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A proprietary herbal extract against hair loss in androgenetic alopecia and telogen effluvium: a placebo-controlled, single-blind, clinical-instrumental study

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Abstract

Introduction: Currently there are only a limited number of drugs available for treatment of androgenetic alopecia and telogen effluvium. However, certain plants and their standardized extracts may provide some clinical benefits against hair loss. We formulated a herbal shampoo and a solution to evaluate their efficacy, safety, and synergy in hair loss.
Methods: We conducted a randomized, placebo-controlled, single-blind, clinical and instrumental study for 6 months on 120 subjects with androgenetic alopecia and telogen effluvium, confirmed by pull test and phototricogram. Each subject was examined monthly. At the end of the study, a self-assessment test was carried out.
Results: Herbal formulations were found to be more effective in preventing and reducing hair loss than placebo at every assessment point. Anagen/telogen ratios improved significantly in the study group. In addition, concomitant use of the shampoo and solution were found to be more effective than single product use.
Conclusion: We interpret this eutrophic effect for scalp hair as the final outcome of the entire content of our herbal formula, which has antiandrogenic, anti-inflammatory, antioxidative, angiogenic, and hair-stimulating features. In combination, these features help prevent hair from falling out and reducing hair loss.

Keywords: herbal extract, hair loss, clinical study

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Introduction

Although hair loss is not a serious problem for general health, it is a matter of concern because it can lower self-confidence and create feelings of inferiority. In general, patients with alopecia have a higher incidence of psychiatric disorders such as depression, anxiety, and social phobia compared to the rest of the population (1).

Although androgenetic alopecia (AGA) is more prevalent in men, it is a widespread dermatological problem that also affects women. Up to 30%, 50%, and 80% of the men affected are over the ages of 30, 50, and 80 respectively. Androgens play an important role that appears to be independent from genetic predisposition, which is considered the main etiologic factor in AGA (2, 3). Testosterone is converted into its more potent form dihydrotestosterone (DHT) by the enzyme 5α-reductase (5α-R). The AGA-prone scalp has high levels of DHT and augmented androgen receptor (AR) expression (4). Inflammatory processes are also increasingly being cited as an integral part in the pathogenesis of AGA (4–8). In AGA, scalp biopsies from both men and women revealed follicular microinflammation and lymphocytic folliculitis, targeting an immunologically driven trigger (7, 8). One of the factors leading to permanent hair loss in AGA may be this continuous inflammation and remodeling of the connective tissue of follicles (4).

Telogen effluvium (TE) is another frequent cause of diffuse hair loss, but the true incidence is not well known because of insufficient data, especially due to subclinical cases (9). Although TE may be one manifestation of various chronic systemic illnesses, an association between stress and hair loss is well accepted among clinicians (10, 11). The sensitivity of human hair follicles to key skin-stress mediators has been demonstrated: organ-cultured hair follicles responded to substance P with premature catagen development and degranulation of mast cells in the connective tissue sheath of hair follicles, indicating a neurogenic inflammation (12). Acute TE due to oxidative stress induced by ultraviolet radiation has also been identified. Photoactivation of porphyrin compounds produced by bacteria in the pilosebaceous duct led to oxidative tissue injury and follicular microinflammation (13).

Currently, although topical minoxidil and oral finasteride are the only medications approved for AGA, cause-oriented treatment is performed for TE. Considering the androgenetic and inflammatory factors in the pathogenesis explained above, we formulated a herbal shampoo and solution containing a mixture of six different herbal extracts (HE) with antiandrogenic, anti-inflammatory, and antioxidative features. Our hypothesis was that, if there is a high enough concentration of relevant flavonoids, vitamins, and trace elements at the site of action (i.e., hair follicles), then inflammatory and aging processes can be slowed down and reversed to a certain extent. This study was performed to assess the efficacy, safety, and synergy of the new cosmetic herbal shampoo and solution for treatment of AGA or TE.

