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Clinical Evidence of Increase in Hair Growth and Decrease in Hair Loss without Adverse Reactions Promoted by the Commercial Lotion ECOHAIR®

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Keywords
ECOHAIR® · Hair growth · Noncicatricial alopecia

Abstract

Background/Aims: Hair exerts protection, sensory functions, thermoregulation, and sexual attractiveness. Hair loss (alopecia) is caused by several diseases, drug intake, hormone imbalance, stress, and infections *(Malassesia furfur)*. Drugs usually used in alopecia produce irreversible systemic and local side effects. An association of extracts of *Coffea arabica* and *Larrea divaricata* (ECOHAIR®) is successfully being commercialized in Argentina for hair growth. The aim of this study was to provide scientific support for the efficacy and innocuousness of ECOHAIR® in patients with noncicatricial alopecia during a 3-month treatment. ***Methods:*** The efficacy was determined through the assessment of an increase in hair volume, improvement in hair looks, growth of new hair, and a decrease in hair loss by the test of hair count and hair traction. The capacity to decrease the amount of dandruff was also evaluated as well as the adverse local effects caused by the treatment. ***Results:*** ECOHAIR® spray improved the overall hair volume and appearance; it increased its thickness, induced hair growth, and decreased hair loss. Besides, no adverse local reactions were observed upon treatment with the product. ***Conclusion:*** This study provides scientific support for the clinical use of ECOHAIR® as a treatment to be used in noncicatricial alopecia.

Introduction

Hair is a protective appendage on the body that is considered an accessory structure of the integument along with sebaceous glands, sweat glands, and nails. Hair plays a role in protection, sensory functions, thermoregulation, and sexual attractiveness [1]. Both men and women suffer from hair loss and/or hair thinning. Many factors contribute to hair loss such as diseases, nutritional deficiency, aging, hormone imbalance, and infections caused by *Malassesia furfur* (dandruff) [2].

The hair is a complex mini-organ made up of terminally differentiated and dead keratinocytes, the matrix cells (which give rise to keratinocytes), and dermal papillae (DP), which contain fibroblasts [3] located in the deepest end of the follicle. Fibroblasts are thought to play an essential role in the induction of new hair follicles and in the maintenance of hair growth [4]. Not only are DP cells (DPCs) essential for hair follicle development through the secretion of growth factors, which stimulate the proliferation and differentiation of the follicular epithelium [5], but they are also a reservoir of cells which can differentiate into a range of therapeutically important cell types [6]. Therefore, the regulation of hair follicle regeneration depends on a complex series of paracrine interactions mediated by DPCs [7]. Moreover, DPC-derived factors have been demonstrated to influence surrounding cells, which in turn contribute to hair growth promotion [8].

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Hair growth is a cyclical process that is divided into 3 phases: anagen (growth phase), catagen (regression phase), and telogen (resting phase) [9]. These cyclic changes involve a rapid restructuring of both epithelial and dermal components of hair follicles [10]. In fact, DPCs, mainly fibroblasts, not only regulate anagen but also catagen and telogen phases. Thus, any factor affecting the functions of DP can influence hair growth; for instance, minoxidil and epigallocatechin-3-gallate [11] stimulate hair growth by exerting antiapoptotic effects on DPCs (through an increase in the Bcl-2 [apoptosis inhibitor]/Bax ratio – an apoptosis promoter). Nevertheless, minoxidil is known to have other mechanisms of action. On the other hand, cisplatin causes hair loss through the induction of apoptosis on DPCs (through a decrease in the Bcl-2/Bax ratio) [12]. Moreover, reactive oxygen species can modulate the balance of the anagen/catagen phase by inducing damage of cellular DNA, proteins, and lipids which lead to cell-cycle arrest [13]. It is known that superoxide anion is involved in hair loss – interfering with nitric oxide (which is indicated as a hair growth factor). Because of that, a major role has been assigned to SOD in the control of the anagen phase and its use in alopecia by local application [14].

Alopecia is a generic term for hair loss, which results in the diminution of visible hair as a consequence of an imbalance between cell proliferation and death. The most common types are androgenetic alopecia or common baldness, telogen effluvium, chemotherapy-induced alopecia, and alopecia areata.

