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Original Research Article

Resolution of oxidative stress on dandruff scalps with Ayurvedic anti-dandruff regime: a clinical study

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ABSTRACT

Background: This study is aimed to evaluate the efficacy and safety of ayurvedic anti-dandruff regime (Indulekha - Svetakutaja oil and dandruff treatment shampoo) in the management of dandruff in healthy, Indian participants suffering from dandruff.
Methods: A total of 53 subjects with clinically diagnosed moderate dandruff were selected based on inclusion and exclusion criteria. Study was conducted in 3 phases, wash-off phase for 2 weeks, treatment phase for 4 weeks, followed by a regression phase of 2 weeks (during which ayurvedic anti-dandruff regime was withdrawn). Participants applied anti-dandruff regime on their scalp as per the instructions provided.
Results: After 4 weeks of treatment, ayurvedic anti-dandruff regime demonstrated a significant reduction in dandruff flakes in 100% of participants, resolution of scalp itch in 100% participants with reduced levels of histamines (by 30%) and oxidative stress marker on scalp as measured by 13-hydroxyoctadecadienoic acid (HODE) by 50%. There were no reported side effects like oiliness/redness/irritation, during treatment and regression phase.
Conclusions: Ayurvedic anti-dandruff regime is a safe and highly effective solution designed to combat dandruff and oxidative stress on scalp, while simultaneously improved overall health of the scalp and hair.

Keywords: Ayurvedic anti-dandruff regime, Ayurvedic scalp treatment, Indulekha-Svetakutaja ayurvedic regime

INTRODUCTION

Dandruff is a chronic and imbalanced desquamating condition of scalp affecting up to 50% of population.[1] This is one of the most common compromised scalp conditions with symptoms like flaking, itching and irritation, often associated with dryness. Role of *Malassezia* (one of the resident microflorae of scalp) overload has been demonstrated by multiple scientific studies, thus making it a suitable target to address this condition with various anti-fungal ingredients like zinc pyrithione, climbazole, and piroctone olamine.[2]

Malassezia is a lipolytic yeast which is a commensal of human scalp.[3] Under aggravated scalp sebum output which is a congenial environment, these organisms outgrow other microflora, secrete the metabolites that penetrate scalp, evoking inflammation and alter the epidermal homeostasis. This leads to hyperproliferation, incomplete corneocyte differentiation, accumulation of oxidative by products and itch provoking histamines on scalp causing all dandruff associated symptoms.[4] Grooming practices, individual susceptibility and exposure to external stressors also aggravate this condition. Considering this multi-factorial causative nature this condition demands to be addressed from the root cause with multi-prong approach.

Ayurveda, an Indian Traditional medicine refers this dandruff as “darunaka” and classified under kshudra roga (minor disease) caused due to vitiated vata and kapha

doshas. Ayurveda recommends use of plants like Svetakutaja, Amla, Neem, Bringha, and Rosemary, processed with coconut or sesame oil as siroabhyanga (scalp massage), in dandruff management to maintain scalp health.[5]

Svetakutaja (*Wrightia tinctoria* Roxb) is historically known to treat inflammatory skin conditions and is commonly recommended ingredient in Siddha and Ayurveda for chronic skin conditions associated with inflammation and is proven for its anti-microbial effects against pathogenic dermatophyte fungi and dandruff causing *Malassezia furfur*.[6,7] Nimba (Neem), is historically used both Ayurveda and natural medicine to treat various skin and scalp conditions. Oxidative stress is one of the hall mark symptoms of chronic inflammatory conditions, and also been reported on dandruff scalps.[8] Amla (*Phyllanthus emblica*), is known for its tridosha samaka potency (pacifies all doshas as per ayurveda) and commonly used all types of scalp problems from dandruff to hair fall and is rich in antioxidants making it an ideal candidate to target redox imbalance. Rosemary is commonly used in various natural and ayurvedic products to improve scalp health and for hair benefits.[9,10] The present clinical study examines the efficacy of the ayurvedic anti-dandruff regime in male and female Indian volunteers suffering from dandruff by siroabhyangana (scalp massage) with the Svetakutaja oil, followed by rinsing scalp and hair with ayurvedic dandruff treatment shampoo (for 4 weeks treatment period).

