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ORIGINAL CONTRIBUTION

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"Miliacin encapsulated by polar lipids stimulates cell proliferation in hair bulb and improves telogen effluvium in women"

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Abstract

Background: Miliacin, the main triterpenoid from millet, is known to stimulate keratinocyte metabolism and proliferation. Polar lipids are able to form vesicles with active compounds and to improve their bioavailability.

Objectives: We aimed to demonstrate potential benefits of a solution of miliacin encapsulated within polar lipids (MePL) on telogen effluvium prevention and hair condition in women.

METHODS: After preliminary cell proliferation studies, a placebo-controlled, multicentric, randomized, double-blind trial was performed on sixty-five nonmenopausal women affected by telogen effluvium, to assess the efficacy of a 12-week oral supplementation with MePL. Telogen and anagen densities were determined by phototrichogram analysis. Scalp dryness and hair brightness were clinically evaluated using a Likert scale.

Results: MePL further enhanced cell proliferation in hair bulb from human scalp than miliacin alone. Compared to the placebo treatment, MePL supplementation significantly reduced telogen density after 12 weeks of treatment. An increase of anagen density was observed in both groups, although there was no significant difference between the two treatments. Scalp dryness was more decreased in the MePL group than in the placebo group. A better improvement of hair brightness was also observed after 12 weeks of supplementation with MePL.

Conclusion: Twelve weeks of MePL supplementation significantly reduced the hair density in the telogen phase and, in parallel, improved scalp dryness and hair condition. These effects could be linked to MePL activity on cell proliferation in hair bulb. MePL is an original association of plant extract that could help to prevent and/or limit hair loss in women.

KEYWORDS

cell culture, cosmeceuticals, female pattern hair loss, hair disorders, hair treatment

1 | INTRODUCTION

Hair loss is a common problem and a major source of aesthetic discomfort and distress for people.[1] A thinning or shedding of hair resulting from the early entry of the hair into the telogen phase corresponding to the resting phase is defined as "telogen effluvium." While androgenic alopecia is the most common type of hair loss in men, women are mainly concerned by telogen effluvium in the premenopausal period. Afterward, the hormonal changes that occur during the menopause may bring about a female pattern hair loss with increased prevalence with age, approaching 40% by 70 years of age in Caucasian populations.[2,3] Hair fiber consists of a visible shaft, a hair bulb under the scalp surface, and a dermal papilla at the base of the bulb. The dermal papilla, highly vascularized, and composed of fibroblasts producing the important extracellular matrix, ensures hair nutrition, irrigation, oxygenation, and cellular waste disposal. It can therefore be considered as the "biological engine" of the hair.

Hair growth is a complex process. The hair follicle (HF) undergoes several life-long cyclic transformations between "resting" (telogen), growth (anagen), and apoptosis-driven regression (catagen). The anagen phase corresponding to the "hair shaft factory" phase is mainly characterized by a massive keratinocyte proliferation phase in the hair matrix and a rapid cellular differentiation followed by pigmentation by follicular melanogenesis. This is the longest period of the hair life cycle, lasting one to 6 years on average. The catagen phase is a regression phase during which the hair stops growing and a deconstruction of the "hair shaft factory" is observed. This phase lasts a few weeks. Finally, the telogen phase, which lasts 3-9 months, corresponds to the "resting" phase during which the hair no longer grows, but remains attached to the HF. At the end of this phase, keratinized hair falls out and a new matrix is gradually formed from stem cells in the basal layer of the outer epithelial root sheath bulge. A new hair starts to grow, and the follicle goes back in the anagen phase. If the hair life cycle is normal, the percentage of hair in the anagen phase is between 85% and 90% of all hair, the catagen phase applies to approximately 1% of hair, and approximately 10 to 20% of hair is in the telogen phase.[4] Growth and differentiation of the matrix cells are under the influence of growth factors produced by the dermal papilla.[5,6] Different growth factor families including the epidermal growth factor (EGF)-related ligands, fibroblast growth factors (FGF), transforming growth factor-beta (TGF-beta), insulin-like growth factor (IGF), hepatocyte growth factor/scatter factor (HGF/SF), and platelet-derived growth factor (PDGF) have been shown to be crucial for the regulation of the hair cycle and hair growth.[7] Numerous treatments for hair loss are available. Several pharmaceutical products, physical and regenerative medical therapies, and hair transplant procedures have mainly been developed to cure serious and pathological cases. Nutraceuticals by providing essential nutriments or specific botanical extracts represent the nonmedical and the nonsurgical strategies to prevent or to treat weak to moderate hair loss.[8]

