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Efficacy of *Oryza Sativa* L. (Black Rice) and Opuntia *Ficus Indica* L. Blend in Men with Androgenetic Alopecia: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

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Abstract

Androgenetic Alopecia (AGA) is a progressive hair loss type affecting up to 80% of men and 50% of women. The putative mechanisms in the pathogenesis of AGA include microinflammation, premature cell senescence, oxidative stress, follicle vascular changes and altered sensitivity of hair follicles to androgens. A randomized, double-blind, placebo-controlled trial was carried out on 86 male subjects with AGA from II to III vertex (Hamilton-Norwood). Product efficacy was measured after 3, 6 months of product intake and 1-month post-supplementation. The hair cycle parameters measurement by phototricogram were integrated by the global photography assessment and the self-assessment questionnaire. The results showed an increase of the total hair density and the % of hair in anagen phase with a simultaneous decrease in the % of hair in telogen phase; as consequence the anagen/telogen ratio was improved of about 45.7% and 85.8% at 3 and 6 mo, respectively. The instrumental data were confirmed by the clinical analysis carried out by a board-certified dermatologist and by the self-assessment questionnaire. The obtained improvements were also maintained 1-month post-supplementation. The intake of 250 mg of Actrisave™ improved the hair life cycle increasing hair growth, density and slowing down hair loss in men with mild-to-moderate androgenetic alopecia.

Keywords: Plant secondary metabolites • Androgenetic slopecia • *Oryza Sativa* • *Opuntia ficus-Indica* • Clinical trial

Introduction

Androgenetic Alopecia (AGA) is the most common progressive hair loss type affecting up to 80% of men and 50% of women [1-3]. The occurrence of AGA depends on the interaction of genetics and endocrine factors. AGA is characterized by a progressive miniaturization of the hair follicle (HF) characterized by a reduction of the hair diameter, length and pigmentation [2,4]. Hair loss in AGA typically starts with a hairline recession around the temples and along the mid-frontal border of the scalp followed by hair thinning or complete hair loss in the vertex region. The AGA-related pattern of hair loss in men was originally described by Ludwig in 1951 [5] and later revised by Norwood in 1975 [6]. The Hamilton-Norwood scale is, to date, the most used and reliable scale to assess the severity and the extent of hair loss in men [7]; by categorizing men into 8 different stages of hair loss, from the least to most severe hair loss – I, II, III, III vertex, IV, V, VI and VII.

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The hair miniaturization in AGA occurs at some point between the late catagen or the early anagen phase. Hair miniaturization consists of a smaller HF with a reduced anagen phase [8] due to the alteration of the dermal papilla and the dermal sheath [9]. This anomaly is usually irreversible, although partial regrowth and some reversal is possible in some instances (e.g., in the early stage of AGA). The putative mechanisms in the pathogenesis of AGA include a) microinflammation infiltrating the follicular bulge inducing progressive fibrosis of the perifollicular area [10], b) abnormal sensitivity of HFs to circulating androgens in androgen-dependent areas [11-13] and c) anomalies in arrector pili muscle [8]. Terminal hair is then replaced by vellus hair in miniaturized HFs. Vellus hair is hypopigmented, nonmedullated, less than 30 μm thick and less than 2-3 mm long [14].

The dermal papilla (DP) is a niche of specialized mesenchymal cells located at the bottom of the HF. The roles of DP in regulating HF activation, including progenitor cells activation and differentiation during follicle growth have been long recognized in the scientific literature [15]. DP cells (DPCs) regulate the hair follicle stem cells' activity through short-range cell-cell contact or paracrine effects [16]. In a recent study, Deng Z, et al. showed that paracrine signaling (mainly TGFβ signaling), mediated by androgen receptors, from DPCs induces apoptosis of microvascular endothelial cells in the DP of the balding scalp of AGA [17].