Methods

Products tested

Herbal shampoo: aqua, sodium laureth sulfate, cocamidopropyl betaine, sodium chloride, mixture of herbal extracts (*Urtica urens* leaf extract, *Urtica dioica* root extract, *Matricaria chamomilla* flower extract, *Achillea millefolium* aerial part extract, *Ceratonia siliqua* fruit extract, *Equisetum arvense* leaf extract), glycerin, benzyl alcohol, perfume/fragrance, PEG/PPG-120/10-trimethylolpropane trioleate, laureth-2, hydroxypropyl guar, hydroxypropyltrimonium

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chloride, polyquaternium-7, amodimethicone, tetrasodium EDTA, menthol, mel, panthenol, C11-15 pareth-7, laureth-9, sodium PCA, butylphenyl methylpropional, trideceth-12, coumarin, hydroxyisohexyl 3-cyclohexene carboxaldehyde, methylchloroisothiazolinone, niacinamide, Panax ginseng root extract, and methylisothiazolinone.

Herbal solution: aqua, mixture of herbal extracts (*Urtica urens* leaf extract, *Urtica dioica* root extract, *Matricaria chamomilla* flower extract, *Achillea millefolium* aerial part extract, *Ceratonia siliqua* fruit extract, *Equisetum arvense* leaf extract), sodium hydroxymethylglycinate, mel, and glycerin.

Placebo shampoo: aqua, sodium laureth sulfate, cocamidopropyl betaine, sodium chloride, E150a (caramel color), glycerin, benzyl alcohol, perfume/fragrance, PEG/PPG-120/10-trimethylolpropane trioleate, laureth-2, hydroxypropyl guar, hydroxypropyltrimonium chloride, polyquaternium-7, amodimethicone, tetrasodium EDTA, menthol, mel, panthenol, C11-15 pareth-7, laureth-9, sodium PCA, butylphenyl methylpropional, Trideceth-12, coumarin, hydroxyisohexyl 3-cyclohexene carboxaldehyde, methylchloroisothiazolinone, niacinamide, Panax ginseng root extract, and methylisothiazolinone.

Placebo solution: aqua, E150a (caramel color), sodium hydroxymethylglycinate, mel, and glycerin. The placebo shampoo and solution did not contain the HE. All of the products tested complied with EU cosmetic regulations and the products were notified in the Cosmetic Products Notification Portal (CPNP) system.

Phytochemical analyses of HE

Vitamin and flavonoid analyses were performed with high-pressure liquid chromatography (HPLC) at Phytolab, Vestenbergsgreuth, Germany. Trace elements analyses were performed with inductively coupled plasma optical emission spectrometry (ICP-OES) at Saniter Lab, Istanbul, Turkey.

Clinical panel and ethical requirements

The sample consisted of otherwise healthy Caucasian subjects (60 males and 60 females) 20 to 55 years old, suffering from AGA grades II and III in accordance with the Hamilton–Norwood scale or TE, enrolled in the trial (54 AGA subjects, 66 TE subjects) with written informed consent. The subjects were chosen under the control of a dermatologist on the basis of the inclusion/exclusion criteria: the subjects were not using any topical or systemic medications, they had no scalp or hair disease except the trial indications, they had no hypersensitivity history, and none of them were pregnant or lactating. All of the trial procedures were performed in line with the ethical principles laid down for medical research (Helsinki Declaration of World Medical Association, 1964, and amendments). In the event of any unexpected or adverse reaction, a medical investigator was on standby for intervention.

Group formation

The products' allocations were randomized in accordance with a randomization list. The randomization list was created using Wei's urn algorithm, which is designed to generate balanced random samples throughout the course of an experiment. The following groups were formed: Group A: 30 subjects that used herbal (active) shampoo, Group B: 30 subjects that used herbal (active) solution, Group C: 30 subjects that used herbal (active) shampoo and herbal (active) solution, and Group D: 30 subjects that used placebo shampoo and placebo solution. In addition to the mean/median ages of the subjects, the gender and clinical distribution in each group are presented in Table 1. There were no differences in the demographic features among the groups.