Millet (*Panicum miliaceum*) and its main compound, miliacin, arouse a lot of interest in dermatological research, especially for its tissue repair and wound healing properties. Miliacin, also called Panicol or Prosol, belongs to the class of organic compounds known as triterpenoids. Miliacin is a white odorless solid crystal practically fat- and water-insoluble. Cellular studies using thymocyte and splenocyte cultures have revealed a protective effect of miliacin from DNA fragmentation and apoptosis.[9] Animal and clinical studies with suppurating wounds in different physiopathological conditions have confirmed and deepened these first results. Thanks to its strong anti-inflammatory properties, topical application of millet oil promoted rapid cleansing of the wounds and significantly activated the reparative processes.[10-13]

More recent studies focusing on miliacin have showed that it improved cellular renewal and proliferation and promoted the process of hair growth. When normal human keratinocytes derived from the foreskin were exposed to miliacin (6 mg/mL), the metabolic capacity of these cells was increased (+162%) and their proliferation was also stimulated (215%).[14]

An original combination of miliacin extracted from whole millet seeds and polar lipids (MePL = Miliacin encapsulated with Polar Lipids) extracted from wheat grain has been developed.[15] Polar lipids, in particular phospholipids, are characterized by a hydrophilic part and represent the main constituents of biological membranes. They are also technically able to form vesicles with active compounds, and thus, they are usually used as vectors to facilitate their intestinal passage and increase their bioavailability.[16-19] We hypothesized that polar lipids could enhance bioavailability of miliacin and, in consequence, could reinforce its efficacy. Microscopic examinations of a mixture containing polar lipids, miliacin in crystal form and water, indicated the formation of vesicles from 5 to 25 µm in diameter.[15] These formed liposomes may improve the bioavailability of miliacin by promoting their intestinal passage, as previously described for other molecules (curcumin, naringenin).[18,19]

In the present article, we put forward the results from ex vivo studies assessing the effects of miliacin alone and miliacin encapsulated with polar lipids (MePL) on cell proliferation in a model of human scalp fragment and those from a double-blind, randomized, placebo-controlled clinical trial evaluating the effectiveness of the association MePL on hair loss and hair beauty. The primary outcomes of the clinical trial were the density of the hair in telogen and anagen phases using the objective phototrichogram method. The secondary endpoints were the clinical evaluation by a dermatologist and the general self-assessment by the volunteers.

2 | MATERIALS & METHODS

2.1 | Ex vivo studies

The effects of miliacin alone and of MePL were studied in two separate experiments, both of them using the same model and methodology.

2.1.1 | Culture of human scalp fragments

Human scalp fragments were obtained from occipital crown before hair transplant surgery on 8 donors for miliacin alone study and on 12 donors for MePL study (80% of men and 20% of women). They were put with the epithelium uppermost, at an air/liquid interface, in culture inserts placed in 12-well plates containing specific growth medium adapted to survival conditions. Miliacin (1.7 µg/mL) or MePL (1.7 µg/mL of miliacin and 1.5 µg/mL of polar lipids) was added to the medium every day during the first three days of culture. At D4, the culture was stopped and the scalp fragments were formalin-fixed for later immunohistochemical analyses. For each donor of scalp fragment, miliacin or MePL was compared to a control condition corresponding to culture medium alone.

The study concentration was determined from the consideration that 20% of the miliacin quantity contained in one MePL capsule were absorbed and reached the scalp which measured approximately 600 cm^2. As the human scalp fragments in this ex vivo study measured 1 cm^2, we divided the quantity of 3 mg of miliacin contained in 300 mg of one MePL capsule by 600 to obtain the final concentration of 1.7 µg/mL.

2.1.2 | Immunohistochemical analyses

The formalin-fixed fragments were included in paraffin in order to perform sections of 4 µm thickness. Epithelial proliferation was studied by immunohistochemistry approach using an antibody anti-Ki67, a nuclear marker of proliferative cells (monoclonal antibody MIB1, DAKO). The immunodetection was performed with an indirect immunoperoxidase technique (CsA kit, DAKO) and revealed in red with an AEC solution (3-amino-9-ethylcarbazole). The number of marked proliferative cells was determined from a total number from 100 to 400 cells.

2.2 | Clinical study

2.2.1 | Study design

A placebo-controlled, multicentric, randomized, double-blind trial was performed on sixty-five nonmenopausal women (mean age of 41 ± 6 years) for 12 weeks, between February and June 2012. Sixty-six subjects were initially preselected, but one of them was finally not included because one exclusion criteria was observed (high proportion of gray hair).