Despite AGA being one of the most traditionally understood, genetically determined and androgen-induced causes of hair loss, a new and alternative definition of AGA is evolving. According to the new definition, AGA is a genetically determined, HF-specific premature aging process with an increased sensitivity to both internal (hormonal and vascular) and external (environmental, inflammatory and dietary) factors [18]. The involvement of premature senescence was shown *in vitro* by Bahta AW, et al. [19] in cultured DPCs from balding and non-balding scalps. The authors demonstrated a decrease in the growth rate of the balding DPCs compared to the DPCs of non-

balding scalps. Interestingly the loss of proliferative capacity was associated with both the expression of oxidative stress and DNA damage suggesting that DPCs are particularly sensitive to environmental stress. The effects of the oxidative stress on balding scalp were further investigated by Upton JH, et al. [20] on *in vitro* cultures of balding and occipital scalp DPCs cultured at atmospheric (21%) or physiologically normal (2%) O_2 concentration. At 21% O_2 DPCs showed flattened morphology, a significant reduction in mobility, increased levels of reactive oxygen species (ROS) and senescence-associated β-Gal activity. Interestingly, despite the higher levels of total glutathione and catalase, balding DPCs showed a decreased ability to handle oxidative stress compared to occipital DPCs. These *in vitro* findings suggest that oxidative stress may have a primary role in the pathogenesis of AGA. Premature cell senescence reduced cell migration rate, DNA damage and secretion of known HF inhibitory factors, pave the way for new AGA treatments beyond the traditional pharmacological approach.

Perifollicular vascularization has been previously observed during the hair cycle and in some human diseases characterized by hair loss [21,22]. A role of Vascular Endothelial Growth Factor (VEGF) in the control of perifollicular vascularization during hair growth and cycling was reported by Yano K, et al. in adult mice [23]. Authors reported a fourfold increase in perifollicular vessel size during the anagen phase and a rapid decrease during catagen and telogen associated with cyclic changes in follicle size and correlated with upregulation of VEGF mRNA expression by follicular keratinocytes of the outer root sheath. Interestingly the transgenic overexpression of VEGF accelerated hair growth after depilation with an increase of the hair follicles. These results clearly identify a role for VEGF and more in general for vascular remodeling and blood supply in hair biology.

Currently, topical minoxidil (for both men and women) and oral finasteride (men only) are the only treatments recognized and approved by the Food and Drug Administration (FDA) for the treatment of AGA [24]. However, they are costly, require lifelong treatment and may have adverse side effects (ASEs). The ASEs of topical minoxidil include irritant and allergic contact dermatitis, scalp irritation, pruritus and facial hypertrichosis [25] with a low incidence. A higher incidence of ASEs is reported for oral finasteride use including orthostatic hypotension, decreased libido and erectile and ejaculatory dysfunctions [26]. Beyond the occurrence of ASEs, both the minoxidil and the finasteride therapies are limited by their incomplete efficacy and by the recurrence after cessation [27,28].

Based on the new mechanistic knowledge reported here above and the side effects of the pharmacological approach, several common botanicals [29] have been investigated for their potential therapeutic effects for alopecia and hair loss disorders in general. Among others a recent work reported a protective effect for AGA in male subjects with a high consumption (≥ 3 times weekly) of raw vegetables or fresh herbs [30], suggesting a role for some food of the Mediterranean diet (rich in phytochemicals such as carotenoids and polyphenols) in reducing AGA spots and delaying its onset. Therefore, there is a need for alternative treatments including cosmetic products and food supplements and the use of herbal extracts [31,32]. On the other hand, there is an increase interest in deepening the investigation of less common natural compounds [33,34] in improving HF diseases.

The aim of this study was to assess for the first time the efficacy of the use of a patented [35] combination of *Oryza Sativa* L. (black rice) and *Opuntia Ficus Indica* L. (prickly pear cactus flowers) extracts in men with AGA. The reason behind our interest in this study was to give evidence of the efficacy of a complementary approach based on natural extracts to counteract hair loss safely. To reach this goal and to protect consumers from claims and marketing, we decided to give evidence of the efficacy of the extract through a randomized clinical trial.

Materials and Methods

Ingredient characterization

Chemicals: High performance liquid chromatography (HPLC) grade solvents, methanol, dimethylformamide, acetonitrile, water, formic acid and phosphoric acid were obtained from Carlo Erba Reagenti (Milano, Italy). Reference compounds (Cyanidin-3-O-glucoside, malvidin-3-O-glucoside, quercetin-3-O-glucoside, isorhamnetin 3-neohesperidoside and isorhamneyin 3-rutinoside) were obtained from PhytoLab GmbH & Co (Vestenbergsgreuth, Germany).

