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Efficacy and Safety of a Natural Keratin (Kera-Diet®) Hydrolysate on Hair and Nails. Randomized, Placebo-and Benchmark-Controlled Clinical Trial on Healthy Females Part 2

Vincenzo Nobile[*], EnzaCestone[1], Renaud Sergheraert[2], Francesco Tursi[1], Joel Duperray[2]

[1]*Complife Italia Srl, Via Mons.Angelini 21, 27028 San Martino Siccomario, Pavia, Italy*

[2]*BCF® Life Sciences, Boisel, 52140 Pleucadeuc, France*

[*]**Corresponding author:** Nobile V, Department of Dermatology, San Martino Siccomario, Pavia, Italy, Tel: 0039 0382 25504, Fax: 0039 0382 536006, E-mail: vincenzo.nobile@complifegroup.com

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Abstract

Telogen effluvium (TE) is one of the commonest occurrences in a trichology clinic, with patients claiming excessive hair shedding. TE is so frequent and worrying as to convey urgently the patient to the dermatologist and to extend the complaint even to social blogs on the web worldwide. In its acute (aTE) form, telogen effluvium clinical course duration does not exceed 6 months. The excessive hair shedding typical of aTE is triggered when a large number of hair in the growing phase of the hair cycle (anagen) prematurely and abruptly enter the resting phase (telogen). The duration of the interruption of the anagen hair growth is not noticed by the patient since the mitotically inactive nature of telogen. Hair shedding is noticed by patients only when hair re-enter the anagen phase and a new hair displace the telogen hair from the follicle. The interruption of mitosis, leading to aTE, may occur on account of chemotherapeutic drugs, acute febrile illness, postpartum hormonal changes, hypothyroidism, iron/zinc deficiency, seasonal variation, crash dieting, low protein intake, and malnutrition. aTE can occur in people of any age and ethnicity and is considered to be a quite common condition in either sex. Women are more likely to have lowered quality of life and restricted social contacts as compared to men as a result of hair loss even if the loss of hair becomes a matter of concern in all individuals irrespective of age and sex. Nail plate fragility is a common condition affecting up to 20% of the population, especially women over 50 years of age, characterized by roughness of the surface of the nail plate, tendency of the nail to peel and split, and fragility of the distal nail. According to causative factor, 2 forms of nail fragility can be distinguished: a primary "idiopathic or brittle nail syndrome" form (BNS) and NF secondary to different causes such as inflammatory nail disorders, infections, systemic diseases and general conditions, traumas and alteration of the nail hydration. Nails affected by BNS appear ragged, thin, and dull. The clinical features of BNS include horizontal splits within the nail plate (onychoschizia) and increased longitudinal ridging or splitting (onychorrhexis): the impairment of intercellular adhesive factors of the nail plate is expressed as onychoschizia; while the involvement of the nail matrix is expressed as onychorrhexis. The majority of subjects experiencing BNS indicate that these nail abnormalities are painful, impair daily activities, and may have a negative impact on occupational abilities. In BNS oral supplementation, trace elements and amino acids (especially cysteine) have been reported to be useful to ameliorate the nail plate condition. BNS is also associated with the presence of depressive disorders, indicating a possible impact on the quality of life of those who experience them, similarly to what occurs with hair loss perception.

Keywords: Telogeneffluvium; Brittle nail syndrome; Onychoschizia; Nail matrix; Hypothyroidism; Anagen; Hair growth; Keratin

Introduction

Hair and nails are daily exposed to challenging conditions, including lifestyle, environmental factors, and adverse medications effects. All these conditions have an impact on both the hair and nails appearance [1]. Although most of the hair and nails altered condition caused by these risk factors are not life threatening, they have a deep impact on the affected subject's wellness and its social relationship. Beyond their biological function, both hair and nails function as a visual advertisement of the subject health, social status and mood. The effects of hair loss on the subject psychology is nowadays well-documented. In 2001, Williamson and collaborators reported a decrease of the quality of the life and restricted social contacts, correlated with symptoms of depression, in subjects of both sexes affected by various forms of hair loss [2] with a more severe psychological impact among women than in men [3, 4]. The

