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Efficacy and Safety of a New Topical Hair Loss-Lotion Containing Oleanolic Acid, Apigenin, Biotinyl Tripeptide-1, Diaminopyrimidine Oxide, Adenosine, Biotin and Ginkgo biloba in Patients with Androgenetic Alopecia and Telogen effluvium: A Six-month Open-Label Prospective Clinical Study

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Abstract

Objective: The causes of hair loss are multifactorial; therefore, there are multiple potential targets for treatment, and a combination of different active ingredients can be helpful in managing the condition. Androgenetic alopecia and telogen effluvium comprise two of the main types of hair loss, and differ in their pathophysiology. The objective of the study was to evaluate, in male and female individuals with androgenetic alopecia and telogen effluvium, the safety and efficacy of a new hair loss lotion containing a combination of cosmetic ingredients: oleanolic acid, apigenin, biotinyl tripeptide-1, 2-4diamino pyrimidine-3-oxide, adenosine, Ginkgo biloba, and biotin.

Methods: 56 patients with androgenetic alopecia (AGA) or telogen effluvium (TE) completed the study. For 6 months, the product was applied once-daily before bed and left on overnight. Efficacy and safety assessments took place at baseline, 3 months and 6 months. Efficacy was assessed in three ways: phototrichogram to count the total number of hairs and number and of hairs in telogen and anagen, visual clinical assessment by a dermatologist using a 7 point scale that evaluated hair volume/thickness, scalp coverage and overall hair appearance, and patient opinion questionnaire on the effects and additional qualities of the product.

Results: In the whole study sample, the total number of hairs and number of anagen hairs increased significantly ($p<0.05$). In the subgroup with TE, there was a significant increase in total hairs, and in the AGA subgroup there was a significant increase in total hairs and anagen hairs. On visual clinical assessment, 35.7% of participants were evaluated as having thicker, more voluminous hair, 37.5% had improved general hair appearance and 39.3% had improved scalp coverage. On patient questionnaire, participants reported a reduction in hair loss (79%), increased confidence regarding their hair (86%) and that their hair problem was less visible (79%). The product was rated positively by 100% of participants for texture, smell, and hair condition. There were no adverse events.

Conclusion: This lotion enriched with a mixture of oleanolic acid, apigenin, biotinyl tripeptide-1, 2-4 diamino pyrimidine-3-oxide, adenosine, Ginkgo biloba, and biotin is safe and effective as a topical hair-loss treatment, as proven by the statistically significant increase in total hair fibers and anagen hairs, the increased thickness and scalp coverage on dermatological assessment, and patient self-assessment.

Keywords: Hair loss; Alopecia, Telogen effluvium; Androgenetic alopecia; Topical treatment; Oleanolic acid; Apigenin; Biotinyl tripeptide-1; Diaminopyrimidine oxide; Adenosine; Biotin

Introduction

As patients and dermatologists will testify, the aesthetic effects of dermato-trichological conditions are often the most devastating. For many men and women, having a full head of hair represents physical attractiveness and youthfulness, and hair loss can have significant negative effects on self-esteem and quality of life [1,2]. Androgenetic alopecia (AGA) and telogen effluvium (TE) are two of the most common causes of hair loss in men and women, with differing pathophysiologies but similar symptoms. To date only two therapeutic drugs have been approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of AGA: oral finasteride and topical minoxidil 2% and 5%.

Systemic treatments or surgical options may be unsuitable or undesirable due to potential side effects, expense, or invasiveness [3]. Topical drugs and cosmetics can provide a safe alternative or adjuvant to systemic drug treatment, reducing the potential side effects. In hair cosmetics, one of the keys to successful treatment is finding the most effective combination of actives and taking advantage of their different mechanisms of action. The right combination, targeting the multifactorial causes of hair loss, can improve penetration and achieve faster results. Amongst the newest actives is the combination of oleanolic acid, apigenin, and biotinyl tripeptide-1; this combination of actives improves cellular metabolism and microcirculation, and

inhibits 5α-reductase [4,5]. Diaminopyrimidine oxide is a compound chemically related to minoxidil that works against perifollicular fibrosis [6,7]. Adenosine promotes expression of several growth factors [8] biotin is a vitamin used frequently in hair and nail disease that assists in numerous metabolic reactions involved in fatty acid synthesis [9] and Ginkgo biloba is a potent antioxidant with anti-inflammatory properties [10] and improves microcirculation [11]. Besides the clinical effects of such treatments, it is also important that they be pleasant to use in terms of additional effects on the condition of the hair and the cosmetic properties: patients are unlikely to adhere to unpleasant treatments.