2.2.2 | Experimental plan

When recruited, volunteers went to the clinical center eight times during the study. Three complete visits took place over two days (week 0, week 6 and week 12) and 2 simple visits for the shaving of the scalp area (week 3 and week 9). During the first day of a complete visit, shaving of the studied area, macrophotographies for phototrichogram analysis, and clinical evaluations were performed. On the second day, macrophotographies and phototrichogram analysis were done (Table 1).

2.2.3 | Studied population

Sixty-five healthy women, aged from 25 to 50 years with an excessive hair loss (percentage of hair in telogen phase between 18% and 35% determined by phototrichogram analysis), were recruited and randomly assigned into two groups: one group receiving the MePL and the other group the placebo for 12 weeks. All of the subjects were instructed to take two capsules per day, one in the morning and one in the evening.

2.2.4 | Study products

The products were dispensed in sealed bottles of 60 softgel capsules for one month of supplementation. The composition of the capsules was described in Table 2.

Each subject received two capsules per day, either 600 mg of soybean oil (placebo capsule) or 300 mg of MePL and 300 mg of soybean oil (MePL capsule).

2.2.5 | Phototrichogram analyses

Telogen and anagen densities were analyzed by phototrichogram at each visit, before the beginning of treatment (W0), and after 6 (W6) and 12 weeks of treatment (W12). The phototrichogram is a well-established noninvasive method which allows a reliable and objective evaluation of the efficacy of an anti-hair loss treatment.[20] At each visit (W0, W6, and W12), macrophotographies of the skin and scalp on a 1.5 cm^2 area were taken thanks to the FotoFinder Mediscope ® system. The numbers of hairs in the telogen (T) and the anagen (A) phases were measured, and their densities were calculated by the number of hairs in telogen or anagen phase (T) divided by the surface of the area (S).

2.2.6 | Clinical assessment by the dermatologist

A dermatologist evaluated the scalp dryness as well as hair beauty and brightness at W0, W6, and W12, using a quotation from 0 to 3 on a Likert scale.

2.2.7 | General assessment and self-assessment by the volunteers

At W0, W6, and W12, each subject appreciated the general efficiency of the product on several categories: extent of hair loss, hair density, and hair brightness. Each category was evaluated using a 10-point scale from 0 (low) to 9 (high).

2.2.8 | Safety and tolerance

Adverse events were noted throughout the study. Product acceptance was evaluated by the dermatologist via collection of the nature and frequency of events.

TABLE 1 Experimental plan of the study

	Recruitment		W0—Inclusion			W6—Intermediate visit			W12—Study end	
	D0	*D2*	*D0*	*D2*	W3	*D42*	*D44*	W9	*D84*	*D86*
Checking of the inclusion and exclusion criteria	x		x							
Volunteer information	x		x							
Informed consent	x		x							
Concomitant treatments	x		x	x		x	x		X	x
Supplementation—bottles of 60 capsules			x			x				
Shaving of the evaluation area	x		x		x	x		x	X	
Macrophotography	x	x	x	x		x	x		X	x
Phototrichogram		x		x			x			x
Self-assessment questionnaire			x				x			x
Acceptability questionnaire				x		x	x		X	x
Tolerance										
Compliance evaluation							x			x

2.3 | Statistical analyses

Statistical analyses were performed with the Statview software. After a normality test (Shapiro-Wilk test) and an equality of variances test (Levene test), two analyses were performed:

Average differences between W6/W12 and W0 allow the measuring of the "time effect" of the products;
A comparative analysis of these differences between the two groups (MePL versus placebo) is performed.

For clinical assessment, a Wilcoxon test was carried out to evaluate the "time effect" and a Mann-Whitney test in order to assess the effect of MePL treatment compared to the placebo treatment. For the self-assessment, descriptive statistics were used to perform the analysis.

3 | RESULTS

3.1 | Ex vivo studies

Compared to the control condition, the treatment of scalp fragments with the miliacin alone at a concentration of 1.7 µg/mL increased significantly the number of proliferative cells in the hair bulb (24.0 ± 9.6% for control and 46.1 ± 11.6% for miliacin, $P < 0.01$) (Figure 1A) and in the outer root sheath (20.9 ± 8.8% for control and 40.6 ± 12.8% for miliacin) (Figure 1B).