same applies to nails. In 1966, DeJong and collaborators, in a study with 1728 patients with psoriasis reported that the the 51.8% the subjects had pain caused by the nail changes and that the most of them were restricted in their daily activities [5]. Elewski showed in 93 patients with onychomycosis that 92% reported negative psychosocial and/or physical effects and 44% had a negative self-image [6]. Acute telogen effluvium (aTE) and the brittle nails syndrome (BNS) are the commonest occurrences in a trichology/dermatology clinic. Subjects affected by aTE claim excessive hair shedding extending their compliant even to social blogs on the web worldwide [7]. aTE can occur in people of any age and ethnicity and is a quite common condition in either sex. The BNS is a common condition involving nail plate fragility. BNS affect up to 20% of the population especially women over 50 years of age. According to causative factor, two forms of nail fragility can be identified: a primary "idiopathic or brittle nail syndrome" form (BNS) and nail fragility (NF) secondary to different causes such as inflammatory nail disorders, infections, systemic diseases and general conditions, traumas and alteration of the nail hydration [8]. The clinical features of BNS include horizontal splits within the nail plate (onychoschizia) and increased longitudinal ridging or splitting (onychorrhexis): the impairment of intercellular adhesive factors of the nail plate is expressed as onychoschizia; while the involvement of the nail matrix is expressed as onychorrhexis [9].

The use of dietary supplements is increasingly reported worldwide, and scientists and health professionals agree that dietary supplements can be under certain conditions beneficial to human health. An online survey on health professionals, conducted online by Ipsos Public Affairs for the Council for Responsible Nutrition (CRN), found that the 66% of the surveyed dermatologist (n=300) recommend dietary supplements to patients [10]. Despite the relationship between the nutrition and the hair and nails has been difficult to substantiate in dermatology, there is reason to believe that a healthy diet can significantly contribute to their appearance; therefore, natural bioactive compounds administered by the oral route can represent an effective way to improve both hair and nails conditions. At this purpose, it can be interesting to consider nutrients which composition is close to the human keratin and also highly bioavailable for the organism. In this placebo- and benchmark-controlled study, 60 participants (n = 20 active group, n = 20 placebo group and n = 20 benchmark group) took 1000 mg of a natural protein hydrolysate (Kera-Diet®) twice a day for 90 days. Anagen/telogen ratio and nail growth rate, as well as hair resistance to traction and hair/nail brightness and a global clinical scoring of brittle nails, were assessed by a board-certified dermatologist. This manuscript is the part 2 of a previous manuscript in which we report the result of the study in comparison with a benchmark product [11].

Methods

This single-site, randomized, double-blind, placebo- and benchmark-controlled study enrolled 60 female subjects aged between 30 and 60 years old with ongoing aTE and BNS (not pathological condition). All the study related procedures were carried out in accordance with the Declaration of Helsinki. The study protocol and the informed consent form were approved by the "Independent Ethical Committee for Non-Pharmacological Clinical trials" during its meeting on December 12th, 2016. Before the initiation of any study related procedures, all the subjects participating in the study provided written informed consent. Subjects were also asked to sign a photo consent. The study took place at Complife Italia dermatological facilities in San Martino Siccomario (PV), Italy. Complife Italia is an independent testing laboratory for in vitro and in vivo safety and efficacy assessment of cosmetics, food supplements and medical devices.

Sample size was calculated with a two-sided 5% significance level and a power of 80% taking into account a 20% variation of the primary endpoints due to both inter-individual human variability and error in the measurement techniques. Sample size was calculated using PASS 11 statistical software (version 11.0.8 for Windows) running on Windows Server 2008 R2 Standard SP1 64-bit edition (Microsoft, USA). A sample size of 20 subjects per group was necessary given an anticipated dropout rate of 20%.

Eligible subjects were enrolled in the study by a board-certified dermatologist. According to the inclusion criteria laid down in the study protocol, the subjects were of general good health, aged between 30 and 60 years old, had no alimentary/eating disorders (i.e. bulimia, psychogenic eating disorders, etc.), and known history of metabolic syndrome. The study excluded food intolerances/allergy, pharmacological treatments known to interfere with the test product or having an effect on metabolism, participation in another similar study, unwillingness or inability to comply with the requirements of the study protocol, history for radiotherapy/chemotherapy treatments, and scalp surgery (e.g. hair transplantation), pregnancy or intention to become pregnant, lactation, The study further excluded subjects using food supplements containing active ingredients having an influence on hair loss/growth and on nail plate, oestrogen-progesterone contraception or hormonal treatment therapies within 3 months before study start, systemic treatments (e.g. retinoids, anti- mitotic, cytotoxic drugs other than antineoplastic, anti-androgens, 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, and systemic or local androgenetic alopecia treatment or product, taken or applied (Minoxidil, Aminexil, Finasteride, Dutasteride, cosmetic solution or capsules with vitamin B, zinc, caffeine) for more than 4 consecutive weeks during the last 24 weeks before the inclusion visit, and subjects having excessive and/or fluctuating hair shedding for more than 6 months. Subjects were asked to refrain from change their hair style and to cut their nails 7 days before and after each checkpoint. Hair dyeing was not allowed during all the study period