Samples preparation, calibration and HPLC/DAD analysis: HPLC analysis were performed in duplicate using an Agilent 1100 Infinity (Agilent Technologies, Inc., California, US), equipped with a diode array detector (DAD) and with a 150 x 4.6 mm id, 2.7 µm Ascentis Express C 18 column maintained at 25°C. The flow was 1 mL/min and the injection volume was 5 µL.

The mobile phases for the determination of Isorhamnetin and derivatives was H_2O/H_3PO_4 (99: 1; solvent A), MeOH/ACN/H_3PO_4 (49.5:49.5:1; solvent B). The gradient used was as follows: solvent A concentration of 85% passing to 77% (13 min), keep 77% (5 min), 74% (25 min), 0% (5 min), keep 0% (3 min), total time 58 min. The profile chromatogram was recorded from 190 to 500 nm and monitored at 350 nm ± 2 nm (Figure 1).

The mobile phases for the determination of Anthocyanins and derivatives was H_2O/HCOOH (90:10; solvent A), ACN/W/HCOOH (50:40:10; solvent B). The gradient used was as follows: solvent A concentration of 88% passing to 70% (25 min), 0% (1 min), maintain 0% (4 min), total time 35 min. The profile chromatogram was recorded from 190 to 700 nm and monitored at 520 nm ± 2 nm (Figure 1).

The stock standard solutions were prepared by dissolving 1 mg of the standard in 5 ml of dimethylformamide/water (9:1) for the Isorhamnetin. For the calibration points, the solutions and peak areas were measured into 6 points from an initial concentration of 200 ppm to 2 ppm. The linearity of the ratio between concentration and peak area was evaluated based on the value of the correlation coefficient. The regression data obtained for the analysis are listed in Table 1.

Clinical trial

Study design description: This was a multicentric, randomized (balanced randomization, [1:1]), double-blind, placebo-controlled, parallel-group study conducted in Italy (3 sites).

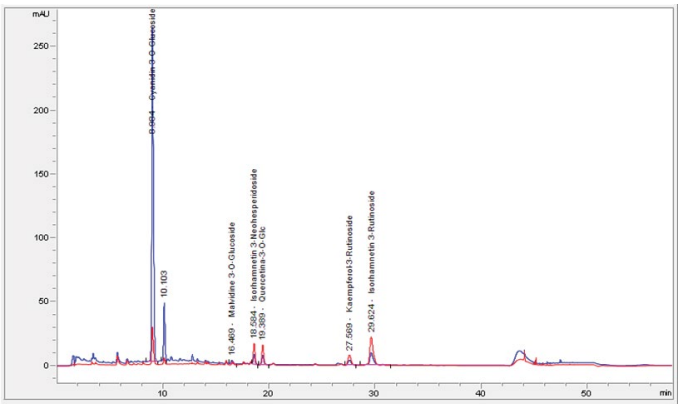

Figure 1. HPLC-DAD chromatogram of Actrisave™ with identification phenolic compound; Ch1 520 nm Anthocyanins and derivatives and Ch2 350 nm Isorhamnetin and derivatives.

Table 1. Chemical characterization results.

Compound (Tr. min)	λ (nm)	Standard	Purity %
8.984	520	Cyanidin 3-O-Glucoside	88
10.103	520	n.d.	n.d.
16.469	520	Malvidine 3-O-Glucoside	89
18.584	350	Isorhamnetin 3-Neohesperidoside	92
19.389	350	Quercetin 3-O-Glucoside	96
27.569	350	Kaempferol -3-Rutinoside	91
29.624	350	Isorhamnetin 3-Rutinoside	92

All the study procedures were conducted in accordance with the World Medical Association's (WMA) Helsinki Declaration and its amendments. Both the study protocol (no. H.E.HU.AL.NHL00.086.01.00_IT0004094/21 version no. 01 by 26/10/2021) and the informed consent form were approved by the "Comitato Etico Indipendente per le indagini Cliniche Non Farmacologiche" (ref. no. 2021/08 by 02.11.2021). The study was registered at isrctn.com, number ISRCTN17383899, https://doi.org/10.1186/ISRCTN17383899 (accessed on 11 November 2022).