Nowadays, there are few drugs for the treatment of alopecia, particularly in women. In this sense, finasteride (the dihydrotestosterone-5-α-reductase inhibitor) and minoxidil (the antihypertensive potassium channel opener) are commonly used. However, due to their limited and transient effectiveness and side effects, novel innocuous pharmacological treatments and agents are on demand.

Previously, a combination of 2 extracts of *Coffea arabica* and *Larrea divaricata* demonstrated to exert an effect on hair growth on C3H mice by inducing the anagen phase [15]. Based in these results, the combination of both extracts has been registered under the name of ECOHAIR®, a trademark of Garre-Guevara laboratory. ECOHAIR® is being successfully used in Argentina for hair recovery.

L. divaricata Cav. (Zygophyllaceae) is a plant that grows in South America, and it is widely distributed in Argentina. It is used in folk medicine for the treatment of many disorders since it has anti-inflammatory and antirheumatic properties [16]. The aqueous extract of its leaves has well-documented antitumoral and immunomodulatory activities [17, 18], antimicrobial properties [19, 20], and antioxidant activity demonstrated by peroxidase secretion of rat salivary glands [21]. Nordihydroguaiaretic acid (NDGA) has been identified as the major compound of *L. divaricata* leaves [22].

C. arabica belongs to the Rubiaceae family. The grains of this plant are used to prepare coffee, which is one of the most popular and widely consumed beverages worldwide due to its pleasant taste and aroma and its stimulant effect. Coffee contains several beneficial antioxidant [23] and antibacterial effects [24]. Chlorogenic acid has been identified as the major compound found in coffee extracts.

Taking into account the in vivo results in mice and the successful use of ECOHAIR® lotion, and to provide scientific support for its effect, the product was assayed in patients with noncicatricial alopecia. The primary objectives of this randomized, cohort, and prospective study were to evaluate the efficacy of treatment with ECOHAIR® spray through (1) the improvement in overall hair volume, thickness, looks, and growth and (2) the effect on hair loss. The secondary objectives were to identify possible signs of adverse local reactions related to the application of ECOHAIR® spray and to evaluate the decrease in dandruff as another signal of efficacy.