All subjects were given a three-month supply of the test product (Kera-Diet®, BCF® Life Sciences, Boisel, 56140Pleucadeuc, France). Subjects were instructed to ingest 4 four capsules per day (1000 mg/day), two at breakfast and two at dinner, for a total period of 90 days. After the basal screening visit subjects were randomized to receive the test product (KE), the placebo product (PL) or the benchmark (BE) product (Table 1). A restricted randomization list was created using PASS 11 (version 11.0.8; PASS, LLC. Kaysville, UT, USA) statistical software running on Windows Server 2008 R2 Standard SP1 64-bit Edition (Microsoft, USA) by a biostatistician and stored in a safe place. Randomization sequence was stratified using biased coin Efron's algorithm with a 1:1:1 allocation ratio. The allocation sequence was concealed from the in-site study director in sequentially numbered, opaque, and sealed envelopes, reporting the unblinded treatment allocation (based on subject entry number in the study). The A4 sheet reporting the unblinded treatment was folded to make the envelope impermeable to intense light. After acceptance of the subject in the study the appropriate numbered envelope was opened. An independent technician dispensed either active or placebo products according to the card inside the envelope. The study adhered to established procedures to maintain separation between the investigator and its collaborators and the staff that delivered the intervention. Investigator and its collaborators who obtained outcome measurements were not informed on the product group assignment. Staff who delivered the intervention did not take outcome measurements. Subjects, investigator and collaborators were kept masked to products assignment. The active and the placebo products were in capsule form and identical in appearance. They were prepacked in blisters and consecutively numbered for each subject according to therandomization schedule. Each subject was assigned an order number and received the capsules in the corresponding prepacked blister.

Primary endpoints were the measurement of anagen/telogen hair and the nail growth speed. Hair resistance to traction (pull testing), hair/nail brightness, and overall hair/nail condition, were secondary efficacy endpoints. The study flow and the schedule of assessments chart is reported in Figure 2.

Hair loss related parameters were investigated by both phototricogram and pull testing. For phototricogram a target area in the mid vertex was clipped evenly using a hair trimmer (Moser, TrichoScan Edition) and short clipped hair were removed by pressing an adhesive strip onto the shaved area. Three days (72 ± 2 hours) after hair clipping, hair was died using a commercially available hair dye (Goldwelltopchic, black 2N, Darmstadt, Germany with Rondo 6% CrèmeOxyd, Coiffeur, Cologne, Germany). After 15 minutes the colored area was thoroughly cleaned with an alcoholic solution (Kodan® Spray, Schülke&Mayr, Vienna, Austria) and digital images were taken using a DermoGenius

camera (DermoScan GmbH, D-93055 Regensburg, Germany). Pictures were then analyzed by TrichoScan® analysis (software version 2.3). Subject repositioning and standardization of close-up picture were achieved using a homemade repositioning device instead of tattoo landmarking. For pull testing, the dermatologist gently wrapped the thumb and index finger around approximately 20-60 hair and pulled, gently but firmly, upwards. Pull testing was repeated in three different scalp areas (frontal, temporal, and occipital region). If more than three hair per each area (or more than then hair over the three areas) were removed, the pull test was considered as positive and suggestive of telogen effluvium.

Digital photographic pictures of the hair (the vertex and frontal level) and the nail plate were taken under standard lighting conditions using a professional digital reflex camera NIKON D300/D600 digital (NitalS.p.A., 10024 Moncalieri, To, Italy) camera equipped with a macro-objective (AF-S Micro NIKKOR 60mm f/2.8G ED), an independent flash system (Kit R1C1) and with cross- and parallel-polarized filters. Hair pictures were scored using a standardized seven-point rating scale (+3 greatly increased; +2 moderately increased; +1 slightly increased; 0 no change; -1 slightly decreased; -2 moderately decreased; -3 greatly decreased) (16). This technique had been demonstrated to have excellent reproducibility (17). Nails plate picture were scored using a 4-point rating scale (1 no effect; 2 mild effect; 3 moderate effect; 4 strong effect). Nail plate growth was measured, using a morphometric image analysis technique, as the difference between the total nail length after cutting and the total nail length after 14 days from cutting.

Hair and nails brightness were measured using a spectrophotometer/colorimeter CM-700D (Konica- Minolta, 20092 Cinisello Balsamo, MI, Italy). The measured parameter was the 8° gloss (specularly reflected light).

Subjects were asked to reply to the questions of a self-assessment questionnaire.