Table 1 | Sex and clinical distributions, means ± standard deviations (SD), and medians of the ages for each group and all subjects.

Group	Male	Female	AGA	TE	Age (mean ± SD)	Age (median)
A	15	15	14	16	36.6 ± 10.6	37.0
B	15	15	13	17	37.3 ± 9.7	37.0
C	15	15	13	17	36.6 ± 9.7	34.5
D	15	15	14	16	37.0 ± 9.6	36.0
Total	60	60	54	66	36.9 ± 9.8	36.5

AGA = androgenetic alopecia, TE = telogen effluvium.

Product use

Shampoo: Every other day, three times a week, apply 5 ml on wet hair, wait for 3 to 4 minutes after foaming, and then rinse well.

Solution: Every day in the morning and in the evening, apply 3 ml on dry hair and massage all over the scalp. Let it stand for at least 4 to 6 hours.

Study design

After the enrollment and the evaluation of the physiological scalp and hair conditions by the dermatologist (T0), the subjects were informed about the principles of the study. At the end of each month, the subjects were evaluated based on the criteria presented in Table 2.

Table 2 | Summary of the study outline (T = Time of evaluation in months).

Component	T0	T1	T2	T3	T4	T5	T6
Enrollment	X						
Inclusion criteria	X	X	X	X	X	X	X
Subject compliance	X	X	X	X	X	X	X
Pull test	X	X	X	X	X	X	X
Phototricogram	X		X		X		X
Dermatological evaluations	X	X	X	X	X	X	X
Self-assessment							X

Pull test

The pull test was used to assess diffuse hair loss. Mild traction was performed on a bunch of hair (approximately 60 fibers) and the number of hairs extracted was counted. Operatively, the dermatologist took a few strands between his thumb and forefinger and pulled them gently. Anagen (growing) hair should remain rooted in place, and telogen (non-growing) hair should come out easily. If the number of lost hairs was greater than six, the pull test was considered positive. Subjects were requested not to wash their hair in the 24 hours before the pull test.

Phototricogram

A targeted area of 1.8 cm^2 was chosen for clipping. This was a zone of transition between normal hair and the balding area in male subjects with AGA, and the middle part of the vertex in TE and female AGA subjects. The shaved hair zone was dyed gray, and photos were taken with a digital close-up camera immediately and 2 days later. By comparing these two consecutive photographs with a software system identifying individual hair fibers, growing (anagen phase) or non-growing (telogen phase) fibers were determined.

Dermatological evaluation

The dermatologist clinically evaluated the presence of dandruff, excess sebum, scalp redness, or the onset of an itching sensation.

Self-assessment

At the end of the study, in month 6, the subjects were given a 10-point questionnaire on which 1 was the lowest and 10 was the highest score in order to rate the benefit of the treatments on certain hair properties. The questionnaire is presented in Table 3.

Table 3 | Questionnaire given to the subjects at the end of the study.

	Questions	Scores
1	Have you noticed a decrease in hair loss?	1 2 3 4 5 6 7 8 9 10
2	Have you noticed the growth of new hair?	1 2 3 4 5 6 7 8 9 10
3	Have you noticed an increase in hair thickness?	1 2 3 4 5 6 7 8 9 10
4	Has your hair grown faster?	1 2 3 4 5 6 7 8 9 10
5	Has the treatment reinforced your hair?	1 2 3 4 5 6 7 8 9 10

Statistical analyses

In intragroup evaluation (time course), the Wilcoxon signed-rank test for non-parametric pull test data and repeated measured analysis of variance followed by Student's *t*-test for parametric phototricogram data were used. In intergroup evaluation (active vs. active vs. placebo), the Wilcoxon Kruskal–Wallis one-way analysis of variance on ranks for non-parametric pull test and self-assessment data, and multivariate analysis of variance followed by Student's t-test for parametric phototricogram data were used.

Results

Phytochemical analyses of HE

The vitamins, flavonoids, and trace elements found in phytochemical analyses of HE are presented in Table 4. The quantities of all of them are expressed as mg/100 g dry extract.