METHODS

Ethics and informed consent

Present clinical study was carried out as per GLP and ICH guidelines in compliance with 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 participants. The study was registered on Clinical Trial Registry of India, under ayurvedic study (CTRI/2023/09/057704) prospectively. Study was executed at MS Clinical Research Pvt Ltd, Bangalore, India between March-2023 to August-2023.

Clinical method

The study was a full head, blind and monocentric, with two test product regime (Svetakutaja oil and ayurvedic dandruff treatment shampoo) and instructed to use the regime thrice a week (by applying oil on scalp and massaging, left on scalp 30 minutes) + rinsing the scalp and hair with the dandruff treatment shampoo). Subjects were recruited as per inclusion and exclusion criteria. Both male and female in the age group of 18-36 years suffering from dandruff were included in the study. 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 were also excluded from the study as per the dermatologist's discretion. Study was conducted under the guidance of ayurvedic doctor (co-investigator). Subjects with moderate dandruff were recruited (post informed consent process) and have undergone wash-out period of 2 weeks with a neutral shampoo. Post wash out, only those subjects meeting the inclusion and exclusion criteria and with total dandruff score ≥40 were proceeded into treatment phase. Total of 53 subjects qualified for treatment phase, and 46 subjects completed the study.

On the baseline visit, participants were acclimatized, followed by dermatologist's visual assessment for adherent scalp flaking score (ASFS) and loose flakes. Scalp sebum was measured using sebumeter, hair fall from roots (with bulb) was assessed using combing test and tape strips were collected from the scalp. Ayurvedic doctor also monitored the participants throughout the study. Study volunteers were restricted from using any hair care product or treatments except the test products provided throughout the study period (during wash-off, treatment and regression).

Ayurvedic anti-dandruff regime were used by volunteers at home as per the instructions provided thrice week. Subjects were instructed not to wash their hair 48 hours before assessment visit. The following were assessment visits: baseline, week 1, week 2, week 4 and regression-1 week (R-week 1) and regression week-2 (R-week 2).

Test products were withdrawn on week 4 assessment, followed by 2 weeks of regression period during which subjects were given a neutral shampoo to use and final assessment was done on week 6 which is a regression assessment (regression-2 week). Tape strips were collected to quantify itch and oxidative stress markers at baseline, week 4 and R-week 2 (which is 2 weeks of regression assessment).

Consumer assessment on product efficacy and sensory was taken during assessment visits. The attributes included overall satisfaction on product efficacy and sensorial like smell, texture, and consistence.

Sample collection method and quantification of histamine

Tape strips were collected from participants scalps at baseline, week 4 and regression week 2 and stored at -80°C till analysis. 30 participants' samples were analysed for histamine quantification. Histamines were extracted from tape strips and quantified by using HIS (Histamine) enzyme linked immunosorbent assay (ELISA) Kit by Elabsciences.

The concentration of histamine from the test samples (tape strips) were calculated using the histamine standard curve generated with histamine stock provided with the kit. 30 participants samples were analysed for histamine quantification.

Sample collection method and quantification of HODE

Tape strips were collected from participants scalps at baseline, week 4, at regression week 2 and stored at -80ºC till analysis. 30 participants' tape stripped samples were analysed for HODE quantification. HODE from tape strips were extracted with methanol by sonication. 13-Hydroxyoctadecadienoic acid (bio-organics- ARK-423-2649) standards were prepared in the range of 0.2 to 209 (ng/ml) and quantitative analysis was performed on a Sciex API 5500 triple quadrupole mass spectrometer and ZORBAX SB C8 column (4.6×100 mm, 3.5 µm) used for chromatographic separation. The mobile phase consisted of the mobile phase consisted of 0.1% formic acid: acetonitrile 40:60 (v/v) ratio, with a flow rate of 0.800 ml/min (with splitter). The total run time was 7.0 minutes. The column oven was maintained at 25 °C, and the autosampler temperature was set at 10 °C. Injected volume of the sample 15 µl.