Statistical analysis was performed using NCSS 10 (version 10.0.12 for Windows; NCCS, LLC. Kaysville, UT, USA) running on Windows Server 2008 R2 Standard SP1 64 bit edition (Microsoft, USA). Data normality was checked using Shapiro-Wilk W normality test and data shape. Intragroup (vs. baseline) statistical analysis was carried out using repeated measures analysis of variance (RM- ANOVA) followed by Tukey-Kramer post-test. Intergroup (between treatments) statistical analysis was carried out using RM-ANOVA followed by tests for two-factor interactions. A p-value <0.05 was considered statistically significant. Statistical analysis output was reported as follows: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Results

The study was conducted between February and July 2017. A total of 60 female subjects were successfully randomized (Figure 3). The population was Caucasian. Demographic and baseline characteristics (Table 2) were similar across treatment arms, indicating an unbiased randomization and the absence of covariates. Subjects attended clinic visits at the time of randomization (baseline) and after 45 and 90 days of product use. Data analysis was intention-to-treat and involved all subjects who were randomly assigned. Subjects' compliance to treatment was assessed by means of product accountability, as follows: at each visit, the expected amount of consumed capsule was compared with the amount dispensed minus the amount the subject returned. No major deviations were observed in the treatment regimen. All subjects were included in the safety analysis data set. All the tested products were well tolerated. No adverse reactions occurred during the study period. A statistically significant increase of hair density was observed both in the KD and BE treatment groups (Table 3a). The hair density was increased in the KD treatment group by 2.1 ± 0.7 and by13.3 ± 1.7, after 45 and 90 days, respectively ($p < 0.001$). A similar efficacy profile was seen for the BE treatment group where hair density was increased by 2.2 ± 0.8 and by 9.6 ± 1.3, after 45 and 90 days, respectively ($p < 0.001$). The variation of hair density was not statistically significant in the placebo group ($p > 0.05$). Both KD and BE hair density variation was statistically significant when compared to the placebo group ($p < 0.05$).

A statistically significant increase of anagen hair was observed both in the KD and BE treatment groups (Table 3b). The percentage anagen hair was increased in the KD treatment group by 3.6 ±0.6 and by 9.1 ± 1.0, after 45 and 90 days, respectively ($p < 0.001$). A similar efficacy profile was seen for the BE treatment group where the percentage of anagen hair was increased by 3.9 ± 0.4% and by 8.6 ± 0.9 after 45 and 90 days, respectively ($p < 0.001$). The variation of percentage of anagen hair was not statistically significant in the placebo group ($p > 0.05$). Both KD and BE variation in the% anagen hair was statistically significant when compared to the placebo group ($p < 0.05$). Specular, but opposite, results were obtained for the percentage of telogen hair variation (Table 3c).

A statistically significant decrease of the number of pulled hair was observed both in the KD and BE treatment groups (Table 3d). The number of pulled hair was decreased in the KD treatment group by26.5 ± 3.7% and by 32.7 ± 3.6%, after 45 and 90 days, respectively ($p < 0.001$). A similar efficacy profile was seen for the BE treatment group where the number of pulled hair was decreased by 22.9± 2.2% and by 33.5 ± 2.7% after 45 and 90 days, respectively ($p < 0.001$). The variation of the number of pulled hair (-10.6 ± 2.6%) was statistically significant in the placebo group after 90 days. Both KD and BE variation in the number of puller hair was statistically significant when compared to the placebo group ($p < 0.05$). Interestingly, both for KD and BE the number of pulled hair after 45 days is not suggestive of telogen effluvium diagnosis.

Figure 4 shows the macroscopic effect of the product in decreasing hair loss. Hair volume and nail conditions were improved both in the KD and BE treatment groups. The subjects showing an improvement of hair volume in the KD treatment group were 40% and 65%, after 45 and 90 days. A similar efficacy profile was seen for the BE treatment group where the subjects improved were 30% and 55%, after 45 and 90 days. The improvement was statistically significant for both the KD and BE treatment groups when compared to the placebo group (5% and 10% of the subjects, after 45 and 90 days). The subjects showing an improvement of nail conditions in the KD treatment group were 30% and 60%, after 45 and 90 days. A similar efficacy profile was seen for the BE treatment group where the subjects improved were 30% and 55%, after 45 and 90 days. The improvement was statistically significant for both the KD and BE treatment groups when compared to the placebo group (15% and 20% of the subjects, after 45 and 90 days).

A statistically significant increase of both hair and nail brightness (Table 4) was observed both in the KD and BE treatment groups. Hair brightness in the KD treatment group was improved by 23.8% and 57.0%, after 45 and 90 days. A similar efficacy profile was seen for the BE treatment group where hair brightness was improved by 13.7% and 41.9%, after 45 and 90 days. Both KD and BE hair brightness variation was statistically significant when compared to the placebo group ($p < 0.05$). Nail brightness in the KD treatment group was improved by 33.1% and 35.7%, after 45 and 90 days. A similar efficacy profile was seen for the BE treatment group where nail brightness was improved by 16.1% and 38.7%, after 45 and 90 days. Both KD and BE nail brightness variation was statistically significant when compared to the placebo group ($p < 0.05$).