Pull test

The comparison between Group A / shampoo (active) vs. Group B / solution (active) vs. Group C / shampoo (active) + solution (active) vs. Group D / shampoo (placebo) + solution (placebo) are presented in Fig. 1, and the graph reports the mean (%) decrease obtained for each product tested. The data are reported as mean (%) variation of hair loss during the pull test for each month (T1–T6) compared to the beginning of the trial (T0). Although the intragroup statistical analyses revealed significant improvement in all groups for all months compared to T0, the intergroup analyses showed that Group C (active shampoo + solution) had the best clinical outcomes and Group D (placebo shampoo + solution) had the worst. The *p* values of the intergroup analyses are presented in Table 5. Fig. 1 also shows that longer usage of products corresponds to better clinical outcomes.

Table 4 | Vitamins, flavonoids, and trace elements found in phytochemical analyses of HE.

Component	(mg/100 g dry extract)
Vitamins	
Thiamin (B_1)	7.4
Riboflavin (B_2)	1.4
Pyridoxin (B_6)	1.2
Ascorbic acid (C)	‹ 0.5
Flavonoids	
Myricetin	1,450
Quercetin	400
Kaempferol	600
Trace elements	
Iron	53.06
Copper	0.645
Zinc	2.048

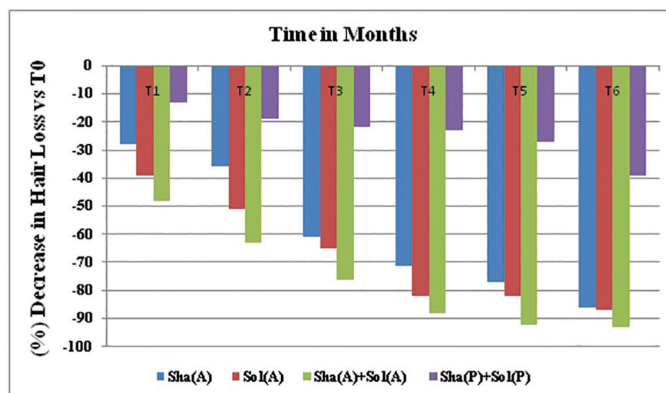

Figure 1 | Summary of pull test results. (%) decrease in hair loss for each month (T1–T6) compared to the beginning of the trial (T0). Group A / shampoo (active) vs. Group B / solution (active) vs. Group C / shampoo (active) + solution (active) vs. Group D / shampoo (placebo) + solution (placebo).

Phototrichogram

Whereas there was no increase in group D (placebo shampoo + solution) during the study, the number of total hairs in groups A (active shampoo), B (active solution), and C (active shampoo + solution) increased significantly in month 4 (T4) and month 6 (T6) compared to the beginning of the trial (T0, Fig. 2).

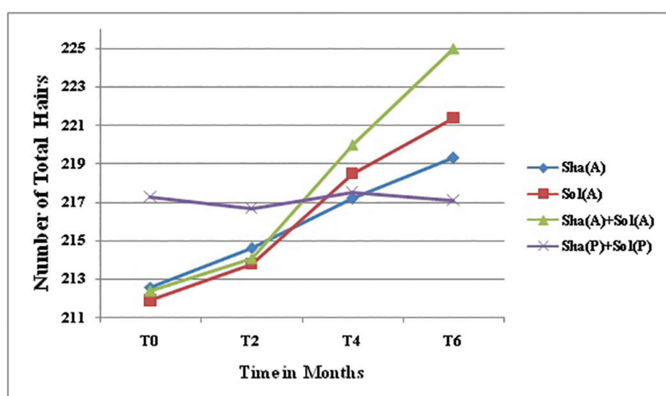

Figure 2 | Increase in number of total hairs from the beginning of the trial (T0) to the month 6 (T6) for every 2 months. The *p* values of Group A / shampoo (active), Group B / solution (active) and Group C / shampoo (active) + solution (active) in T4 and T6 were ‹ 0.001; whereas T2 was › 0.05 compared to T0. All of the values (i.e., T2–T6) of Group D / shampoo (placebo) + solution (placebo) were › 0.05 compared to T0.