Eligibility criteria for participants: Eligible participants were all male healthy adults aged between 18 and 55 years old (extremes included) showing mild-to-moderate androgenetic alopecia degree (from II to III vertex on Hamilton-Norwood scale). The study further included subjects with all types of scalp and hair, registered with health social security or health social insurance, certifying the truth of the personal information declared to the Investigator, able to understand the language used in the investigation center and the information given, able to comply with the protocol and to follow protocol's constraints and specific requirements, agreement to preserve a length of hair longer than 5 cm during the study, agreement to have a zone of about 2 cm^2 shaved on the scalp, willingness to use the same shampoo during all the study period.

Exclusion criteria were as follows: participation to another clinical study during the study period (in the same or in another investigation center), participation to another clinical study with anti-hair loss products or treatment within the last 24 weeks before the inclusion visit, acute, chronic or progressive illness liable to interfere with the study data or considered by the Investigator hazardous for the subject or incompatible with the study requirements, a long-treatment considered by the Investigator liable to interfere with the study data or incompatible with the study requirements, a skin condition liable to interfere with the study data or considered by the Investigator hazardous for the subject or incompatible with the study requirements, personal history of cosmetic, drug or domestic products irritative reactions, inflammatory skin disease or progressive skin lesion on the scalp (psoriasis, seborrheic dermatitis, severe erythema, severe excoriation, severe sunburn, etc.), scalp lesion in relief which may be traumatized, history of hypersensitivity or intolerance to any of the ingredient in product formula, systemic treatments (retinoids, anti-mitotic, cytotoxic drugs other than antineoplastic, anti-androgens [spironolactone, flutamide], androgens, anti-epileptic agents, interferon alpha) affecting the hair growth taken for more than 4 consecutive weeks during the last 24 weeks before inclusion visit, systemic or local androgenetic alopecia treatment or product, taken or applied (minoxidil, aminexil, finasteride, dutasteride, cosmetic solution or capsules with vitamin B, zinc, caffeine, etc.) for more than 4 consecutive weeks during the last 24 weeks before the inclusion visit, any other local treatment applied on the scalp (non-steroidal anti-inflammatory, ketoconazole, etc.) within the last 2 weeks before the inclusion visit, any hair care product applied on the scalp between the last shampoo and the inclusion visit (e.g., gel, hairspray, wax, foam, etc.), radiotherapy, chemotherapy at any time, scalp surgery (hair transplants, laser) at any time.

Eligibility criteria for participants: The study took place at Complife Italia Srl facilities (Biella, San Martino Siccomario and Milan) from September 2021 to September 2022.

Intervention: The test product was a capsule containing 250 mg Actrisave™ (Bionap Srl, 95032 Piano Tavola Belpasso, CT, Italy), 100 mg microcrystalline cellulose, 100 mg maltodextrin and 2 mg titanium dioxide. Actrisave™ is a blend of *Oryza Sativa* L. (black rice) and *Opuntia Ficus Indica* L. (prickly pear cactus flowers) extracts. The extract was characterized for the black rice-derived anthocyanins content and for the prickly pear cactus flowers flavonoids content, as follows (w/w): 4.5-5.5% anthocyanins (as cyanidin-3-glucoside) and 1.0-2.0% isorhamnetin and derivates (isorhamnetin-3-rutinoside). The placebo capsule contained 350 mg maltodextrin, 100 mg microcrystalline cellulose and 2 mg titanium dioxide. The posology for both the active and the placebo product was 1 capsule per day after lunch or dinner.

The subject's compliance to treatment was assessed by product accountability as follows: (number of capsules intake/total number of capsules to intake) x 100. A cut-off value by 80% was needed to include the subjects in the per-protocol analysis.

Randomization and masking: A randomization sequence was created using PASS 11 (version 11.0.8, PASS, LLC. Kaysville, UT, USA) statistical software. The "Efron's biased coin" algorithm was used to stratify by center with a 1:1 (active:placebo) allocation.

Both the active and the placebo products were identical in appearance. They were prepacked by the study Sponsor and each pack was consecutively numbered according to the randomization sequence. Each subject was assigned to a randomization number and received the capsule in the corresponding numbered pack.