A statistically significant increase of nail growth rate was observed both in the KD and BE treatment groups (Figure 5). The nail growth rate in the KD treatment group was 0.07 ± 0.01 mm/14days and 0.14± 0.01 mm/14days, after 45 and 90 days, respectively ($p < 0.001$). A similar efficacy profile was seen for the BE treatment group where nail growth rate was 0.04 ± 0.00 mm/14days and 0.07 ± 0.01 mm/14days, after 45 and 90 days, respectively ($p < 0.001$). The nail growth rate was not statistically significant in the placebo group ($p > 0.05$). Both KD and BE nail growth rate was statistically significant when compared to the placebo group ($p < 0.05$). Moreover, KD group nail growth rate was statistically higher than those of BE group ($P<0.05$).

The complete results of the self-assessment questionnaire are reported in figure 6. Both KD and BE were perceived more effective than PL. Subjects' answers after 90 days product use are very positive with 75% global satisfaction for both KD and BE group for hair and nails. On the other hand, placebo effect is only 35 %. This underlines that all improvement of objectives criteria is enough visible to be perceived by women of KD and BE groups.

Discussion and Conclusion

Hair and nails are two specialized keratinous skin appendages growing at fixed rate and with complete replacement over time. The growth of

both hair and nails relies on the metabolic activity of the hair follicle and the nail matrix, respectively. It is then easy to understand why, virtually, every nutritional deficiency can affect the growth of the nail in some manner.

The connection between nutrition and skin (and its appendages) conditions is a common interesting research field for both scientists and humans from ancient time to nowadays. It is nowadays clear that the nutrients of diet have a direct impact on the structure and growth of both hair and nails. To confirm that, in recent years the use of food supplements has increased both in Europe and in the USA with many physicians recommending them (12,13).

In our study we demonstrated the role of a food supplement containing a natural extensively hydrolysed keratin in improving both hair and nails conditions after a 3-months intake period.

An improvement of hair conditions was seen after 90 days of treatment. This was demonstrated by the decrease of hair shedding and telogen hair. The active group showed a significant decrease of telogen hair after 45- and 90-days treatment. The percentage of hair in the telogen phase was decreased, resulting in a decrease of the number of hair loss on pull testing. Interestingly the diagnosis of aTE by pull testing was negative after 45 days treatment. Also, the hair fiber showed a positive improvement, demonstrated by the increase of its ability to reflect the light (radiance). This phenomenon relies on a healthy hair cuticle structure.

Nails also improved their conditions after 45 and 90 days of treatment. After 90 days product use the 60% of the subjects showed an improvement of nails condition. This was also demonstrated by the increase of the nail growth rate by 0.14 mm/14 days.

The active product was also scored positively by most of the subjects participating in the study. The subjects at the end of the study perceived their hair and nails conditions as improved.

The efficacy of KD was comparable to the efficacy of the benchmark product.

The putative mechanism of action of the Kera-Diet® in improving hair and nails conditions can be attributed to its association with traced elements and specific vitamins and also to the bioavailability of amino acids from Kera-Diet® which is compounded of more than 92 % of free amino acids.

Therefore, and more generally, this study demonstrates that Kera-Diet® associated with traced elements and specific vitamins at the right dosage can enhance hair and nails conditions, even though human nutrition is more and more balanced. The study, furthermore, demonstrates the role of nutrients in both aTE and BNS.

	KD 250 mg	BE 250 mg	PL
	Kera-Diet®	benchmark product	Placebo
Ingredients			
Maltodextrin	250	250	524.3
Kera-Diet®	250		---
Other keratin (benchmark ingredient)	---	250	---
Magnesium stearate	14	14	14
Zinc sulphateheptahydrate (22% Zn)	11.36	11.36	---
Silice	5	5	5
Vitamin B3 (nicotinamide)	4.5	4.5	---
Vitamin B5 (D-Calcium pantothenate)	3.72	3.72	---
Dry extract of aerial part of *Equisetum Arvense*	2.5	2.5	---
Copper sulphatepentahydrate	1.48	1.48	---
Vitamin B6 (pyridoxine hydrochloride)	0.6326	0.6326	---
Vitamin B8 (biotin)	0.15	0.15	---

Table 1: Capsules composition. Quantities are reported in mg.