Demographics of the study volunteers

53 subjects were enrolled and 46 subjects completed the study. Efficacy of the test products in reducing dandruff was assessed by dermatological visual assessment of both adherent scalp flaking score (ASFS) and Loose flakes. Additionally, scalp oiliness was measured by sebumeter, whereas hair fall was assessed by combing test. Ayurvedic doctor reviewed the study throughout along with dermatologist, the primary investigator. Demographic characteristics of the subjects are depicted in Table 1.

Table 1: Demographics and volunteers characteristics at baseline.

Characteristics	Frequency
Age range (years)	18-45
Gender category	
Number of participants (ratio of male: female)	53 (1:2)
Total dandruff score at baseline as per dermatologist's assessment	≥40 (ASFS+ loose flakes)

Statistical analysis

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

RESULTS

Efficacy in reducing adherent scalp flakes

Dandruff scales were assessed based on ASFS. The scalp area was divided into 8 parts, and each partition was assessed individually on a 1-10 scale and added to get total ASFS score. Ayurvedic anti-dandruff regime showed significant reduction in ASFS from week 2 onwards compared to baseline (Figure 1). The present anti-dandruff treatment reduced ASFS by 14.8 units by the end of 4 weeks and sustained the same below baseline levels till the end of 2-weeks regression (R-week 2).

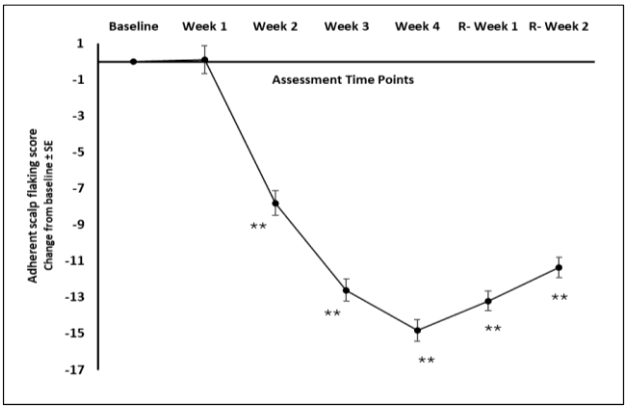

Figure 1: Adherent scalp flaking score: data expressed as change from baseline±SE; **p<0.05 versus baseline).

Efficacy in reducing loose flakes

Participant's scalp was evaluated by dermatologist for the presence of non-adherent loose dandruff flakes by partitioning scalp into 6 parts, using a scale from 0 to 10 for each partition. Ayurvedic anti-dandruff regime showed significant reduction in loose flakes from week 2 onwards compared to baseline (Figure 2) with sustained reduction of loose flakes by 15 units at the end of 4 weeks and maintained the same below baseline levels till the end of 2 weeks regression (R-week 2).

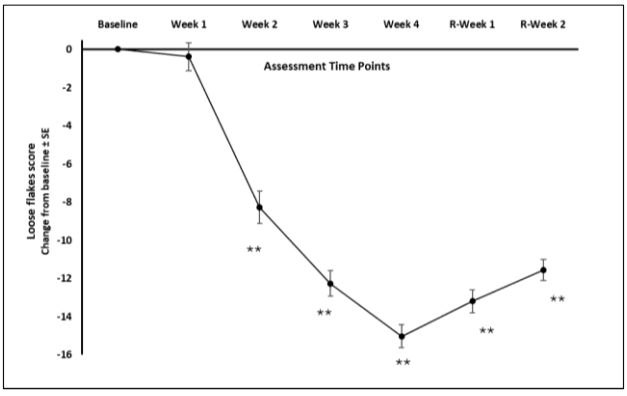

Figure 2: Loose flakes score: data expressed as change from baseline±SE; **p<0.05 versus baseline).

Efficacy in reducing scalp sebum levels

Scalp sebum levels were measured to quantify the sebum on scalp to assess the effect of treatment. The ayurvedic anti-dandruff regime showed significant reduction in scalp sebum levels from week 1 onwards with sustained reduction till weeks 4 treatment (versus baseline). The

ayurvedic anti-dandruff regime demonstrated significant sebum reduction by 64% compared to baseline by the end of 4 weeks treatment phase (Figure 3).