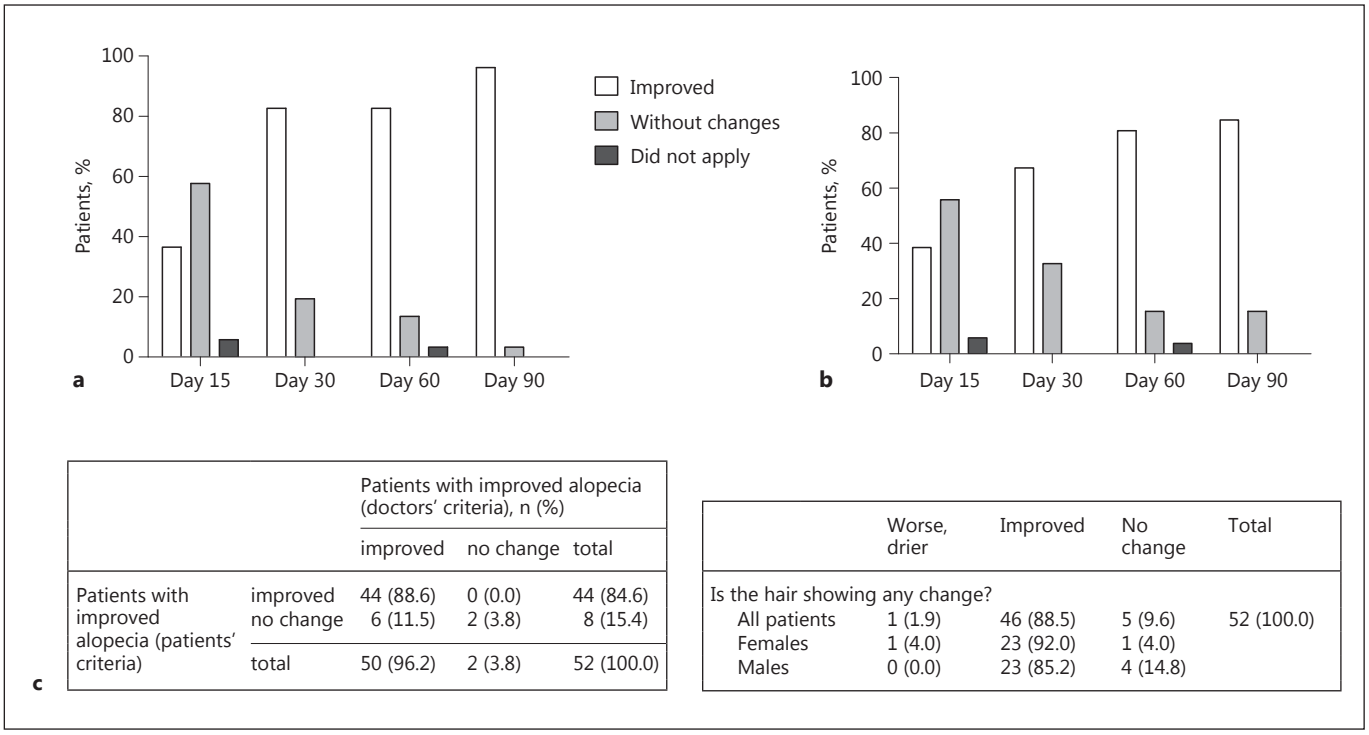
Methods

Study Design

An open, prospective, cohort (and with a unique branch phase IV) study was performed to evaluate the efficacy of ECOHAIR® spray lotion in men and women with noncicatricial alopecia during a daily administration period of 3 months in accordance with the product prospectus. The study was performed in accordance to the Comité de Ética en Investigación Clinica (CEIC; Ethical Clinical Research Committee), Ministerio de Salud de la Ciudad de Buenos Aires, under the protocol EH – 001. The study was performed at the Instituto de Hematología y Medicina Clínica Dr Rubén Dávoli, Laprida 1061 Rosario, Argentina, by Dr. Marina Rodriguez under IQUIMEFA-UBA-CONICET and LAT Research SRL guidance and supervision.

Patients

Patients were recruited during a period of 10 months (October 2013 to August 2014). Initially, the study sample comprised 72 volunteer patients. As 20 patients were withdrawn from the study due to a lack of adherence, the sample size was then reduced to 52 patients – 25 women (48.1%) and 27 men (51.9%). The distribution of patient ages was as follows: women had a mean age of 54.96 years (95% CI: 49.96–60.53, median: 60, variance: 181.873, range: 30–78). Men had a mean age of 41.44 years (95% CI: 35.64–47.25, median: 39, variance: 215.487, range: 21–71).



c

		Patients with improved alopecia (doctors' criteria), n (%)		
		improved	no change	total
Patients with improved alopecia (patients' criteria)	improved	44 (88.6)	0 (0.0)	44 (84.6)
	no change	6 (11.5)	2 (3.8)	8 (15.4)
	total	50 (96.2)	2 (3.8)	52 (100.0)

	Worse, drier	Improved	No change	Total
Is the hair showing any change?				
All patients	1 (1.9)	46 (88.5)	5 (9.6)	52 (100.0)
Females	1 (4.0)	23 (92.0)	1 (4.0)	
Males	0 (0.0)	23 (85.2)	4 (14.8)	

Fig. 1. Changes in alopecia assessed by doctors and patients. The graphics were constructed taking into account the percentage of patients with improved hair look during the treatment (on days 15, 30, 60, 90) according to doctors (**a**) and patients (**b**). **c** Table insert: statistical study of the conclusions obtained by doctors and patients on alopecia improvement and hair looks at the end of treatment. These results were obtained from questionnaires given to patients and doctors.

Before enrolling in the trial, all patients were subjected to a complete clinical revision. The following inclusion criteria were employed: (1) age older than 18 years, (2) dermatological diagnosis of noncicatricial alopecia, (3) signed informed consent form. Exclusion criteria were as follows: (1) the appearance of any acute or chronic medical condition indicative of treatment discontinuation, (2) known records of hypersensitivity to any of the excipients present in the product, (3) nonobservance of the clinical trial proceedings, (4) treatment with minoxidil or finasteride during the month previous to the clinical trial, and (5) participation in any clinical trial within a month previous to the study.

Study Objectives and Assessments

Patients were treated with ECOHAIR® spray on a daily basis, once a day, during 90 consecutive days.

Two main variables were studied: efficacy in relation to hair growth promotion, thickness, overall hair volume, the effect on hair loss, and security and tolerability. The efficacy was analyzed on days 15, 30, 60, and 90 after treatment. To this end, patients and doctors fulfilled self-assessment questionnaires about the overall volume, appearance, and thickness of hair, and signals of hair growth at different times during the treatment period.