	KD	BE	PL
Sex			
Female	100%	100%	100%

Phototricogram			
% telogen	21.3±0.6	20.7±0.7	21.0±0.8
% anagen	78.7±0.6	79.3±0.7	79.0±0.8
Hair density (hair no/cm^2)	192.7±6.7	195.7±7.1	193.2±7.4
Pull test	12.3±0.3	12.6±0.4	12.3±0.3
Hair radiance	3.36±0.41	3.31±0.38	3.33±0.35
Nail growth rate (mm/14days)	1.24±0.07	1.28±0.06	1.26±0.06
Nail brightness	7.32±0.72	7.41±0.50	7.28±0.51

Table 2: Demographic and baseline characteristics. Data are means ± SE. KD Kera-Diet®, BE Benchmark, PL Placebo.

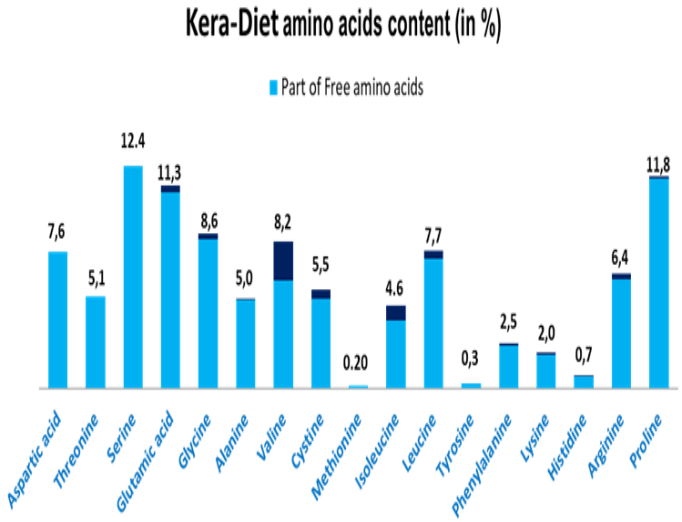


Figure 1: Kera-Diet®aminoacidic composition. Kera-Diet® is a hydrolysate of natural keratin 250 mg (Kera-Diet®), obtained from a non-human source (feathers), having an amino acid profile similar to the hair and containing a high level of free amino acids (>92%).

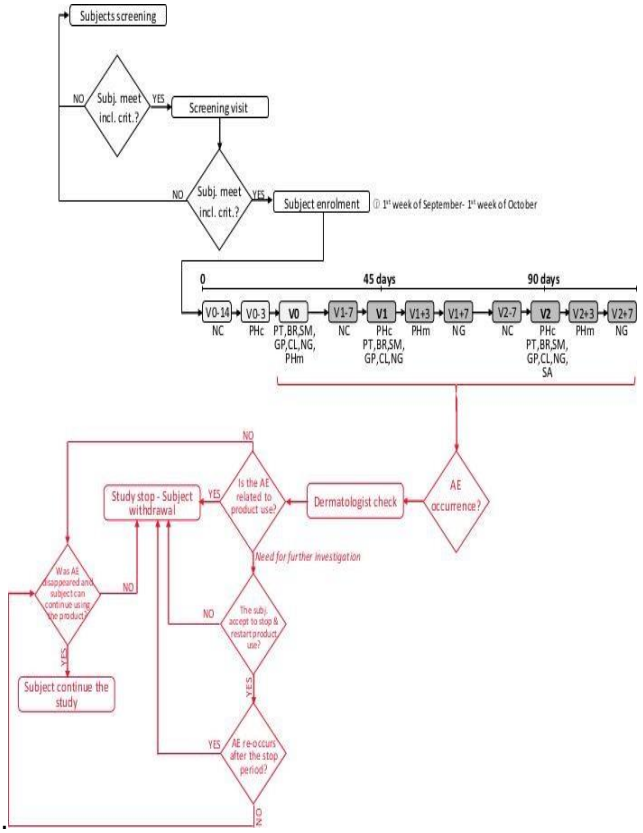


Figure 2: Study flow and schedule of assessment chart. Legend. NC nail cut, PHcPhotricogram hair cutting, PT pull testing, BR Brightness measurement, GP Global photography, CL Global photography scoring, NG Nail growth rate, PHmPhotricogram hair measurement, SA Self- assessment questionnaire.