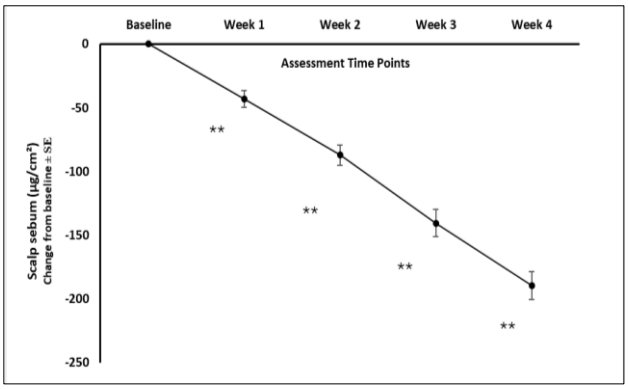

Figure 3: Sebum levels: data expressed as change from baseline±SE; **p<0.05 versus baseline).

Efficacy in reducing scalp itch

Dandruff scalps are often associated with itch and irritation. Hence itch was assessed based on subject's self-perception scores. The present ayurvedic anti-dandruff regime showed significant reduction in itch perception from week 1 onwards compared baseline (Figure 4). Itch resolved completely by the end of 4 weeks treatment and no relapse of itch was observed till the end of 2 weeks regression (R-week 2) indicating the long-lasting itch control benefit of the regime.

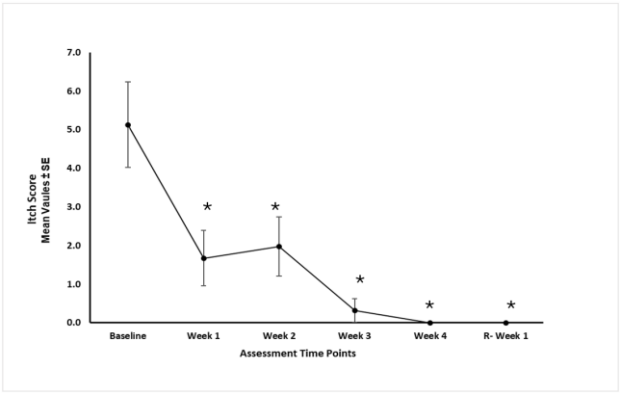

Figure 4: Itch score: data expressed as mean score±SE; **p<0.05 versus baseline).

Efficacy in reducing hair fall

Hair fall is also one the problems associated with dandruff. Hair fall from roots, with bulb was assessed using combing test from baseline to regression week. The present ayurvedic anti-dandruff regime showed significant reduction in hair fall (as measured by number of hairs fallen with roots, with bulb) from week 3 onwards compared baseline (Figure 5) and maintained the efficacy below baseline till end of 2-week regression period (R-week 2).

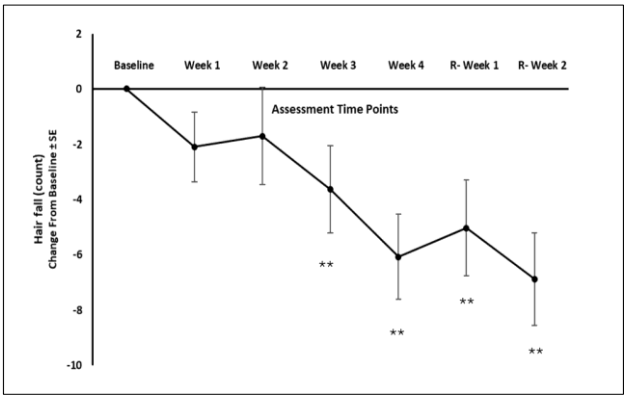

Figure 5: Hair fall: data expressed as change from baseline±SE; **p<0.05 versus baseline).

Efficacy in reducing histamine levels

Histamines from tape strips were extracted and quantified using ELISA kit. High level of histamines was observed on dandruff scalps at baseline. Ayurvedic anti-dandruff regime showed significant reduction in histamine levels (30%) by the end of 4 weeks treatment phase (Figure 6) with sustained low levels even at the end of 2-weeks regression period (R-week 2). There is no significant reduction compared to 4-weeks treatment assessment time point versus 2-weeks regression period indicating the long-lasting treatment benefit.