The effect on hair growth was determined by ocular inspection employing a magnifying glass to determine the number of terminal hairs in the target area of the scalp.

To determine the effect on hair loss, patients were trained during the first visit of trial to count the hair lost after brushing for 1 min during 3 consecutive days with a fine-tooth comb. The number of fallen hairs was registered in the charts that were given to each patient. Day 15 was considered the basal value. Therefore, to calculate the decrease in hair loss, the recounting of fallen hair between days 15 and 90 was considered.

Safety measures included spontaneous reports of adverse events and any adverse events disclosed during clinical evaluations. The incidence and type of adverse local effects were registered, as well as the severity and causality accounting for a lack of adherence to the protocol. Safety data were correlated to demographic data such as age and sex, clinical information such as acute or chronic health conditions, and concomitant treatment with other drugs. The percentage of patients with adverse reactions was determined. The percentage of adverse reactions was correlated with those found in the literature, reported as frequent (1–10%) and very frequent (>10%).

Statistical Analysis

Several statistical tests were used: the Wilcoxon test for nonparametric median distribution of related samples, the Mann-Whitney U test, the Kolmogorov-Smirnov test, and the median test of independent samples.

The Wilcoxon test was used to analyze the medians of counted fallen hairs on day 15 and day 90, and to analyze the medians of

Table 1. Demographics and clinical characteristics of patients

a Gender distribution

	Females (*n* =52)	Males (*n* =52)	Total (*n* =52)
Age categories, *n* of patients (%)			
<31	2 (3.8)	10 (19.2)	12 (23.1)
31–45	5 (9.6)	6 (11.5)	11 (21.2)
>45	18 (34.6)	11 (21.2)	29 (55.8)
Total	25 (48.0)	27 (51.9)	52 (100.0)

b Presence of dandruff

	Females		Males		Total	
	no	yes	no	yes	no	yes
Greasy hair						
Brown	0	0	4	0	4	0
Gray	0	0	1	0	1	0
Black	0	1	2	2	2	3
Blonde	0	1	0	0	0	1
Total	0	2	7	2	7	4
Normal hair						
Brown	10	0	7	1	17	1
Gray	1	0	3	1	4	1
Black	3	0	2	0	5	0
Blonde	2	0	2	0	4	0
Total	16	0	14	2	30	2
Dry hair						
Brown	2	1	1	0	3	1
Gray	0	0	0	0	0	0
Black	2	0	1	0	3	0
Blonde	2	0	0	0	2	0
Total	6	1	2	0	8	1
Total						
Brown	12	1	12	1	24	2
Gray	1	0	4	1	5	1
Black	5	1	5	2	10	3
Blonde	4	1	2	0	6	1
Total	22	3	23	4	45	7

c Noncicatricial alopecia subtypes

	Subtypes			
	Androgenic	Areata	Telogen effluvium	Total
Sex, *n* of patients (%)				
Female	18 (72.0)	3 (12.0)	4 (16.0)	25 (100.0)
Male	26 (96.3)	1 (3.7)	0 (0.0)	27 (100.0)
Total	44 (84.6)	4 (7.7)	4 (7.7)	52 (100.0)

Table 1 (continued)

d Subtypes of androgenic alopecia

	Subtypes					
	1	2	3	4	5	Total
Sex, *n* of patients (%)						
Female	14 (77.8)	4 (22.2)	–	–	–	18 (100.0)
Male	7 (26.9)	7 (26.9)	6 (23.1)	1 (3.8)	5 (19.2)	26 (100.0)

the fallen hairs after brushing for 1 min during 3 consecutive days in women and men, and among individuals belonging to any age category.

The Mann-Whitney U test, the Kolmogorov-Smirnov test, and the median test of independent samples were used to analyze the differences between medians of counted fallen hairs of both women and men on day 90 and day 15, and among individuals belonging to any age category. p values <0.05 were considered significant.

