticotropin-releasing hormone, and proven to inhibit proliferation of human hair dermal papillary cells through cell cycle arrest and induced the accumulation of reactive

oxygen species providing a convincing role of emotional stress in hair fall. In Ayurveda, brahmi is known to treat stress/anxiety and boost memory.[37,38] Several research articles suggest the role of bacaopside I isolated form *Bacopa monnieri* as HPA axis modulator.[39] Ayurveda uses brahmi as one of the actives in classical formulations to treat hair fall. Using Brahmi is an entirely distinctive strategy in ayurveda that could address hair fall mediated by stress. Oxidative stress in aging hair follicles is one of the irrefutable mechanisms in the pathogenesis alopecia. Extracts of *Bacopa monnieri* were proven to inhibit lipid peroxidation and enhanced antioxidant enzymes in experimental models, such benefits compells the use of this plant in the present scalp leave-on treatment.[40]

Healthy hair follicles stay in anagen which is an actively growing phase and demands adequate supply of nutrients through enhanced vascular supply. Rosemary (*Rosmarinus officinalis*) is widely used in cosmetic formulations for hair growth. Essential oils from rosemary were proven to protect dermal papillary cells from oxidative stress along with increased levels of VEGF and IGF-1.[41] Several clinical studies with multi herbal formulations with rosemary as one of the actives have proven to improve hair density, hair fall control justifies the use of rosemary as one of the active ingredients in the present scalp leave-on treatment.[42]

Pudina *(Mentha piperita)* is referred in Ayurveda to treat kapha and vata doshas, the imbalance of which leads to destruction of hair follicles leading to hair loss.[43,44] As per Acharya Charaka excessive Pitta along with Vata disturbs hair follicle strength, while Kapha and Rakta obstruct the hair roots, inhibiting the hair growth.[45] Hence it is quiet logical to use pudina which balances these two doshas to treat hair fall. Active constituents of Pundina (menthol) were reported to increase blood flow up on topical treatment via increased nitric oxide response.[46] A randomized controlled clinical study with mint oil in subjects suffering from hair loss was proven to be effective in hair fall control and new hair growth compared to placebo group.[47] Continuous supply of nutrients is essential for healthy growth of hair follicle and to prolong the anagen phase to maintain the normal density of hair on the scalp. Formulations with minoxidil are known to improve blood supply to hair follicles is widely recommended by dermatologists and trichologists for hair loss treatment.[48] Actives from Pudina, with proven scientific evidence of improving blood flow along with above mentioned ingredients with differentiated mechanism of actions provides a logical justification of incorporation in the present scalp leave-on treatment.

Limitations

The limited sample size and non-comparative study design are the two drawbacks of the study. Additionally, a comparative study design with a greater sample size having severe hair fall and a wider population range can increase the scientific value of our clinical investigation.

CONCLUSION

In the present study we have demonstrated significant upregulation of VEGF (by DPC) upon the treatment with bringha+complex, providing the mechanistic explanation of the present leave on treatment, and clinically proven to improve hair density and anagen: telogen ratio indicating the hair cycle balance with reduced hair fall by the end of the treatment phase. 100% of the study participants experienced improvement in hair thickness, 97% felt product delivers hair fall control and most of the users liked sensory of the leave-on treatment. There were no side effects (skin irritation, and dryness) reported throughout the study and participants did not report any oily or greasy feel, indicating that the leave-on treatment developed with multi-prong approach is not only efficacious in boosting hair density but also safe to use in both men and women.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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