We studied the effects of a topical lotion containing a combination of oleanolic acid, apigenin and biotinyl tripeptide-1, diaminopyrimidine oxide, adenosine, biotin, and Ginkgo biloba for the treatment of telogen effluvium and androgenetic alopecia.

Methods

Participants: The study included 56 male and female patients aged 25-50 yrs. Diagnosed by a dermatologist with as having androgenetic alopecia or telogen effluvium by a dermatologist (Figure 1). Severity of hair loss was assessed in patients with androgenetic alopecia using the Hamilton scale for men and the Ludwig scale for women; severity of telogen effluvium was not assessed. Participants were otherwise healthy, with healthy skin at the test region and no history of allergy or atopy, and who washed their hair at least three times per week. The study excluded individuals using hair extensions, or hair straightening within 3 months prior to the study, as well as those with any immunodeficiency, those using systemic or topical steroids or retinoids, and pregnant or breastfeeding women. None of the participants were taking conventional or alternative treatments for hair loss, including 5-reductase inhibitors, minoxidil, or nutritional supplements. All participants provided signed informed consent. The study was conducted in accordance with Brazilian legislation, following Brazilian resolution No. 466/2012, adhering to good clinical practice guidelines (ICH E6 R1 (CPMP/ICH/135/95)) and the Declaration of Helsinki and its successive updates. The internal ethics committee at the research institution (IPclin Instituto de Pesquisa Integrada Ltda. Jundiai, Brazil) approved the study.

To simulate normal use, participants applied the product to their scalp at home, in accordance with the product's instructions for use: the product was applied once per day, before bed, and left on overnight. During the study, participants were instructed to not use any hair treatments that could affect hair loss, and not to change their usual hair hygiene habits, diet, exercise routine, or contraceptive method. Anti-inflammatories, antihistamines, immunosuppressive and retinoids were prohibited during the study.

A medical assessment for adverse events was performed at D0, D90 and D180. A dermatologist assessed for erythema, edema, vesicles, papules, macules, crust, dryness, and dyschromia, and participants were questioned about any feelings of discomfort, such as burning or stinging.

Efficacy assessment involved three methods: digital phototrichogram, visual clinical assessment by a dermatologist, and patient questionnaire. For phototrichogram assessment, each patient had a 2 cm^2 area of scalp shaved to leave a maximum of 1 mm hair length, and the central 1 cm^2 was assessed.

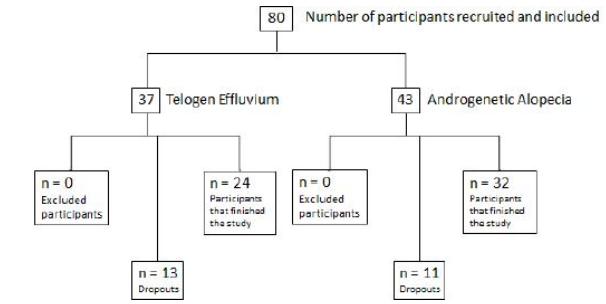

Figure 1: Flowchart of number of patients who were enrolled and completed the study.

The same area was shaved at D0, D30, D90, D120, D150 and D180. This avoided excessive hair growth so the same area could be assessed by phototrichogram at all experimental times. Images were taken using the computerized trichogram Tricho Scan (Dermoscan GmbH, Regensburg, Germany) within a maximum of 2 D after shaving, at D2, D92 and D182 (Figure 2). If this time was exceeded, the participant had their hair shaved again with a corresponding new phototrichogram image taken 48 h later.

Figure 2: Phototrichogram images for one patient at baseline, D92 and D182. Total number of hair fibers increased from 117 to 155 to 167.