Results

Demographics and Clinical Characteristics

The distribution of patients according to sex and age is shown in Table 1a. Hair analysis as regards the presence or absence of dandruff and the type of hair of each patient is shown in Table 1b. The more frequent subtypes of noncicatricial alopecia observed in patients are shown in Table 1c. A high percentage of women (72%) and men (96.3%) presented androgenic alopecia. Finally, the subtype of androgenic alopecia in women and men is shown in Table 1d. The more frequent subtypes of androgenic alopecia in women and men were types 1, 2, and 3.

Efficacy

Evaluation of Efficacy: Overall Hair Volume, Appearance, Thickness, and Growth

To study the efficacy of ECOHAIR® spray, patients were treated during 90 days with the spray and evaluated on days 15, 30, 60, and 90. An increase in volume and thickness and an improvement in hair looks were considered changes of alopecia and analyzed by both the doctor and the patient.

When medical criteria were considered (Fig. 1a), on day 15, 30 patients (57.7%) did not show any progress, while 19 patients (36.5%) showed some degree of improvement; 3 patients (5.8%) were not checked. On day 30, all patients were evaluated: 43 patients (82.7%) showed improvement, and 9 patients (17.3%) did not show any change. On day 60, 43 patients (82.7%) improved, 7 patients (13.5%) did not show any change, and 2 patients (3.8%) were not evaluated. On day 90, 50 patients (96.2%) improved and 2 patients (3.8%) did not show any improvement.

When patients' criteria were considered (Fig. 1b), on day 15, 20 patients (38.5%) improved, and 29 patients (55.8%) did not show any change; 3 patients (5.8%) were not evaluated. On day 30, all patients were evaluated: 35 patients (67.3%) improved, and 17 patients (32.7%) did not demonstrate any change. On day 60, 42 patients (80.8%) improved, and 8 patients (15.4%) did not demonstrate any change; 2 patients (3.8%) were not evaluated. On day 90, 44 patients (84.6%) improved, and 8 patients (15.4%) did not show any improvement.

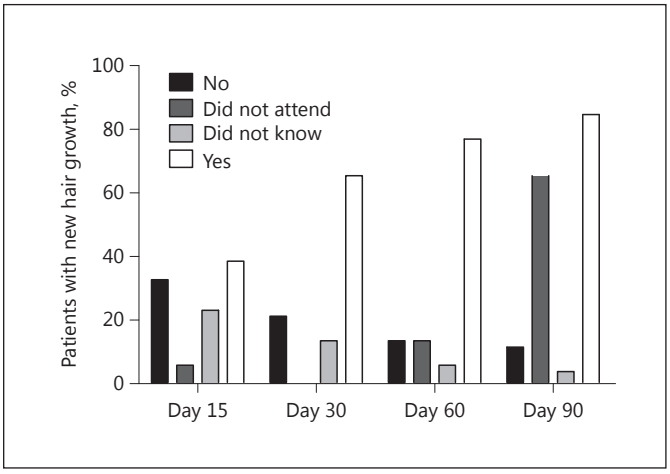
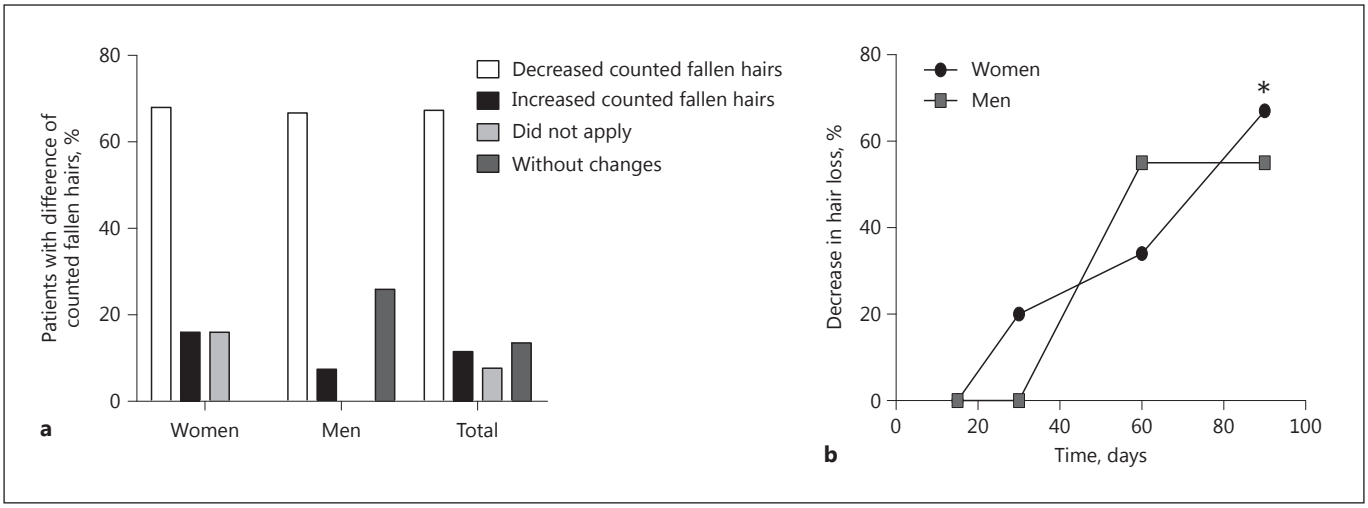