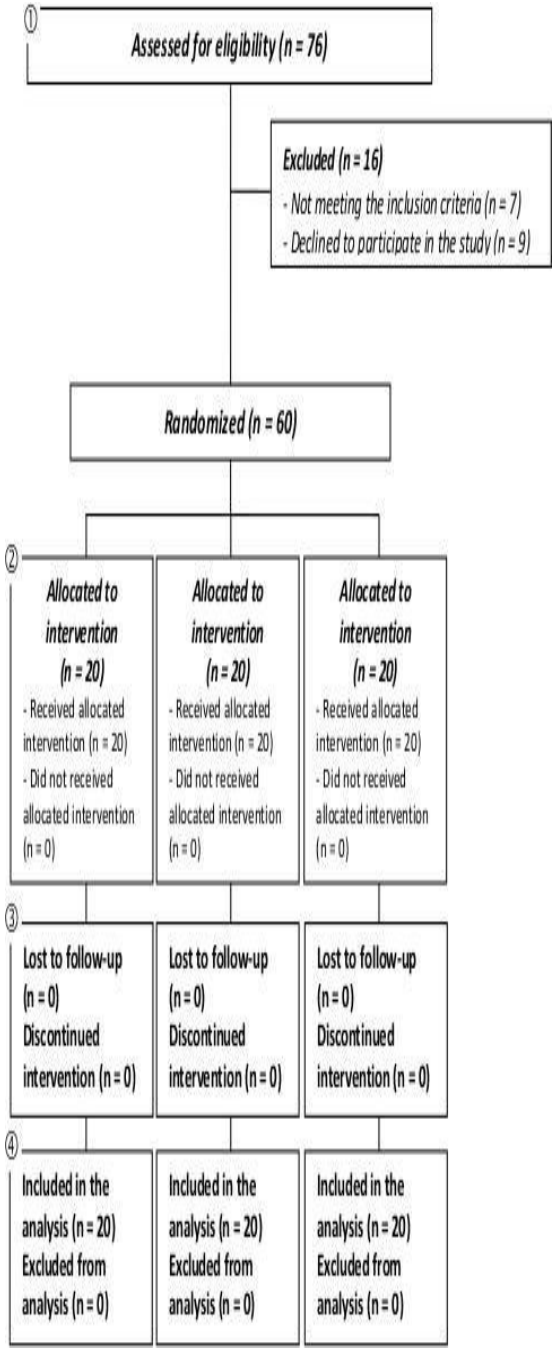


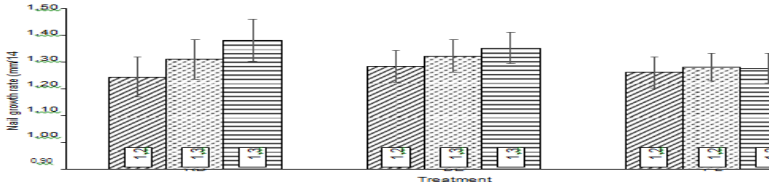
Figure3:CONSORT2010flowdiagram.Legend.
① Enrolment, ② Allocation, ③ Follow-up, ④

a) Hair density (number/cm2)				Mean ± SE			Min ÷ Max	
		n	KD	BE	PL	KD	BE	PL
Day 0		20	192.7±6.7a	195.7±7.1a	193.2±7.4a	155.0÷250.6	152.7÷252.3	138.3÷2
Day 45		20	194.7±6.7b	197.9±7.1b	194.0±7.1a	160.2÷251.2	160.1÷258.5	142.2÷2
Day 90		20	205.9±6.4c	205.4±6.7c	195.0±7.2a	172.3÷266.3	165.2÷268.3	145.7÷2
D45-D0		20	2.1±0.7†	2.2±0.8†	0.8±0.8	-3.2÷7.2	-6.2÷12.9	-5.5÷5.9
D90-D0		20	13.3±1.7†	9.6±1.3†	1.8±0.9	-6.8÷21.2	-3.4÷40.2	-2.8÷10
b) Anagen (%)	hair			Mean ± SE			Min ÷ Max	
		n	KD	BE	PL	KD	BE	PL
Day 0		20	78.7±0.6a	79.3±0.7a	79.0±0.8a	72.8÷82.5	71.1÷84.5	71.6÷83
Day 45		20	82.3±0.7b	83.2±0.7b	78.6±0.7a	74.9÷89.8	77.9÷88.8	71.1÷84
Day 90		20	87.7±0.6c	88.0±0.6				
D45-D0		20	3.6±0.6†	3.9±0.4†				
D90-D0		20	9.1±1.0†	8.6±0.9†				
c) Telogen hair Mean ± SE								
(%) n KD BE PL								
Day 0		20	21.3±0.6c	20.7±0.7				
Day 45		20	17.8±0.7b	16.8±0.7				
Day 90		20	12.3±0.6c	12.0±0.6				
D45-D0		20	-3.6±0.6	-3.9±0.4				
D90-D0		20	-9.1±1.0†	-8.6±0.9				
Pulled hair (hair no.)				Mean ± S				
		n	KD	BE				
Day 0		20	12.3±0.3c	12.6±0.4				
Day 45		20	8.9±0.4b	9.7±0.5b				
Day 90		20	8.1±0.4a	8.3±0.4a				
D45-D0		20	-3.4±0.6†	-2.9±0.3				
D90-D0		20	-4.2±0.6†	-4.3±0.4				

Table 3: Phototricogram and pull testing results. a) Hair density. b) Anagen hair. c) Telogen hair. d) Pull test results. Significantly different from D0: a<b<c, $p < 0.05$. RM-ANOVA followed by Tukey- Kramer post-test. † Significantly different ($p < 0.05$) from Placebo. RM-ANOVA followed by tests for two-factor interactions.