TABLE 2 Composition of the capsules

	Placebo capsule	MePL capsule
Refined soybean oil	300 mg	150 mg
MePL	0 mg	150 mg

The treatment of the scalp with the combination MePL, at a concentration of 150 µg/mL (containing 1.7 µg/mL of miliacin and 1.5 µg/mL of polar lipids), significantly and strongly increased

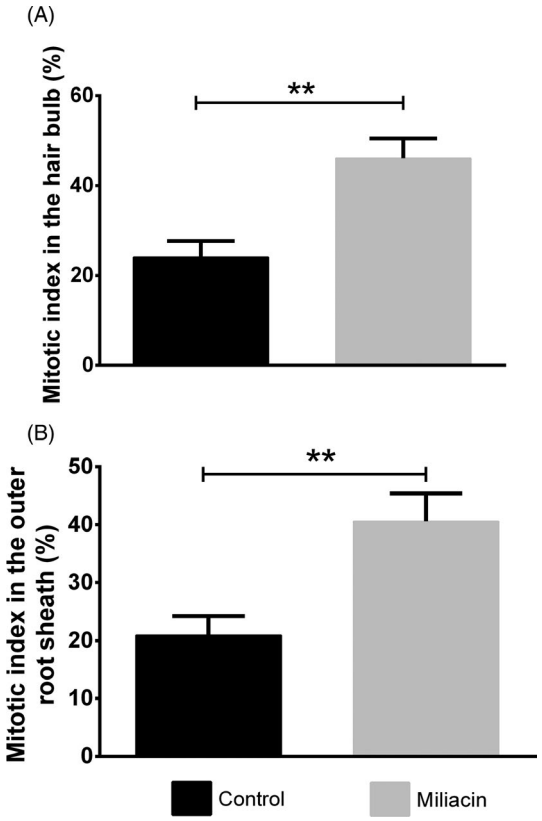

FIGURE 1 Effects of miliacin on cellular proliferation of hair bulb and of outer root sheath. Mitotic index evolution was measured on human scalp by the quantification of cells in mitosis with immunohistochemical analysis of Ki67 on the hair bulb (A) and on the outer root sheath (B). Results are expressed in percentage of mitotic index ± SEM (%). N = 8 for both groups. $^{**}P < 0.01$

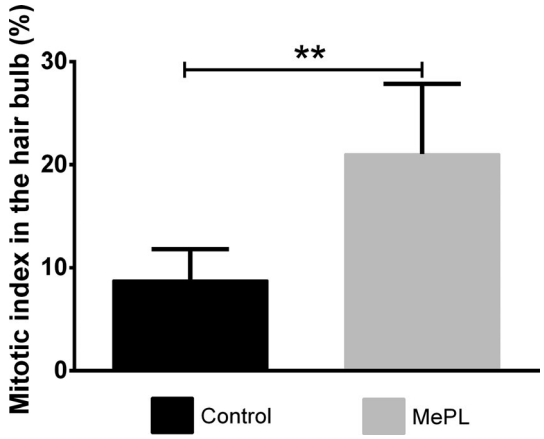

FIGURE 2 Effects of MePL on cellular proliferation in hair bulb. Mitotic index evolution was measured on human scalp by the quantification of cells in mitosis with immunohistochemical analysis of Ki67 on the hair bulb. Results are expressed in percentage of mitotic index ± SEM (%). N = 12 for both groups. $^{**}P < 0.01$

($P < 0.01$) the number of proliferative cells of hair bulb compared to the control condition (8.8 ± 9.9% for control and 21 ± 22.4% for MePL) (Figure 2).

When we compare the results from these two ex vivo studies, we observed that miliacin, at 1.7 µg/mL, increased about 92% in hair bulb and 94% in the outer root sheath the number of cells in proliferation in comparison with the control condition while MePL, at 150 µg/mL, increased about 140% the number of proliferative cells in the hair bulb suggesting a better efficacy of the combination MePL than miliacin alone.

3.2 | Clinical study

Among the 65 volunteers, one subject from the placebo group dropped out of all analyses because of noncompliance. Thus, in general, the values of 64 subjects were considered for the statistical analyses. Phototrichogram analyses were carried out on 60 volunteers (30 in each group). The data of 4 volunteers were not analyzed because one of them presented aberrant results, another one showed a high proportion of gray hair making the determination of the hair formula unreliable, and for two of them, the analyzed area was lost from one visit to another. The scalp dryness statistics were done on 49 volunteers (25 in the placebo group and 24 in the MePL group): 15 volunteers were removed from the analysis (7 of the placebo group and 8 of the MePL group) because they did not present scalp dryness at the beginning of the study.