Fig. 2. Effect of the lotion on hair growth during the time of treatment. The percentage of patients that showed new terminal hair as a signal of hair growth during the treatment was determined (no: no growth observed; yes: hair growth observed).



Visit		Female	Male	Total	Visit		Female	Male	Total
Day 15	Counted hair				Day 60	Counted hair			
	Mean	24.5	13.9	18.5		Mean	17.5	10.2	13.8
	Median	15.0	9.0	12.5		Median	10.0	4.0	9.0
	Lost	4	0	4		Lost	1	2	3
	Maximum	87	60	87		Maximum	90	35	90
	Minimum	2	2	2		Minimum	1	2	1
	Recounting	25	27	52		Recounting	25	27	52
Day 30	Counted hair				Day 90	Counted hair			
	Mean	20.1	12.2	15.9		Mean	13.2	8.1	10.5
	Median	12.0	10.0	10.0		Median	5.0	4.0	5.0
	Lost	1	0	1		Lost	0	0	0
	Maximum	64	53	64		Maximum	65	34	65
	Minimum	1	2	1		Minimum	2	2	2
	Recounting	25	27	52		Recounting	25	27	52

c

Fig. 3. Analysis of the decrease in hair loss in relation to sex. **a** Percentage of men and women that showed differences of counted fallen hairs between days 15 and 90 as a signal of a decrease in hair loss during the treatment. **b** Decrease in hair loss (%) in men and women during treatment. **c** Table insert: counted hair (mean of 3 determinants). * $p < 0.05$, significantly different (Wilcoxon test of nonparametric median distribution of related samples).

On day 90, the conclusions obtained by doctors and patients were statistically analyzed (median test for independent samples). As can be observed in Figure 1c (table insert), patients and doctors agreed on the facts that the treatment was effective in 84.6% of patients and that there was an improvement in hair features. The null hypothesis that both agreed was affirmative ($p = 0.056$).

Furthermore, the variable hair looks at the end of treatment was analyzed. For this latter analysis, sex was taken into account to find that women and men improved their hair appearance by 92 and 85.2%, respectively (Fig. 1c, table insert).

In order to determine whether ECOHAIR® induced hair growth, the number of new hairs over time was determined. To this end, an ocular inspection was done with a magnifying glass in order to determine the number of terminal hairs in the target area of the scalp. Moreover, the percentage of patients with new hairs was scored. The results of these observations are summarized in Figure 2. The number of patients with new terminal hairs increased over time, reaching 84.6% at the end of treatment.