Table 5 | The *p* values of Group C / shampoo (active) + solution (active) vs. Group A / shampoo (active), Group B / solution (active), and Group D / shampoo (placebo) + solution (placebo) for months 1 through 6, each month (T1–T6) compared to the beginning of the trial (T0).

Group	T1	T2	T3	T4	T5	T6
Sha(A) + Sol(A) vs. Sha(A)	0.000568	0.000042	0.001583	0.000194	0.000002	0.013762
Sha(A) + Sol(A) vs. Sol(A)	0.741908	0.046241	0.037569	0.070589	0.010426	0.049356
Sha(A) + Sol(A) vs. Sha(P) + Sol(P)	0.000001	0.000001	‹ 0.000001	‹ 0 .000001	‹ 0.000001	‹ 0.000001

Sha = shampoo, Sol = solution, (A) = active, (P) = placebo.

The decrease in telogen hairs and increase in anagen hairs were also significant in groups A, B, and C in T4 and T6 compared to T0 (Figs. 3–5). The (%) changes in telogen and anagen hairs through T2–T6 in group D were not significant (Fig. 6).

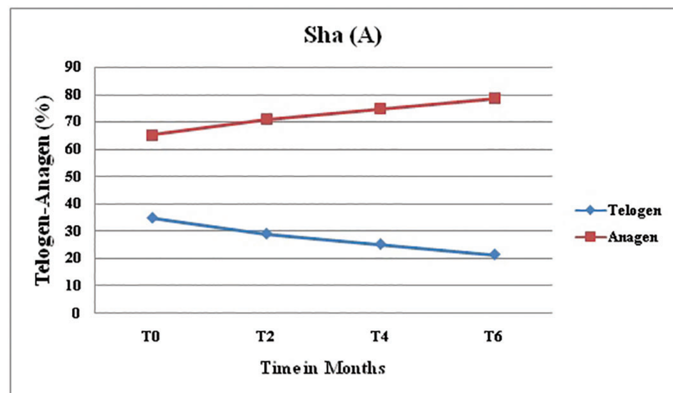

Figure 3 | Telogen-anagen (%) changes for Group A / shampoo (active) for every 2 months. The p values for both telogen and anagen changes in T2–T6 were < 0.001 compared to T0.

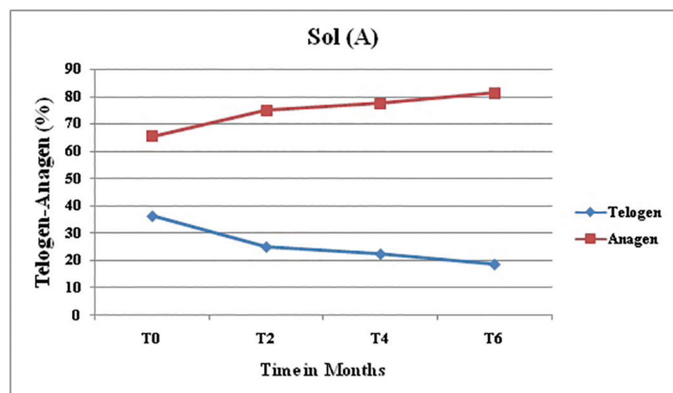

Figure 4 | Telogen-anagen (%) changes for Group B / solution (active) for every 2 months. The p values for both telogen and anagen changes in T2–T6 were < 0.001 compared to T0.