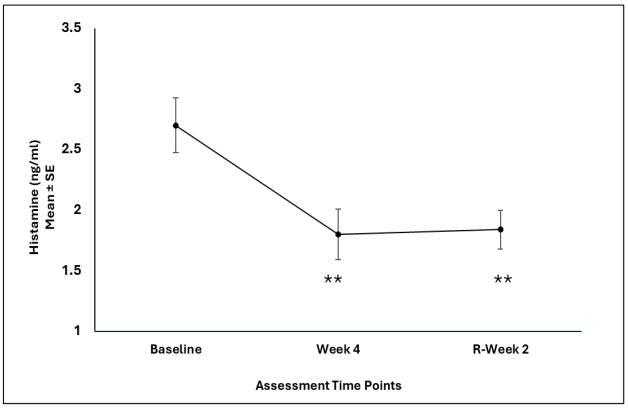

Figure 6: Histamines: data expressed as mean histamine levels (ng/ml) ±SE; **p<0.05 versus baseline).

Efficacy in reducing HODE levels

High level of HODE was detected on dandruff scalps (estimated from tape strips) at baseline. Ayurvedic anti-dandruff regime showed significant reduction in HODE levels (by 50%) at the end of 4 weeks of treatment phase (Figure 7), with sustained low levels even at the end of 2-weeks regression period (R-week 2). Moreover, there is no significant difference observed in HODE levels compared

to 4-weeks treatment assessment time point versus 2 weeks regression indicating the long-lasting treatment benefit.

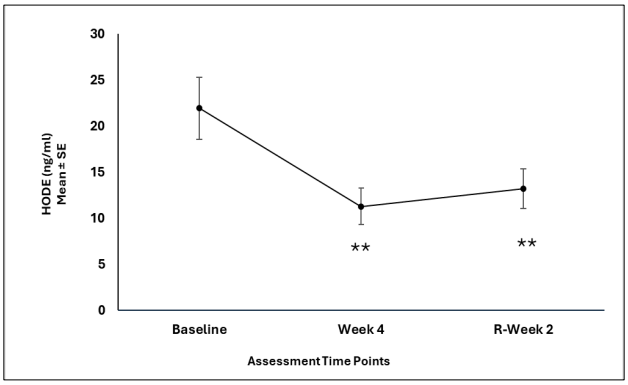

Figure 7: HODE: data expressed as mean HODE (ng/ml) ±SE; **p<0.05 versus baseline).

DISCUSSION

Redox imbalance is a highly studied pathological event in etiopathology of various human diseases starting from simple inflammatory condition to cancer tracing its origin from the cellular level to various external stressors.[11] Even healthy cells, during various metabolic pathways produce highly reactive oxygen species or free radicals which are quenched by innate antioxidant defense enzymes like catalase, superoxide dismutase (SOD) to tri-peptides like glutathione along with vitamin E.[12,13] Human skin and scalp are the first line defense and acts as a barrier to protect internal organs. In healthy scalp redox status is balanced with optimum levels of innate antioxidant systems. However, when exposed to constant internal and external stressors redox state shifts in favor of prooxidants due to impaired antioxidant systems leading to tissue damage. At molecular levels this causes protein oxidation or lipid peroxidation, draws inflammation leading to loss of structural and functional properties of cell/tissue and impairs tissue homeostasis of skin or scalp.[14,15]

Dandruff is a scalp scaling condition with altered epidermal homeostasis and oxidative stress on scalp. Multiple factors have been implicated in the development of dandruff condition like excessive sebum, high load of Malassezia species, with altered scalp microbiome, individual susceptibility and grooming practices.[16] One in every two individuals are known to suffer from dandruff at some point of time in their life. Owing to high prevalence multiple products and formats are available in the market space and developed with anti-fungal actives like zinc pyrithione, climbazole, and piroctone olamine.[17] To address this multifactorial causative nature, the present anti-dandruff regime is developed with multi-ingredient approach as per ancient system of Indian medicine, The Ayurveda using authentic ayurvedic processes like tailapak and arka vidhi.