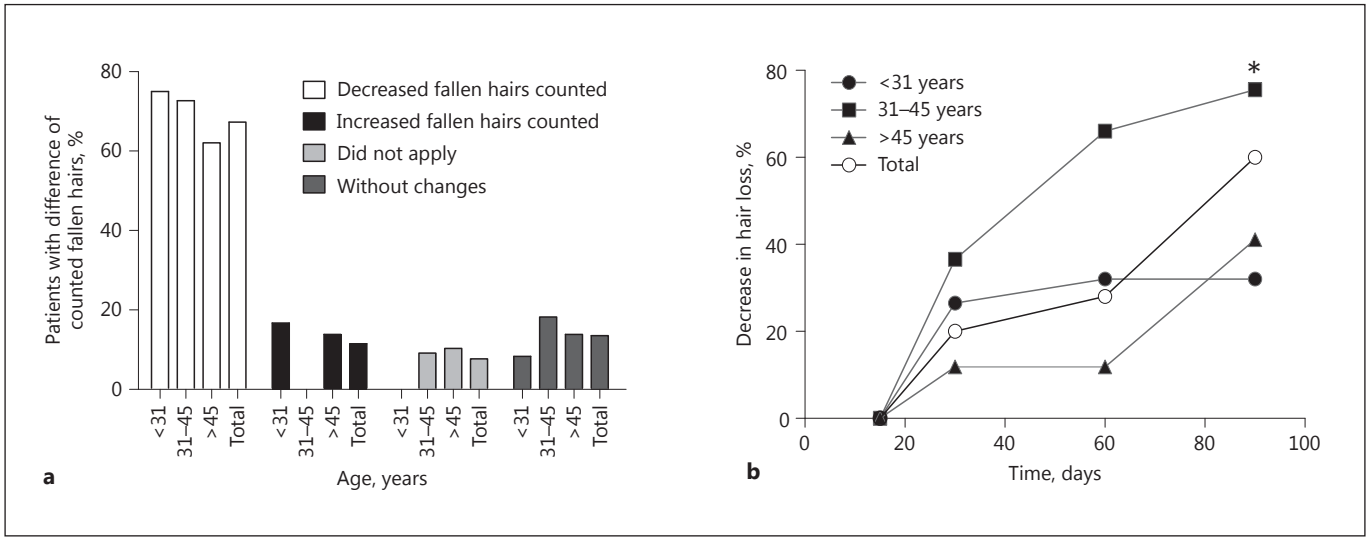


Fig. 4. Analysis of the decrease in hair loss in relation to age. **a** Percentage of patients <31, 31–45, and >45 years that showed differences between counted fallen hairs on days 15 and 90 during the treatment. **b** Decrease in hair loss (%) in patients with different range of ages during treatment. * $p < 0.05$, significantly different (Wilcoxon test for nonparametric median distribution of related samples).

Evaluation of Efficacy: Effect on Hair Loss in Relation to Sex

The percentage of patients (women and men) who presented an increase or decrease in hair loss was studied. The percentage of patients who presented a decrease in hair loss was higher than in those who had an increase in hair loss or other categories (Fig. 3a). However, no significant differences were observed between the 2 groups analyzed.

The number of fallen hairs between day 15 and day 90 was significantly different ($p < 0.05$; Wilcoxon test for nonparametric median distribution of related samples); on day 90 the number was lower.

In order to determine whether the decrease in hair loss induced by the treatment was sex related, statistical analyses were carried out employing the Mann-Whitney U test, the Kolmogorov-Smirnov test, and the median test of independent samples. No differences among the group medians were observed ($p = 0.061$, $p = 0.241$, and $p = 0.128$, respectively). However, when the fallen hairs, after brushing for 1 min during 3 consecutive days (see Study Objectives and Assessments), were subjected to statistical analysis, significant differences ($p < 0.05$; Wilcoxon test for nonparametric median distribution of related samples) were observed in the medians of counted fallen hairs of women, who underwent a higher decrease (21%) compared with men (Fig. 3b, c, table insert).

Evaluation of Efficacy: Effect on Hair Loss in Relation to Age

The effect of the lotion on hair loss was studied in relation to age (<31 years, 31–45 years, and >45 years), evaluating the parameters explained above. The percentage of patients with a decrease in hair loss was higher than the percentage of patients with an increase in hair loss between day 15 and day 90; however, no significant differences were found between age categories (Fig. 4a). It is important to note that the number of fallen hairs between day 15 and day 90 was significantly different ($p < 0.05$; Wilcoxon test for nonparametric median distribution of related samples); on day 90 the number was lower.

In order to determine whether the decrease in the number of fallen hair was higher among individuals belonging to any age category, the median test of independent samples was applied. No differences among the group medians were observed ($p = 0.238$). However, when the fallen hairs, after brushing for 1 min during 3 consecutive days (see Study Objectives and Assessments), were subjected to statistical analysis, significant differences ($p < 0.05$; Wilcoxon test for nonparametric median distribution of related samples) were observed in the medians of counted fallen hairs of the subgroup 31–45 years, showing a higher decrease in hair loss on day 90 (75%) (Fig. 4b).