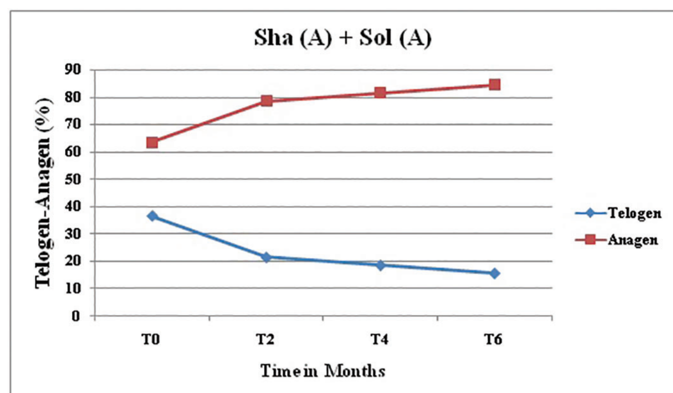

Figure 5 | Telogen-anagen (%) changes for Group C / shampoo (active) + solution (active) for every 2 months. The p values for both telogen and anagen changes in T2–T6 were < 0.001 compared to T0.

Self-assessment

Fig. 7 presents the mean scores of self-assessment questions. The highest scores are from Group C (active shampoo + solution), and

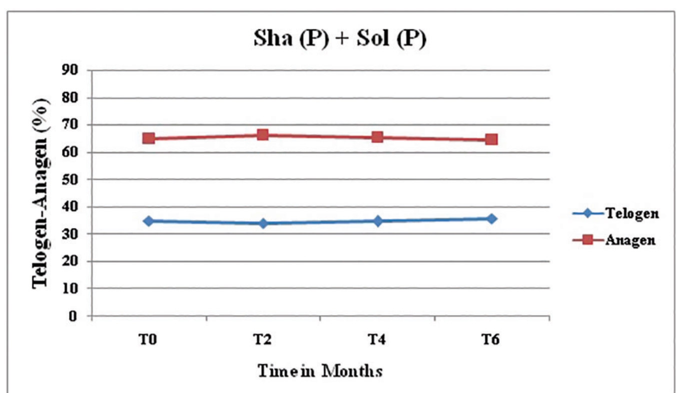

Figure 6 | Telogen-anagen (%) changes for Group D / shampoo (placebo) + solution (placebo) for every 2 months. The p values for both telogen and anagen changes in T2–T6 were > 0.05 compared to T0.

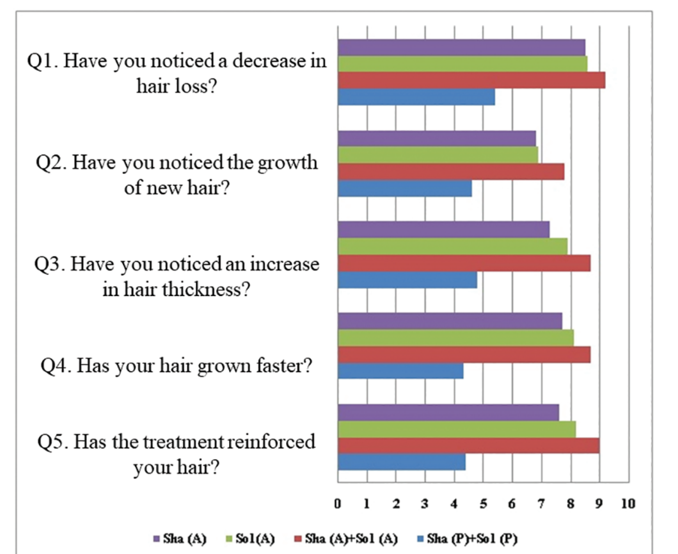

Figure 7 | Results of self-assessment questions for the treatments and placebo.

the lowest scores are from Group D (placebo shampoo + solution). In statistical evaluation of the self-assessment data, all of the questions showed statistically significant results based on the difference from placebo. Here Group C also has the best clinical outcomes and Group D the worst. The p values of intergroup analyses for each question are presented in Table 6.