The TrichoScan device obtains images of the shaved area on the scalp, and the software counts the number of hair fibers in each phase of the hair growth cycle, thus enabling the acquisition of a quantitative data concerning hair growth improvement during treatment. The data obtained with the equipment was: total number of fibers, percentage of hairs in anagen (hair growth phase), and percentage of hairs in telogen (death phase). With these data we were able to calculate the absolute number of anagen and telogen hairs. Statistical analysis was performed with a paired t-test to compare before and after data for each parameter measured on trichogram.

For the visual clinical assessment, a dermatologist assessed each patient's hair and scalp on D0, D90, and D180 for three aspects: hair volume/thickness, general hair appearance, and scalp coverage. Each aspect was assessed on a 7 point scale ranging from -3 (very thin or very bad) to 3 (very thick or very good).

Patient questionnaires were completed at D90 and D180. Participants assessed the product's effects and cosmetic qualities *via* closed questions and a 4-point agree/disagree scale (1=agree completely, 2=agree somewhat, 3=disagree somewhat, 4=disagree completely), and yes/no questions on satisfaction: whether they would buy the product and whether they would recommend it.

Results

Eighty participants were enrolled in the study; 56 completed the study and were included in the analysis (Figure 1). Of those who completed the study, twenty-four had telogen effluvium (TE), and 32 had androgenetic alopecia (AGA). The mean age was 35.6 yrs. (min 25-max 50). Patient demographics are shown in (Table 1). Of the 24 dropouts, 22 did not return for the day 90 visit, so only baseline data was available; two patients did not return for the day 180 visits. Only patients with complete data were included in the analysis. All patient dropouts were reported as being for personal reasons and further follow-up was not performed.

None of the participants reported discomfort and no adverse reactions were detected on examination at D90 and D180.

On phototrichogram analysis, in the study sample as a whole (AGA plus TE), there was a statistically significant increase ($p<0.05$) in the number of anagen hairs and the total number of hairs after 90 and 180 days of treatment (mean 183 hairs per 1 cm^2 test area at D0, 191 at D90, and 194 at D180). As a percentage improvement, there was a median 5% (range, -23% to 81%) increase in total number of hairs and median 9% (range, -20% to 56%) increase in number of anagen hairs. When extrapolated from the test area to the whole scalp area (taken to be 500 cm^2) [12] this increase represented 5598.2 more hairs per patient at the end of the study. When analyzed according to etiology, in patients with androgenetic alopecia, there was a statistically significant increase in the number of anagen hairs as well as in the total number of hair fibers after 90 and 180 days of treatment ($p<0.05$).

Whole study	**N=56**
Age, y	35.6 (min 25-max 50)
Female, No. (%)	30 (53.6)
Diagnosis	
Telogen effluvium	24 (42.9%)
Female	20 (83.3% of TE)
Male	4 (16.7% of TE)
Androgenetic alopecia	32 (57.1%)
Female	10 (31.3% of AGA)
Male	22 (68.8% of AGA)
Severity	
Ludwig scale (women)	
Stage I	6
Stage II	4
Hamilton scale (men)	
Stage I	1
Stage II	15
Stage III	6

Table 1: Baseline characteristics of the participants who completed the study.

In patients with telogen effluvium, there was a statistically significant increase only in the number of anagen hairs after 90 and 180 days of treatment (Table 2) contains numerical phototrichogram data on total hair number and number of anagen hairs, which were the statistically significant parameters.

Whole study sample	**Total number of hair fibers**							
	day	N	Mean	SD	Median	Min	Max	p
	2	56	183	46	185	77	264	n/a
	92	56	191	45	191	103	265	0.001
	182	56	194	47	192	101	282	0.003
	Number of anagen hair fibers							
	2	56	124	35	122	58	190	n/a
	92	56	132	33	132	68	204	<0.001
	182	56	134	34	133	61	210	<0.001
Telogen effluvium	**Number of anagen hair fibers**							
	2	24	137	25	139	85	190	n/a
	92	24	146	26	143	85	204	0.002
	182	24	144	31	138	84	210	0.049
Androgenetic alopecia	**Total number of hair fibers**							
	day	N	Mean	SD	Median	Min	Max	p
	2	32	168	53	163	77	264	n/a
	92	32	177	48	172	103	265	0.01
	182	32	185	48	175	101	275	0.009
	Number of anagen hair fibers							
	2	32	114	38	113	58	184	n/a
	92	32	122	34	127	68	176	0.002
	182	32	126	35	130	61	183	0.001

Table 2: Phototrichogram data for statistically significant parameters: total number of hair fibers and number of hairs in anagen. Data for the whole study sample and by etiology at D90 and D180 of treatment, N=number of patients in sample; SD=standard deviation.