Figure 4: Global photography assessment a) KD group b) BE group c) PL group.

Table 4: Hair and nail brightness. Significantly different from D0: a<b<c, p < 0.05. RM-ANOVA followed by Tukey-Kramer post-test. † Significantly different (p < 0.05) from Placebo. RM-ANOVA followed by tests for two-factor interactions.



Growth rate (mm/14dd)			Mean ± SE	
	n	KD	BE	PL
Day 0	20	1.24±0.07a	1.28±0.06a	1.26±0.0
Day 45	20	1.31±0.07b	1.32±0.06b	1.28±0.0
Day 90	20	1.38±0.08c	1.35±0.06c	1.28±0.0
D45-D0	20	+0.07†	+0.04†	0.02
D90-D0	20	+0.14†∫	+0.07†	0.01

Figure 5: Nail growth rate. Significantly different from D0: a < b < c, p < 0.05. RM-ANOVA followed by Tukey-Kramer post-test. † Significantly different (p < 0.05) from Placebo. ∫ Significantly different (p< 0.05) from Benchmark. RM-ANOVA followed by tests for two-factor interactions. Data aremeans ± SE.

Hair brightness			Mean ± SE			Min ÷ Max		
(au)	n	KD	BE	PL	KD	BE	PL	
Day 0	20	3.36±0.41a	3.31±0.38a	3.33±0.35a	0.64÷6.57	1.45÷8.17	1.04÷6.71	
Day 45	20	3.98±0.45b	3.74±0.42a	3.28±0.36a	1.16÷7.44	1.36÷8.98	1.08÷6.33	
Day 90	20	4.99±0.53c	4.68±0.55b	3.53±0.39a	1.20÷8.94	1.91÷10.7	1.17÷7.63	
D45-D0	20	+23.8%†	+13.7%†	-1.90%	-12.2%÷81.3%	-8.1%÷47.7%	-24.6%÷18.3%	
D90-D0	20	+57.0%†	+41.9%†	6.40%	-6.5%÷106.3%	-1.1%÷88.7%	-22.0%÷36.5%	
Nail brightness			Mean ± SE			Min ÷ Max		
(au)	n	KD	BE	PL	KD	BE	PL	
Day 0	20	7.32±0.72a	7.41±0.50a	7.28±0.51a	2.17÷12.47	3.32÷10.84	4.82÷12.66	
Day 45	20	9.57±0.93b	8.63±0.70b	7.76±0.59a	3.10÷17.10	4.02÷16.61	5.00÷13.45	
Day 90	20	9.57±0.86b	10.07±0.65c	8.45±0.69b	3.44÷15.15	4.51÷16.05	5.11÷14.85	
D45-D0	20	+33.1%†	+16.1%†	6.70%	1.0%÷83.9%	2.1%÷84.0%	-16.1%÷33.9%	
D90-D0	20	+35.7%†	+38.7%†	17.20%	9.8%÷70.5%	5.9%÷88.1%	-24.1%÷66.1%	

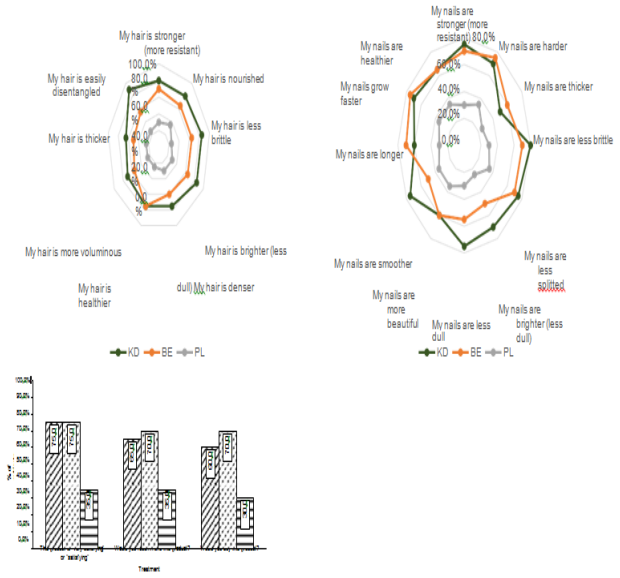


Figure 6: Self-assessment questionnaire. a) Hair self-assessment questionnaire. b) Hair self-assessment questionnaire. c) Overall Self-assessment.

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Conflict of interest

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