General considerations for clinical-instrumental results

The products were well tolerated and no side effects were recorded during the study. The herbal/active shampoo and solution helped ameliorate the baseline conditions of subjects suffering from AGA or TE. In this 6-month study, the regular use of the herbal shampoo and/or solution decreased hair loss and increased the number of anagen hairs. These effects, clinically or instrumentally confirmed, were also perceived by the subjects participating in the study and significantly delineated in the statistical analyses of self-assessment results. The results obtained in the group that used both active products (shampoo and solution) were sig-

Table 6 | The p values of Group C / shampoo (active) + solution (active) vs. Group A / shampoo (active), Group B / solution (active), and Group D / shampoo (placebo) + solution (placebo) for each self-assessment question. All of the values are statistically significant.

Group	Q1	Q2	Q3	Q4	Q5
Sha(A) + Sol(A) vs. Sha(A)	0.031921	0.017811	0.000336	0.001156	0.000034
Sha(A) + Sol(A) vs. Sol(A)	0.027737	0.024259	0.027002	0.028522	0.013295
Sha(A) + Sol(A) vs. Sha(P) + Sol(P)	< 0.000001	< 0.000001	< 0.000001	< 0.000001	< 0.000001

Sha = shampoo, Sol = solution, (A) = active, (P) = placebo.

nificantly better than those obtained in the groups that used only one active product (shampoo or solution). The concomitant use of herbal shampoo and herbal solution had a synergistic effect in improving the parameters measured.

Discussion

The causes for the clear significant positive results of the products tested should be sought in the interaction between the properties of the plants in our HE and etiopathogenetic factors in the hair loss types included in this study.

Among the plants used in the formulation, *Urtica dioica* (Ud) is the most widely studied. The use of Ud root extract in symptomatic benign prostate hyperplasia (BPH) and lower urinary tract symptoms has been investigated in numerous clinical trials and found effective (14–17). This indication is mainly due to its 5α-R inhibition activity (18, 19). Inhibition of 5α-R hampers the formation of DHT from testosterone. An excess amount of DHT is related to BPH (17). The same pathogenesis is also true for AGA (4, 5), and this mechanism of action may be the main cause of the success of our formulation in patients with AGA. Although Ud leaves have traditionally been used for hair loss, confirmatory clinical trials are still lacking (17). In a study performed with a combination of herbal extracts including Ud, it was shown that the combination enhanced human dermal papilla cell proliferation at concentrations between 1.5% and 4.5% (20).

As stated in the introduction to this report, apart from the pathogenesis of AGA related to androgen metabolism, inflammatory and oxidative processes are the basic common pathways in the etiopathogenesis of both AGA and TE (4–13). Polysaccharides and caffeic malic acid (CMA) both exist to some extent in every part of Ud. *Urtica* polysaccharides and CMA present anti-inflammatory activity by inhibiting cyclooxigenase and lipoxygenase (21, 22). β sitosterol, also a component of Ud, stimulates angiogenesis by increasing vascular endothelial growth factor (VEGF) synthesis and supports new hair growth (23). This molecule also suppresses testosterone synthesis and contributes to decreased DHT levels (24).

The proximate analysis results showed that leaf extract of *Urtica urens* (Uu) contains a high amount of total phenolics, flavonoids, tannins, ortho-diphenols, and flavonols (25, 26). It has been shown to decrease paw edema after carrageenin administration in rats and to improve the activities of catalase, superoxide dismutase, glutathione peroxidase, and malondialdehyde, exhibiting powerful antioxidant and anti-inflammatory activity (26).

In a study performed with *Equisetum arvense* (Ea) alone and with a combination of some other plants, Ea suppressed superoxide anion levels in the xanthine/xanthine oxidase system and eliminated the hydroxyl radical. Ea also reduced reactive oxygen species (ROS) in neutrophils that were stimulated by phorbol myristate acetate. In the same study, although carrageenin-induced paw swelling in rats was significantly inhibited by the plant mixture, this inhibition could not be achieved by the components on an individual basis (27). This phenomenon could explain why our plant mixture also had a synergistic effect in obtaining the good outcomes in the study. In addition to various studies depicting its antioxidative features (28–30), Ea exhibited significant diabetic wound healing by showing higher epidermal and dermal regeneration, angiogenesis, and granulation tissue thickness in diabetic rats (31). Ea is one of the highest silicon accumulators among plant species. Silicon penetrates the hair follicles, enters in the hair matrix, and makes the hair fibers thicker. Therefore, the higher the silicon quantity in the hair, the lower the extent of hair loss (32, 33).