On clinical assessment by the dermatologist, in the whole study sample, after 90 D of product use, 23.2% of the participants had thicker, more voluminous hair; 25.0% had improved general hair appearance and 35.7% had improved scalp coverage. After 180 D of product use, 35.7% had thicker, more voluminous hair; 37.5% had improved general hair appearance and 39.3% had improved scalp coverage. When assessed according to etiology, patients with androgenetic alopecia had more striking results than those with telogen effluvium: at D90, 25.0% had thicker hair (*vs.* 20.8% for TE), 31.3% had improved general appearance of hair (16.7% for TE), and 43.8% had improved scalp coverage (25.0% for TE). At D180, 46.9%

had thicker hair (*vs.* 20.8% in TE), 50.0% had improved general appearance (20.8% in TE), and 50% had improved scalp coverage (25.0% in TE).

On participant self-assessment questionnaire the product was rated highly both for efficacy and cosmetic qualities. At D180, 79% of participants said their hair problem was less visible, 84% said hair growth had increased, 79% reported reduced hair loss, 79% said their hair was stronger, 86% were more confident about their hair, 86% were satisfied with the results, 86% would recommend the product, and 86% said they would buy the product regardless of the price. In general, in the group with androgenetic alopecia, these percentages were slightly higher than in those with telogen effluvium. Full results broken down by question, timing, and alopecia etiology are given in (Table 3). Cosmetically, the product was rated very highly: 100% of participants judged it as having a non-greasy, non-sticky texture and pleasant smell. This was regardless of etiology, i.e. 100% in both groups.

Question/statement	% of participants in agreement					
	Whole sample		Androgenetic alopecia		Telogen effluvium	
	D90	D180	D90	D180	D90	D180
Cosmetic qualities						
The product is pleasant	100	100	100	100	100	100
The texture of the product is nice	100	100	100	100	100	100
The product has a pleasant smell	100	100	100	100	100	100
The product is non-greasy	100	100	100	100	100	100
The product is non-sticky	100	100	100	100	100	100
Efficacy						
The product makes my hair problem less visible	77	79	78	81	75	75
The product makes my hair more voluminous	57	59	59	63	54	54
The product makes my hair grow	82	84	84	88	79	79
Hair loss was reduced	79	79	78	78	79	79
My hair is thicker	48	48	50	50	46	46
My hair is stronger	79	79	81	81	75	75
New hairs grow faster than before	79	79	78	78	79	79
The appearance of hair loss is reduced	54	54	56	56	50	50
The product helps regulate excess oily hair	59	61	59	63	58	58
My hair looks healthier	82	82	84	84	79	79
My hair is more manageable and easier to comb	55	57	56	59	54	54
Within a month of using the product my hair is already improving	50	50	53	53	46	46
At the end of the treatment my hair has improved	82	86	84	88	79	83
This product makes me more confident about my hair	82	86	84	88	79	83
I would prefer to use this product instead of a costly hair transplant	77	77	78	78	75	75
Since using the product I am less worried about hair loss	71	71	75	75	67	67
I feel my image has improved	82	84	84	84	79	83
I feel more confident about my image	82	82	84	84	79	79
Are you satisfied with the results?	82	86	84	87.5	79	83
Would you recommend this product	82	86	84	87.5	79	83
Would you buy this product regardless of the price?	82	86	84	87.5	79	83

Table 3: Results of participant questionnaires on efficacy and cosmetic qualities of the product at day 90 and day 180.

Discussion

The results show that normal use of this product resulted in a significant increase in the total number of hair fibers and number of anagen hairs in patients with AGA and an increase in the number of anagen hairs in patients with TE. On assessment by a dermatologist, 39% of participants had visibly improved scalp coverage at the end of the study. The majority of patients found the product to be effective and wished to continue using it.