The unblinded randomization sequence was concealed from the study director (V.N.) in sequentially numbered, opaque, sealed envelopes. V.N. was not involved in the clinical part of the study. The sheet reporting the unblinded randomization sequence was folded to render the envelope impermeable to intense light. The envelopes were stored, in a centralized and safe place, by the study director (V.N.) and were not accessible to others study staff team.

Neither the staff who delivered the inventions nor the outcome assessor were aware of the allocated treatment. All investigators, staff and participants were kept masked to outcome measurements and to the study results.

Primary and secondary objectives and outcome measures: The primary objective of the study was the assessment of the efficacy of the test product in decreasing the hair loss after 3, 6 months (mo) of product intake and 1 mo after the last product intake. The primary outcome measure was the evaluation of the following hair cycle parameters: anagen, telogen and hair density.

The secondary objective was the assessment of the clinical and self-perceived effects. The secondary outcome measures were the clinical scoring of hair growth carried out by the dermatologist on digital pictures and the subjects scoring by a self-assessment questionnaire.

The study flow and the schedule of assessment chart are reported in Table 2.

Phototricogram

Anagen, telogen and hair density were estimated in a target area of (0.59 cm^2 diameter) on a transitional area near the bald spot with hair clipped less than 1 mm. A DermoGenius ultra-polarized (DermoScan GmbH, Regensburg, Germany) dermatoscope was used to capture magnified images of the target area two days after clipping. Before picture taking hair were dyed to enhance hair contrast. Anagen, telogen and hair density are automatically calculated by TrichoScan® (Tricholog GmbH & Datinf GmbH, Freiburg, Germany) software.

Global photography assessment

The clinical scoring of hair growth was performed by the dermatologist on standardized pictures. Pictures were scored using a 7-point scale: Greatly decreased (-3), Moderately decreased (-2), Slightly decreased (-1), No change (0), Slightly increased (+1), Moderately increased (+2), Greatly increased (+3). This technique has been demonstrated to have excellent reproducibility [36].

Self-assessment questionnaire

Subjects were asked to score the product efficacy in decreasing the AGA spot size, in increasing hair growth and in slowing down hair loss.

Statistical analysis

Statistical analysis was performed using NCSS 8 (version 8.0.4 for Windows; NCSS, Kaysville, UT, USA) running on Windows Server 2008 R2 Standard SP1 64-bit edition (Microsoft, USA). A two-way t test of Student was performed for normally distributed data (instrumental measurements) while a Wilcoxon or a Mann-Whitney U test was performed for non-parametric data (clinical analysis). A $p < 0.05$ was considered statistically significant. Statistical analysis output was reported as follows: *p<0.05, **p<0.01 and ***p<0.001.

Results

Chemical characterization

The analyses of the extract identified 6 compounds as shown in Table 1

Table 2. Study flow and schedule of assessment chart.

	<---------- Treatment period ---------->										Follow-up
	M0-2	MO	M1	M2	M3-2	M3	M4	M5	M6-2	M6	M7-2	M7
Informed consent	√											
Informed consent signature and photograph authorisation form signature	√											
Inclusion and non-inclusion criteria check	√					√				√		√
Demographics data/Medical history	√		☎	☎		√	☎	☎		√		√
Prior and concomitant pharmacological treatments	√		☎	☎		√	☎	☎		√		√
Assessment of physical and functional signs by dermatologist	√					√				√		√
Enrolment and randomization	√											
Hair clipping for phototricogram	√				√				√		√	
Phototricogram		√				√				√		√
Expert scoring		√				√				√		√
Self-assessment questionnaire						√				√		√
Dispensing of daily log (alimentary habits)		√				√						
Dispensing of investigational products		√				√						
Product intake at home		<---------- From M0 to M6 ---------->										
Daily log return*						√				√		
Investigational product return*						√				√		
Compliance to treatment check			☎	☎		√	☎	☎		√		
Recording reactions (AE, SAE)			☎	☎		√	☎	☎		√		√

* Data are used to check subjects' compliance to treatment.

and Figure 1. In particular, cyanidin 3-O-glucoside, malvidine 3-O-glucoside, isorhamnetin 3-neohesperidoside, quercetin 3-O-glucoside, kaempferol -3-rutinoside, isorhamnetin 3-rutinoside were identified. The identification was performed through the comparison of chromatographic behavior and UV-Vis spectra with commercially available standard compounds.