3.2.1 | Telogen and anagen densities

The telogen density decreased significantly ($P < 0.001$) for the two groups (44.87 ± 2.86 hair/cm^2 at W0, 30.17 ± 2.66 hair/cm^2 at W6, and 27.82 ± 2.53 hair/cm^2 at W12 with the placebo treatment versus 48.1 ± 2.19 hair/cm^2 at W0, 32.25 ± 2.35 hair/cm^2 at W6, and 24.37 ± 1.98 hair/cm^2 at W12 with the MePL treatment) (Figure 3A).

The anagen density increased significantly ($P < 0.001$) at W6 and W12 for the placebo group (135.67 ± 5.69 hair/cm^2 at W0, 166.34 ± 6.65 hair/cm^2 at W6, and 158.97 ± 6.96 hair/cm^2 at W12) and for the MePL group (139.24 ± 4.12 hair/cm^2 at W0, 160.31 ± 3.99 hair/cm^2 at W6, and 165.08 ± 4.31 hair/cm^2 at W12) (Figure 3B). Moreover, when comparing the variation of telogen density from W12 to W0, this difference was significantly higher in the MePL group than the placebo group (Figure 3C) (−17.04 ± 2.65 hair/cm^2 for the placebo group and −23.73 ± 2.0 hair/cm^2 for the MePL group). No significant difference was observed for the W6-W0 variations of telogen density between the two groups (−14.69 ± 2.51 hair/cm^2 for the placebo group and −15.85 ± 2.17 hair/cm^2 for the MePL group). In the same way, the anagen density variations between W6-W0 and W12-W0 did not differ significantly between the two groups (Figure 3D).

3.2.2 | Scalp dryness

Scalp dryness significantly decreased for the MePL group after 6 and 12 weeks of supplementation ($P < 0.001$) (1.66 ± 0.17 AU at W0, 0.75 ± 0.12 AU at W6, and 0.37 ± 0.12 AU at W12) (Figure 4A). There was no significant difference for the placebo group after 6 and 12 weeks of treatment (1.76 ± 0.17 AU at W0, 1.20 ± 0.14 AU at W6, and 1.16 ± 0.14 AU at W12). Moreover, the comparison of the variations between W12 and W0 showed a significant difference between the two groups ($P < 0.05$) (−0.60 ± 0.19 AU for the placebo group and −1.29 ± 0.16 AU for the MePL group) with a superior effect for MePL (Figure 4B). This significant difference between the two groups was not observed for the W6-W0 variation (−0.56 ± 0.16 AU for the placebo and −0.91 ± 0.15 for the MePL).

3.2.3 | Hair brightness and beauty assessed by the dermatologist

Hair brightness and beauty increased significantly for both groups ($P < 0.001$) (for the placebo group, 0.81 ± 0.11 AU at W0, 1.53 ± 0.12 AU at W6, and 1.71 ± 0.10 AU at W12, and for the MePL group, 0.97 ± 0.10 AU at W0, 1.87 ± 0.10 AU at W6, and 2.34 ± 0.10 at W12) (Figure 5A). Considering the score 0 for dull hair and 3 for bright and beautiful hair, MePL and placebo treatment had a significant time effect on hair brightness and beauty. The comparison of the variations between the two groups showed a significant and superior effect for the MePL group between W12 and W0 ($P < 0.05$) (0.90 ± 0.12 AU for the placebo group and 1.37 ± 0.11 AU for the MePL group), whereas there was no significant difference between W6 and W0 (0.74 ± 0.10 AU for the placebo group and 0.90 ± 0.15 for the MePL group) (Figure 5B).

3.2.4 | General and self-assessment and tolerance to the products

After 12 weeks of treatment, the subjects in the MePL group noticed several improvements of their hair: 91% observed a reduction of the hair loss (versus 78% in the placebo group), 78% noticed a