The existing scientific literature contains evidence on the efficacy and mechanism of action of some of the individual ingredients in this product. The key ingredients in this product are oleanolic acid, apigenin, biotinyl tripepdide-1, diaminopyrimidine, and adenosine, biotin, and Ginkgo biloba. As briefly mentioned above, the combination of oleanolic acid, apigenin, and biotinyl tripepdide-1 acts against the principal causes of hair loss, namely poor scalp microcirculation, follicle ageing, and follicle atrophy caused by dihydrotestosterone. Previous studies in the literature have shown that biotinyl-GHK (a member of the matrikine family) improves cellular metabolism [13]. While apigenin, a citrus flavonoid improves microcirculation [5] and oleanolic acid inhibits DHT (dihydrotestosterone) [4] *via* 5-alpha reductase inhibition. This study adds clinical evidence on the efficacy of this particular combination of

ingredients in normal use conditions (Table 4). summarizes the known mechanism of action of each of the actives in this product.

Active	Mechanism of action
Oleanolic acid	Improves cellular metabolism, stimulates hair matrix cell proliferation [4]
Apigenin	Stimulates microcirculation [5]
Biotinyl tripeptide-1	Inhibits 5α-reductase [13]
Diaminopyrimidine oxide	Inhibits perifollicular fibrosis, similar mechanism of action to minoxidil [6,7]
Adenosine	Promotes expression of several growth factors, stimulates growth of dermal papilla, lengthens anagen [8]
Biotin	Vitamin that assists in numerous metabolic reactions involved in fatty acid synthesis [9]
Ginkgo biloba	Antioxidant+anti-inflammatory. Improves microcirculation [10,11]

Table 4: Active ingredients in the product studied and their mechanism of action against hair loss.

In 2008, a double-blind, randomized, placebo-controlled study of 30 women with female pattern hair loss found that twice-daily use of adenosine lotion for 12 months improved hair growth and thickness in women [14] as assessed on dermatological clinical examination and phototrichogram. In the intervention group, there was a significant increase in anagen hair growth and hair thickness, superior to placebo, but no significant difference in anagen hair ratio. Our study results differ slightly from those of Oura et al, in that we found increased anagen hair number; however, we did not directly measure growth rate or individual hair fiber thickness. More recently, a 2015 study from Japan concluded that topical adenosine increases the proportion of thick hair in Caucasian men with androgenetic alopecia [15]. In that study, individual hair fiber thickness was assessed and found to increase significantly, but on dermatologist assessment of overall hair appearance there was no significant difference between the intervention group and placebo group. In our study, dermatologist assessment found that 35.7% of participants had some improvement in hair thickness appearance at the end of the study, although our study design did not include a control group. This could be an area for further study, to compare this product against a placebo.

In addition to the direct effects of adenosine on hair growth, it has also been demonstrated to mediate the effects of minoxidil against hair loss. A 2001 study in dermal papilla cells found that adenosine triggered intracellular signal transduction, thus increasing minoxidil's effect on hair growth [16]. This concept of synergistic interaction is of great importance when trying to establish the optimal combination of ingredients. In this study we have demonstrated that the combination of ingredients in the product tested is clinically effective against hair loss.

The strengths of this study are that it assessed clinical effects in multiple ways: the objective and validated phototrichogram assessment [17] the clinical impression according to the dermatologist, and patient opinion, which although subjective by definition, is fundamentally important to any medical or cosmetic treatment. The patient questionnaire was a commonly-used questionnaire for subjective evaluation of cosmetic products, but is not a validated questionnaire, and as such, constitutes a weakness of the study. The study could have been further improved by use of a control group and blinding. Collecting information on time since diagnosis, and time since last pregnancy (if applicable) would provide more complete data (pregnancy was an exclusion criteria). Future studies, looking at female patients in particular, in which systematic treatment is limited due to side effects, would be an interesting focus. A larger sample size would make the data from such studies more robust.

Conclusion

Once-daily use of this product for 6-12 months is effective and safe as a topical hair-loss treatment, as demonstrated by increased total and anagen hairs, increased overall thickness and scalp coverage, and patient-reported reduction in hair-loss. This lotion would be suitable for use as an adjuvant to systemic treatments or in patients who are not suitable for or decline systemic or invasive treatments.

Acknowledgement

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Conflicts of interest

A Garre, G Martinez, and C Trullas are employees of ISDIN, who sponsored the study. J Piquero is an external medical advisor to ISDIN.

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