Participants and product tolerability: A total of 86 subjects were successfully randomized to receive the active (n=43) or the placebo (n=43) treatment (Figure 2). Subjects attended clinic visits at baseline and after 3 and 6 months of product use; an additional visit was planned 1 month after the last product intake. The population was male subjects aged between 19 and 55 years old. Demographic and baseline characteristics (Table 3) were similar across treatment arms. No covariates were identified between the two treatment groups. Two subjects per each treatment arm interrupted prematurely (at month 3) the study (for personal reasons not related to the product intake). The subject's compliance to treatment was 93.2% (from 83.9% to 100%) and 94.3% (from 85.6% to 100.0%), for the active and the placebo product, respectively. Forty-one (n=41) subjects per treatment group were included in the efficacy analysis dataset. All the enrolled subjects (n=86) were included in the safety dataset. Both the active and the placebo products were well tolerated. No adverse events were reported during the study period.

Phototricogram: The mean change in the total hair density after 3 mo and 6 mo of supplementation was significantly greater in the active group (+5.5 hair/cm^2 at 3 mo and +13.9 hair/cm^2 at 6 mo) compared to no significant change in the placebo group (+1.3 hair/cm^2 at 3 mo and +0.5 hair/cm^2 at 6 mo) (Figure 3). The results obtained at 6 mo in the active group were maintained (+12.7 hair/cm^2) after 1-month post-supplementation. Presented as percent changes, Actrisave™ increased total hair density by 3.8% and 9.5% at 3 mo and 6 mo, respectively compared to 0.9% and 0.3% in the placebo group.

Actrisave™ also significantly increased anagen hair density, increasing it from 100.0 ± 2.4 hair/cm^2 at baseline to 115.1 ± 2.4 hair/cm^2 at 3 mo ($p<0.0001$) and 128.1 ± 3.8 hair/cm^2 at 6 mo ($p<0.0001$); increasing anagen hair density 15.1% and 28.2% at 3 and 6 mo, respectively. This significant increase in anagen hair density was maintained (+26.6 hair/cm^2) after 1 mo post-supplementation. In addition, telogen hair density decreased 21.0% and 31.1% at 3 mo and 6 mo; an effect that was maintained 1 mo post-supplementation

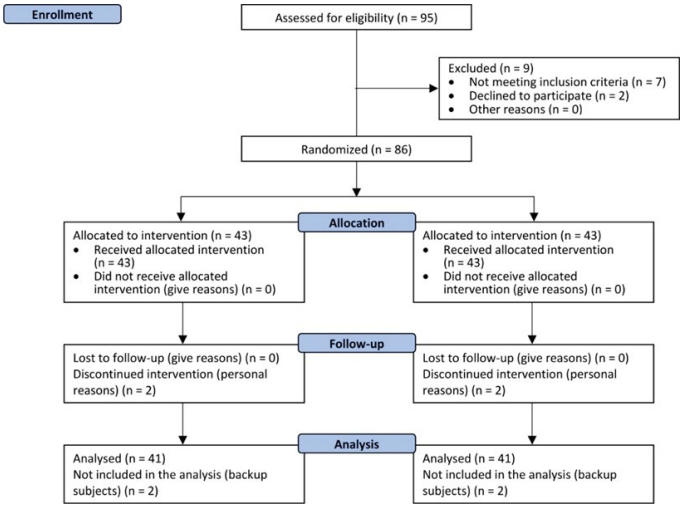

Figure 2. Participants flow diagram.

Table 3. Baseline and demographic characteristics. Data are mean ± SE.

		Active	Placebo	Units
Sex	Male	100% (41)	100% (n=41)	% (no.)
	Age	42.1 ± 1.6	41.8 ± 1.7	Years
AGA scoring	II	31.7% (13)	26.8% (11)	% (no.)
	III	31.7% (13)	26.8% (11)	% (no.)
	III vertex	36.6% (15)	46.3% (19)	% (no.)
Scalp type	Dry	12.2% (5)	17.1% (7)	% (no.)
	Normal	48.8% (20)	56.1% (23)	% (no.)
	Oily	39.0% (16)	26.8% (11)	% (no.)
Phototricogram	Anagen	100.0 ± 2.4 (68.8 ± 0.8%)	100.4 ± 3.35 (69.6 ± 0.7%)	no. (%)
	Telogen	45.7 ± 1.9 (31.2 ± 0.8%)	43.5 ± 1.6 (30.4 ± 0.7%)	no. (%)
	Hair density	145.7 ± 3.6	143.8 ± 4.3	no. (%)