Leaves and flowers of *Achillea millefolium* (Am) have been used for centuries for their anti-inflammatory effect in conditions such as rheumatism, wound healing, and skin inflammation (34). In an *in vivo* study performed with Am oil extract on artificially irritated skin, the parameters tested (i.e., pH, capacitance, and erythema index) were restored to basal values after 3 to 7 days of treatment (35). Am extract showed an augmentation in cytokeratin-10, transglutaminase-1, and filaggrin expressions, together with an increase in the thickness of epidermis in cultured skin biopsies. In addition, a 2-month topical application of 2% Am extract significantly improved the outlook of wrinkles and pores *in vivo* when compared with placebo (36).

Historically, oil of *Matricaria chamomilla* (Mc, German chamomile) has been used in the treatment of some inflammatory dermatoses. It contains three major sesquiterpene compounds (i.e., azulene, bisabolol, and farnesene) with anti-inflammatory or antihistamine effects. Among them, α-bisabolol has the strongest anti-inflammatory effect (37, 38). In a study performed with 3% Mc oil topically on the skin of mice showing its immunoregulatory potential, it alleviated atopic dermatitis through influencing helper T cell 2 (Th2) activation (39). Mc extract applied topically also showed wound healing potential in a linear incisional model in rats (40). A pharmacologically active flavonoid apigenin that is contained by both Mc and Am supports hair growth by suppressing transforming growth factor-β1 (TGF-β1), which stimulates the catagen phase in the hair growth cycle (41, 42).

Ceratonia siliqua (Cs) pod extract presents antioxidant features due to its catechin, epicatechin, epigallocatechin, epigallocatechin gallate, and epicatechin gallate contents, along with simpler phenolics, such as phloroglucinol, pyrogallol, catechol, and gallic acid (43–45). In addition, Cs fibers exhibit higher antioxidant capacity than many edible products rich in polyphenols, such as blueberries, grapes, or red wine (46, 47).

On the other hand, the flavonoids, vitamins, and trace elements we found in the phytochemical analyses of our formulation may offer more specific support for the wellbeing of hair physiology. Myricetin, quercetin, kaempferol, and copper keep the hair follicle in the anagen phase longer by inhibiting 5α-R type 2 and preventing DHT formation (48, 49). Vitamin B complex and vitamin C (ascorbic acid) enhance blood vessel formation and increase blood flow in the scalp by stimulating the synthesis of VEGF (50). It was reported that mice that were exposed to high amounts of environmental cigarette smoke developed alopecia, and this was precluded by administration of l-cystine and vitamin B_6 (pyridoxine) combination (51). B-group vitamins and l-cystine mixtures are also customarily used in OTC products for hair loss (52). Vitamin B_2 (riboflavin) is known to enhance the metabolism of vitamin B_6 (53, 54). Vitamin C might improve the efficacy of therapeutic angiogenesis by cell transplantation (55). Some derivatives of vitamin C were also found to be promising in treating and preventing DHT-induced balding (56). Iron is a crucial cofactor for the enzymes implicated in energy metabolism and DNA synthesis. It is a fundamental element for healthy skin, mucous membranes, hair, and nails. In the case of scalp hair, iron deficiency leads to dryness and fragility (57). Zinc is found in the enzyme systems affecting hair formation, and local zinc ions stimulate scalp cellular formation (50). Zinc metabolism disturbances play a key role in hair loss, especially in AGA and TE (58).

The results obtained in this study demonstrate that the products tested have a eutrophic effect on scalp hair, and we interpret this effect as the final outcome of the entire content of the HE formulation, which has antiandrogenic, anti-inflammatory, antioxidant, angiogenic, and hair-stimulating features. These features together help prevent "hair fall" and reduce hair loss.

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