Table 2. Effect of ECOHAIR® on dandruff

Summary of processed data	Counted	Lost	Total
Patients with improved dandruff, n (%)			
Patients' criteria	7 (13.5)	45 (85.5)	52 (100.0)
Doctors' criteria	7 (13.5)	45 (86.5)	52 (100.0)

Counted: patients who presented dandruff at the beginning of the study and improved at the end. Lost: patients who did not present dandruff at the beginning of the study. Total: total number of patients in the study.

Table 3. Adverse effects

Visits	Patients, n (%)
Day 15	
No	46 (88.5)
Did not apply	3 (5.8)
Yes, slight burning	1 (1.9)
Yes, slight itching	1 (1.9)
Yes, temporal itching	1 (1.9)
Total	52 (100.0)
Day 30	
No	52 (100.0)
Total	52 (100.0)
Day 60	
No	50 (96.2)
Did not apply	2 (3.8)
Total	52 (100.0)
Day 90	
No	52 (100.0)
Total	52 (100.0)

Evaluation of Efficacy: Effect on Dandruff Quantity at the End of Treatment

To determine the effect on dandruff quantity, each patient's head was subjected to ocular inspection on day 90. As shown in Table 2, a low number of patients presented dandruff at the beginning of the study (only 7), but all of them had improved signs and symptoms at the end of the treatment.

Safety and Tolerability

Some slight adverse reactions such as burning and itching were observed only on day 15 in a few patients. The adverse reactions are presented in Table 3.

Discussion

In this study, the effect of ECOHAIR® spray on the improvement of alopecia in men and women, mainly suffering from androgenic alopecia varieties, is demonstrated. ECOHAIR® induced hair growth, improved hair appearance, decreased hair loss, and eliminated dandruff.

The stimulatory action exerted by ECOHAIR® in humans could be explained by the capacity of the 2 extracts present in the product (*L. divaricata* and *C. arabica*) to promote hair growth, as was demonstrated in mice; in this experiment the extracts were able to promote hair growth by the induction of the anagen phase [15]. In addition, the extract from *C. arabica* demonstrated a fungicidal effect in vitro on *M. furfur*, the fungus causative of dandruff and related to alopecia [25]. Taken together, these effects could provide a partial explanation for the effect of the extracts on human hair growth promotion.

The improvement in hair looks (volume and thickness) was analyzed by both patients and doctors, and the statistical conclusions established that 84.6% of patients had an improvement in hair looks at the end of treatment. The latter finding could be related to the increase in hair growth demonstrated by the new terminal hairs observed and the decrease in hair loss exerted by the lotion. It is noteworthy that the effect was gradual and that a daily continuous use was required. Even though the lotion was effective in both men and women, it could be hypothesized that the sex variable had some influence in the results, as 92% of women improved their hair appearance compared with 85.2% of men. When age was considered, the lotion was efficient in all groups, but the subgroup of 31–45 years was more sensitive to the treatment. Finally, the lotion decreased the amount of dandruff, which could be related to the fact that the extract of *C. arabica* present in the lotion has an inhibitory effect on the growth of *M. furfur* [25].

Moreover, the lotion was safe, as only slight adverse reactions were observed in a few patients at the beginning of treatment. It is noteworthy that these adverse reactions did not last over time, as assessed by the dermatologist. All these results support the rational use of ECOHAIR® to promote hair growth and to prevent hair loss.

Conclusions

It can be concluded that:

– ECOHAIR® spray was demonstrated to improve hair looks in 84.6% of patients after 90 days of treatment.

– The lotion decreased hair loss from the beginning of treatment. This effect was more marked in women. Best results were obtained in patients with androgenic alopecia subtypes 1 and 2.
– ECOHAIR® was demonstrated to eliminate dandruff and was well tolerated.

Acknowledgments

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Statement of Ethics

This study was conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

This study protocol and informed consent were reviewed and approved by an institutional review board. Informed consent was obtained from all individual participants included in the study.

This study was conducted in accordance with applicable guidelines for the protection of human subjects for research as outlined by the Comité de Ética en Investigación Clínica (CEIC) Argentina – Ministerio de Salud del Gobierno de la Ciudad de Buenos Aires (Acreditación 031 Disposición DI-2012-135-DGDOIN, Registro Nacional de Investigaciones en Salud [RENIS] Código CE000022, Office for Human Research Protection [OHRP] IRB Registration 00001678, USA).

Disclosure Statement

The authors declare no conflicts of interest.

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