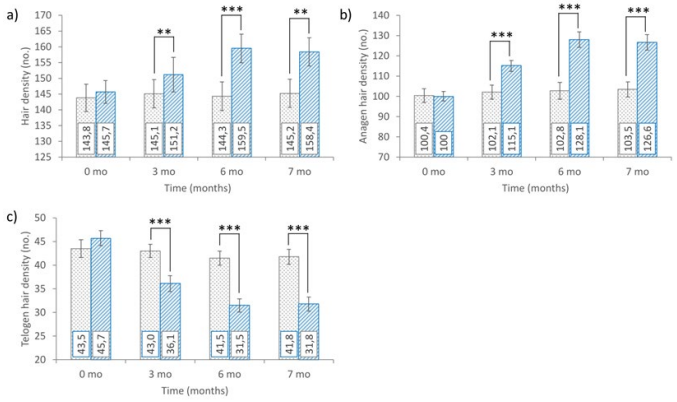

Figure 3. (a) Total hair density, **(b)** Anagen hair density and **(c)** Telogen hair density. Data are average (± standard error). The intergroup statistical analysis is reported above the bar as follows: ** p<0.01, and *** p<0.001.

Table 4. Global photography assessment of hair growth.

	Active			Placebo		
Score	**3 mo**	**6 mo**	**7 mo**	**3 mo**	**6 mo**	**7 mo**
Greatly decreased	0.0%	0.0%	0.0%	0.0	0.0	0.0
Moderately decreased	0.0%	0.0%	0.0%	0.0	2.4	4.9
Slightly decreased	0.0%	0.0%	0.0%	12.2	9.8	7.3
No change	36.6%	4.9%	12.2%	63.4	53.7	63.4
Slight increased	56.1%	43.9%	36.6%	24.4	31.7	24.4
Moderately increased	7.3%	48.8%	48.8%	0.0	2.4	0.0
Greatly increased	0.0%	2.4%	2.4%	0.0	0.0	0.0
% Positive scores	63.4%	95.1%	87.8%	24.4%	34.1%	24.4%

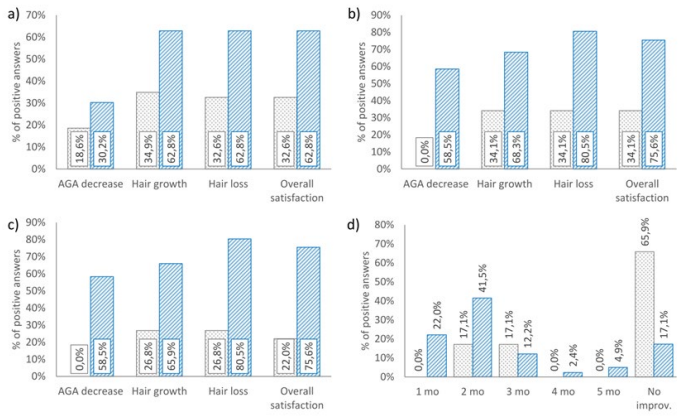

Figure 4. (a) Self-assessment questionnaire after 3 months, **(b)** Self-assessment questionnaire after 6 months, **(c)** Self-assessment questionnaire 1-month post-supplementation (7 months) and **(d)** Self-assessment questionnaire 1-month post-supplementation (7 months), question "When you started to notice the first results?".

at 30.4% reduction from baseline. In absolute terms, telogen hair density was decreased significantly by 9.6 hair/cm^2 and 14.3 hair/cm^2 from a baseline value of 45.7 ± 1.9 hair/cm^2 to 36.1 ± 1.4 hair/cm^2 at 3 mo (p<0.0001) and to 31.5 ± 1.5 hair/cm^2 (p<0.0001). The results obtained at 6 mo in the active group were maintained (-14.0 hair/cm^2) after 1 mo post-supplementation.