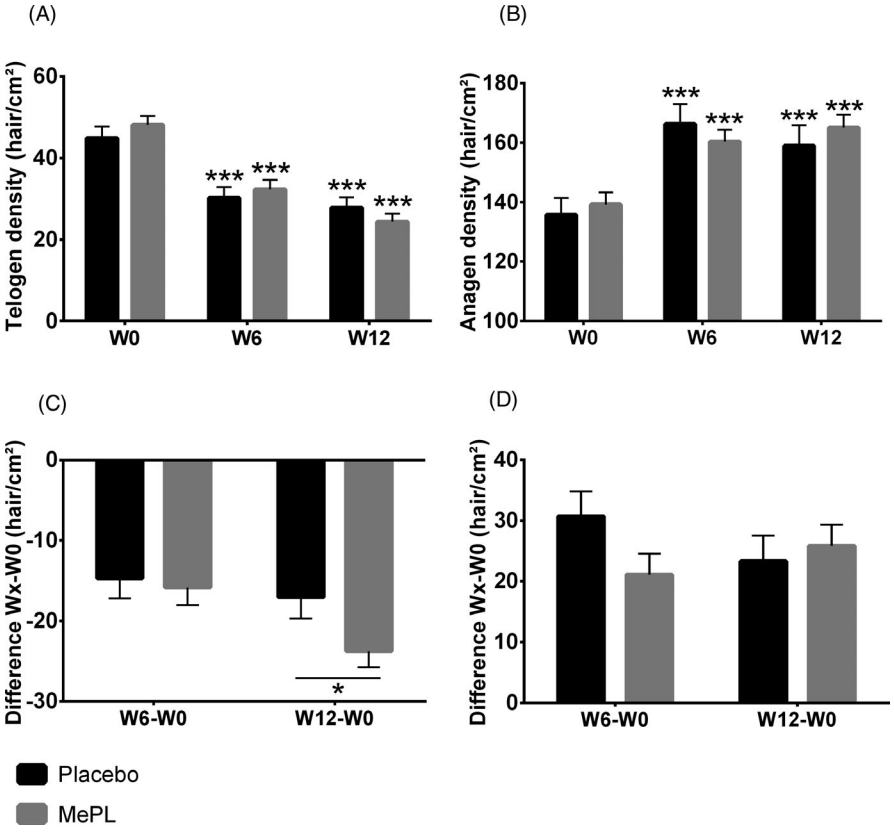

FIGURE 3 Telogen and anagen densities. Telogen and anagen densities were analyzed by phototrichogram. Comparison of the telogen (A) and anagen (B) densities at W6 and W12 to W0. Comparative analysis of the telogen (C) and anagen (D) density variations between MEPL and placebo (W6-W0 and W12-W0). All the results are represented as means ± SEM (D) for each group. N = 30; * $P < 0.05$, *** $P < 0.001$

faster growth (versus 65% in the placebo group), and 75% observed an increase of hair brightness and beauty (versus 69% in the placebo group).

Moreover, 88% of the subjects in the MePL group had a good general impression of the product.

Cutaneous and digestive tolerance to the MePL and the placebo was considered as excellent by the investigator and the volunteers.

4 | DISCUSSION

Telogen effluvium is a physiological form of hair loss that usually lasts for a given period. This phenomenon can become chronic if periods of hair shedding last for more than 6 months. During telogen effluvium, disturbances of the hair cycle are observed and, notably, an increased proportion of hair shifts from the growing phase (anagen) to the shedding phase (telogen). Normally, only 10% to 20% of the scalp hair is in the telogen phase, but in telogen effluvium, this increases to 30% or more. Telogen effluvium is generally reversible and is different from the permanent hair loss disorder called alopecia which is mainly caused by hormonal imbalance.

However, most of the treatments developed these last years target pathological and hormonal hair loss and, notably, the activity of the 5-alpha-reductase which converts testosterone to dihydrotestosterone (DHT), the specific androgen involved in the pathogenesis of androgenic alopecia. None of these treatments are adapted to the physiological and nonhormonal telogen effluvium in women, and moreover, they are often associated with side effects. In this study, we propose an innovative, alternative, and nutritional solution for hair loss.

Preliminary studies performed on human scalp fragments have allowed us to confirm that miliacin alone stimulated cell proliferation in hair bulb and in outer root sheath[14] and to highlight that the combination MePL had a stronger proliferative effect than the miliacin alone. The hair bulb is the invisible part of hair fiber under the scalp surface containing dividing keratinocytes to form the hair shaft and the surrounding root sheaths: the inner root sheath necessary for hair growth and the outer root sheath hosting the stem cells needed for cyclic regeneration of the hair follicle. Thus, we can suggest that in a superior way compared to miliacin alone, MePL could stimulate the proliferation of keratinocytes present in hair bulb, and as a consequence, MePL could promote hair shaft formation and hair growth while strengthening the hair anchorage in the scalp. Obviously, complementary preclinical studies are needed to confirm and deepen these speculations. Anyway, these preliminary results lead us to evaluate the potential clinical efficacy of MePL against hair loss.