Ayurveda recommends multiple ingredients to treat this dandruff condition. Dandruff, is referred to as darunaka and is classified under kshudra rogas (minor diseases), with symptoms like kesha boomi sputana (scaling), kandu (itching) which is caused due to vitiated vata and kapha doshas.[18-20] Multiple ayurvedic ingredients like Svetakutaja, Amla, Nimba, and Rosemary are recommended to treat dharunaka in various ayurvedic texts.[21-23] Svetakutaja with proven efficacy against pathogenic dermatophyte fungi and dandruff causing *Malassezia furfur* and is also commonly used to treat inflammatory skin and scalp condition like psoriasis.[24] Neem is popularly referred for various skin conditions, to promote scalp health for anti-itch benefits and scientifically proven to have anti-fungal properties.[25] Additionally, there is an accumulated clinical evidence of using Neem in resolving dandruff, compelling its use in anti-dandruff formulations.[26] This present ayurvedic anti-dandruff regime is thoughtfully crafted with ayurvedic ingredients (Neem, Svetakutaja, Bringha, and Rosmary) to deliver anti-dandruff benefits and is clinically proven to reduce dandruff flakes and scalp sebum levels significantly, in 100% of the study participants as early as two weeks of treatment.

Incomplete differentiation of keratinocytes into corneocytes and defective desquamation leads to build up scales leading to visible dandruff flakes.[27] Histamines have been implicated in altering the differentiation of keratinocytes and high levels histamines are reported in dandruff scalps which could be one of the reasons for imbalance in corneocyte differentiation causing flaking disorders.[28,29] Adding to the above, histamines have a proven role in evoking itch response, thus confirming the role of histamines in flaking and itch in dandruff scalps.[30] High levels of histamines have been reported in dandruff scalps and resolution of the same was observed up on treatment with anti-dandruff treatments developed with anti-fungal actives.[31] In the present clinical study ayurvedic anti-dandruff regime showed itch control from week one onwards and resolved itch completely by week 4 along with reduction of itch markers (histamines) by the of end of treatment (4 weeks) with sustained low levels even after 2 weeks of withdrawal of the anti-dandruff regime.

Redox imbalance has been reported on scalps of people suffering from dandruff compared to healthy scalps.[32] In general dandruff scalps secrete higher levels of sebum and constituents of which are highly prone to oxidation.[33] With low antioxidant defense systems, vicious cycle of oxidative reactions causing damage to lipids and proteins on the scalps and induce inflammatory reactions. High levels of HODE, squalene peroxides and malondialdehyde were estimated from the dandruff scalps providing compelling evidence of oxidative stress.[34-36] HODE is a oxidatively modified fatty acid and have been an established oxidative stress maker in various inflammatory condition, metabolites of HODE were proven to evoke inflammatory pain in experimental models.[37] Hence reducing the oxidative stress on dandruff scalps is a strategic target to treat root cause of dandruff symptoms effectively. Rosemary is known for its anti-dandruff and antioxidant benefits and have been used at functional levels in the present ayurvedic anti-dandruff regime.

High levels of the HODE as detected at baseline are indications of redox imbalance on dandruff scalps in the present study, which were reduced by 50% by the end of 4 week treatment period, and reduced levels were maintained in spite of withdrawing the regime for two week, indicating that products not just works at surface levels but penetrates deep to treat the root cause and bringing back the redox balance. The present ayurvedic anti-dandruff regime is developed with Svetakutaja, Amla and Rosemary and other actives that helped in reducing oxidative stress (on dandruff scalps) to restore scalp health.

Limitations

The study is limited by its non-comparative design and small sample size. Future comparative studies with larger, more diverse samples could strengthen the evidence.

CONCLUSION

The present ayurvedic anti-dandruff regime is crafted with unique combination of ayurvedic ingredients with multiple mechanisms of actions to deliver the end benefit of long-lasting anti-dandruff efficacy. Treatment was effective in reducing dandruff flakes, hair fall from root (with bulb) and sebum levels along with itch and oxidative stress markers on scalp. Scalp itch is a hallmark symptom of dandruff, and in the present study, it was completely resolved, with the significant reduction in histamine levels observed in these dandruff sufferers.

89 % of the study subjects participating in the study felt the product improves scalp health and reduced dandruff. There were no adverse events reported throughout the study. The present clinical study concludes the efficacy of ayurvedic anti-dandruff regime in treating dandruff and associated symptoms effectively. Regime is safe to use in both men and women without any undesirable product sensory like greasy/tacky/oiliness on scalp.

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