The variation of the percentage of hair in the anagen phase was increased significantly with Actrisave™ supplementation by 11% and 17% at 3 mo and 6 mo, respectively: increasing from 68.6% at baseline to 76.1% and 80.3% at 3 and 6 mo. In the active group, the ratio between anagen and telogen hair was positively shifted toward anagen by increasing the ratio of about 45.7% after 3 mo and 85.8% after 6 mo; the increase was maintained 1 mo post-supplementation (82%).

The increase in% of hair in anagen phase demonstrates a reversal of the reduction in% anagen hair density that typically occurs with aging and even more in AGA. There were no significant changes in total hair density, total or% anagen or telogen hair density in the placebo group. Importantly, the beneficial effects on hair parameters resulting from Actrisave™ supplementation were highly statistically significant at every time point and measured parameter compared to placebo (p<0.0001).

Global photography assessment: The number of subjects with hair growth positive scoring was higher in the active group (Table 4). The responders to the treatment were 63.4%, 95.1% and 87.8%, after 3, 6 and 7 months of product use. A low percentage of responders was seen in the placebo group.

Self-assessment questionnaire: The number of subjects scoring in a positive way the tested product was higher in the active group (Figure 4). It is worth noting that 63.5% of the men did notice the first results within the first two months of ingesting Actrisave™ (Figure 4).

Discussion and Conclusion

During this 6 mo intervention plus 1 mo follow-up clinical trial, Actrisave™ was well tolerated by all the subjects participating in the study, with a safety profile comparable to the one of placebo product.

Both the active and the placebo groups were similar at baseline, differences between the active and the placebo group were not statistically significant indicating an unbiased randomization. The outcomes were, therefore, not affected by covariates such as age, ethnicity, sex, or AGA score.

The results of the study showed that the supplementation with Actrisave™ in men with AGA was effective compared to the placebo product. The hair density, the anagen hair and the telogen hair parameters measured by phototricogram were all statistically significantly improved with a positive impact on hair growth. The improvement was supported also by a progressive and visible clinical finding as well as by the self-assessment carried out by the subjects participating in the study. Adult men usually have 80–90% of hair follicles in anagen phase, while those experiencing hair loss (AGA) tend to have a low anagen percentage [37]. With 6 mo Actrisave™ supplementation, the anagen percentage arises up to 80.3%. In AGA, the duration of anagen decreases with each cycle, while the length of telogen remains constant or is prolonged; this results in a reduction of the anagen to telogen ratio [38]. The test product demonstrated to improve the anagen/telogen ratio at each of the time point measured increasing it from 45% up to 85.8%.

Subjects in the placebo group did not show any significant improvement of their AGA condition for any parameter measured in the placebo treatment arm.

The mechanism of action besides Opuntia flowers flavonoids and the black rice anthocyanins on AGA is still under study. However, literature data highlight the anti-oxidant and the improvement of the scavenger activities and the anti-inflammatory activity of the bioactive molecules contained in Opuntia flowers (isorhamnetin and derivatives) and in black rice (anthocyanins and derivatives) together with other properties that might be important for the hair life cycle such as the improvement of microcirculation, the endothelial vascular protective effect against insult from oxidative stress, protection from DNA damage [20-22,39] could contribute to the measured efficacy. In the study carried out by Jonas A, et al., [40] has been observed that extracts from cactus flowers act as inhibitors of the 5-α-reductase enzyme. Moreover, the two ingredients of the tested blend showed a synergic activity *in vitro* if used in combination, instead to be used alone, boosting the viability of DPCs of human HFs as shown in the patent [35].

These scientific evidences taken together with the results of this clinical trial might sustain the protective activity of these ingredients on DPCs increasing their viability and keeping hairs and HFs healthier compared to the placebo product.

The intake of 250 mg of Actrisave™ improved the hair life cycle increasing hair growth, density and slowing down hair loss in men with mild-to-moderate androgenetic alopecia.

Patents

Bonina A.F., Bonina C. Compositions based on plant extracts for inhibition of the 5-alpha reductase. WO 2015/132755 Al. Sept. 11, 2015.

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Conflict of Interest

V.Z. and F.P. are Bionap srl employee. This does not alter the author's adherence to all the journal policies on sharing data and materials. The other authors declare no conflicts of interest. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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