In this clinical study conducted on women with telogen effluvium, we demonstrated that, in comparison with a placebo treatment, a 12-week supplementation of MePL significantly reduced the number of hairs in the telogen phase and improved scalp dryness and hair brightness and beauty. We also highlighted a significant increase of anagen density for both groups after 12 weeks of treatment. However, we did not observe significant differences for the anagen density, probably because of the short duration of the study. A latency period from 4 to 7 months is observed between

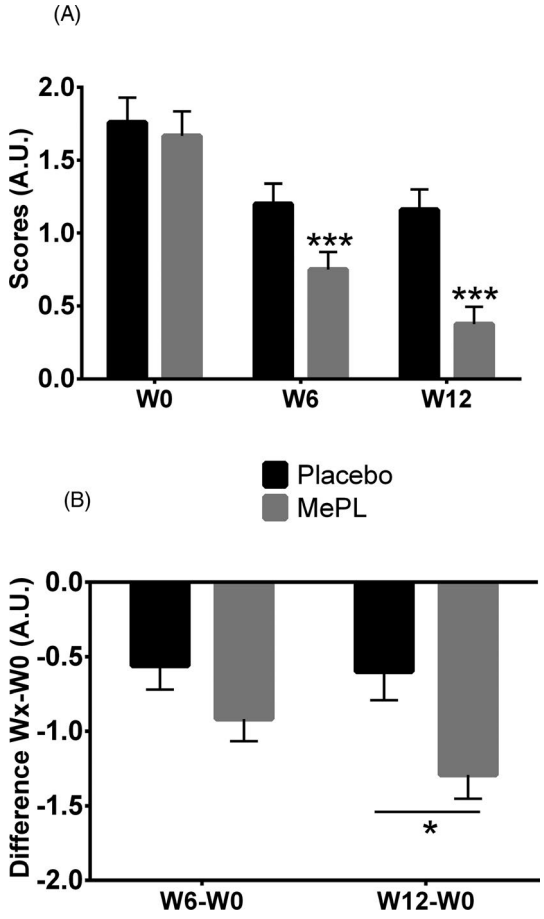

FIGURE 4 Scalp dryness. Scalp dryness was evaluated by a dermatologist during the clinical assessment with the following quotation: 0 = hydrated scalp, 1 = slightly dry scalp, 2 = moderately dry scalp, and 3 = very dry scalp. A, Evolution of the scalp dryness score at W6 and W12 compared to W0. B, Comparative analysis of scalp dryness score variations between MEPL and placebo (W6-W0 and W12-W0). All the results are represented as means ± SEM (AU) for each group. N = 25 for the placebo group and N = 24 for the MEPL group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

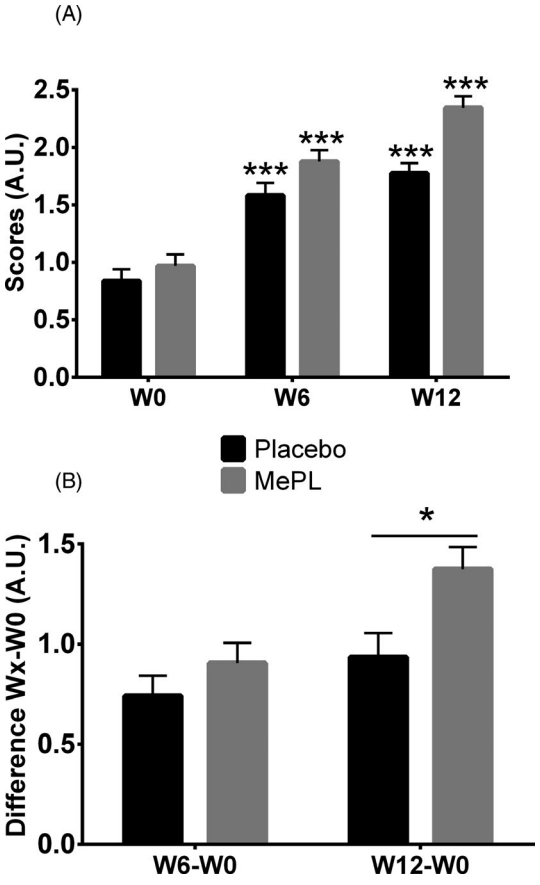

FIGURE 5 Brightness and hair beauty. Brightness and hair beauty were evaluated by the dermatologist during a clinical assessment with the following quotation: 0 = dull, 1 = slightly dull, 2 = moderately bright, and 3 = bright. A, Evolution of the brightness and hair beauty score at W6 and W12 compared to W0. B, Comparative analysis of the brightness and hair beauty score variations between MEPL and placebo (W6-W0 and W12-W0). All the results are represented as means ± SEM (AU) for each group. N = 32 for the placebo group and N = 32 for the MEPL group; $^{*}P < 0.05$, $^{***}P < 0.001$

the moment the hair in telogen phase falls and the moment when the hair in anagen appears visibly. Indeed, the hair in anagen phase begins its growth under the surface of the scalp and is invisible.[21]

The phototrichogram test that we used in our study did not detect the hair when it is in its early growth phase. Thus, considering this latency period, the reduction of hair in telogen phase could be observed earlier than the increase of hair in anagen phase. We suggest that if we continued the study beyond 3 months, the phototrichogram device could have detected more hair in anagen phase. Given the latency phase after the fall of the hair, it would be wiser to extend the time of the supplementation and of the assay to show significant positive effects on the density of anagen hairs.

Mechanistically, preliminary studies based on the fact that the growth factor, IGF-1 (insulin-like growth factor-1), is well known as a hair growing factor have been conducted. It could represent a biological target for MePL. In fact, a new theory concerning IGF-1 synthesis emerged, mainly from the researches from the K. Okajima team, and IGF-1 is known to be a very important growth factor with pleiotropic actions for the body homeostasis. In the skin, it plays critical roles in promoting cell proliferation, differentiation, survival, and function. Moreover, it helps to boost the immune system and wound healing. Thus, it represents a major molecular actor for tissue remodeling and maintenance of tissue integrity.[22] In the dermis, it would be exclusively expressed by mesenchymal cells, and in the hair, it would be produced in the follicle and would promote hair growth by proliferating keratinocytes. In fact, it was shown that an administration of capsaicin and isoflavones increased IGF-1 production in hair follicles, thereby increasing hair growth in mice and in volunteers with alopecia.[23,24] Both of these nutritional components were shown to promote increased tissue levels of IGF-1 via stimulation of sensory neurons and subsequent increased calcitonin gene-related peptide (CGRP) release. Moreover, the circulating level of IGF-1 was shown to decrease continuously with advancing age, a factor stimulating hair loss.[25]

An ex vivo study using a model of human hair scalp fragments containing hair follicles and maintained in survival conditions for 7 days has demonstrated that MePL significantly induced an increased secretion of IGF-1, associated with an important and significant renewal of keratinocytes in the hair bulb.[26] As some studies have highlighted a higher expression of IGF-1 during the early inflammatory phase in animal healing models, and a positive role in the proliferation and migration of fibroblasts and to subsequently, in the collagen production,[27] these functions were also investigated in this same ex vivo study and we observed a significant increase of extracellular matrix, essentially collagen, thickness in the connective tissue sheath of the hair in contact with MePL.[26]

Our results have been supported by more recent clinical data from the Leiden Longevity study which has shown that in a random subgroup of 323 middle-aged women (mean age 61 ± 5 years), women ranked according to the severity of their hair loss, low HDL cholesterol, and IGF-1 were associated with a higher risk of hair loss in women.[28] The first preclinical studies need to be further explored and confirmed with clinical data showing an increase in IGF-1 circulating level with MePL supplementation in subjects affected by hair loss. Therefore, MePL could represent a nutritional and safe method for restoring the circulating levels of IGF-1 which appears to be critical for the normal hair cycle and for the hair growth.

Thanks to its positive effect on IGF-1 production, and on subsequent hair growth, but also to its potential complementary healing power and capacity to boost collagen synthesis, MePL could help improve the results/success of hair restoration procedures. So, in some future clinical studies performed on specific populations with pronounced hair loss, it would be interesting to investigate whether MePL can improve and optimize the effects of the existing pharmaceutical products or surgical methods. In this study, we presented the results of an oral approach to MePL. We could also hypothesize that MePL may have similar or superior efficacy through a topical application like a serum or hair lotion. Indeed, a clinical study on eyelashes which, like hair, grow under an independent cycle has been performed with MePL. MePL applied in the form of an eyeliner twice daily for two months significantly increased eyelash length compared to the placebo (Data S1). A further study should be performed on hair to confirm these preliminary results on eyelashes. MePL represents a new and original combination of plant extracts with no side effects which offers great promises for helping prevent and/or limit telogen effluvium in women.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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