Active Beauty
Darkenyl™
Advanced hair pigmentation recovery

Crafted by white and green technologies

Givaudan

engage your senses

Focus on the product

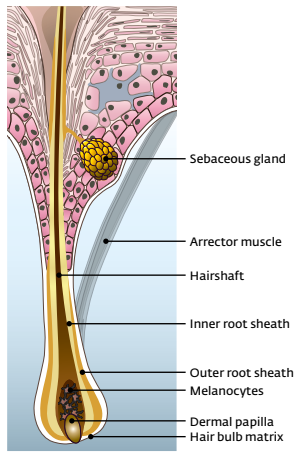
Hair greying in our society

The loss of pigmentation in the hair shaft (hair greying or *canitie*) is one of the most obvious signs of ageing, and one of the main beauty concerns of ageing. Indeed, between 45 and 65 years old, 74% of the worldwide population is affected by greying hair[1]. This loss of pigmentation progressively appears between 35 and 45, depending on ethnicity[2].

Structure of hair and pigmentation mechanism

A human hair shaft is formed by three different layers: the **cuticle** (external shell), **the cortex** (internal part, containing melanin granules, responsible for hair colour), and the **medulla** (soft core only present in mature white hair). Melanin production in the hair is controlled by melanocytes located in the hair bulb matrix[3]. Their activity is regulated by the normal hair cycle:

- During the anagen (growing) phase, the melanin produced by active melanocytes is transferred into cortical keratinocytes resulting in pigmentation of the entire hair shaft.
- During the catagen phase, the melanocytes will enter in apoptosis and disappear during telogen phase[4].
- In order to produce a pigmented hair during the new anagen phase a new pool of melanocytes will migrate and differenciate from the hair follicle stem cells reservoir (ORSc) to the hair bulb to naturally pigment the new hair[5].



Loss of pigmentation main causes: ageing and oxidative stress

The hair greying is explained by age related functional changes in the stimulation and migration of the stem cells from the bulge, but also by environmental factors. Indeed, **the accumulation of reactive oxygen species (ROS)** into melanocytes upon ageing will lead to mutations, decrease of antioxidant protection system, inflammation, hair falling and greying[6] through two main actions:

- Decrease of the melanogenesis (lower melanin production by melanocytes).
- Decrease of the melanocytes number (less melanocytes to produce melanin).

Three types of hair can thus be identified when pigmentation loss starts to occur: coloured hair (high content in melanin), grey hair (low melanocytes number and diluted content of melanin), and white hair (lacking melanocytes).

Darkenyl™: acting on stem cells to repigment the hair shaft

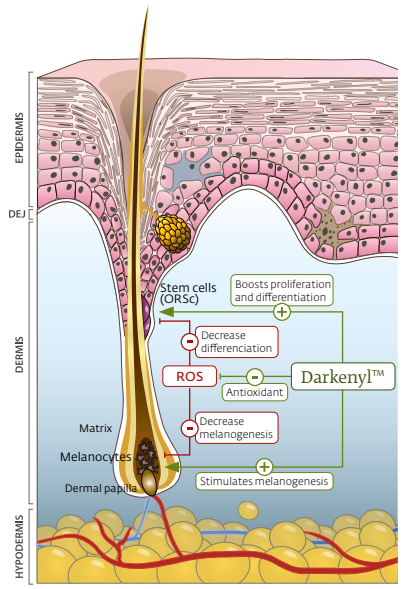
Darkenyl™ is a smart combination of **taxifolin glucoside** (a stabilised antioxidant, acting as a stimulator of stem cells proliferation and maintenance) and **N-acetyl-tyrosine** (a precursor of melanin).

Darkenyl™ fights against white hair through the following mechanism:

- Protects hair follicles from oxidation (ROS).
- Stimulates hair follicle stem cells to create new melanocytes.
- Activates the melanogenesis to increase melanin production.

This mode of action is completely independent from gender, hair type or hair colour, making Darkenyl™ an ideal solution to universally target hair greying.

In 4 months, Darkenyl™ significantly reduces the proportion and density of white hair (down to 50%).



[1] S. Panhard, and al. - Br J Dermatol. 2012 Oct;167(4):865-73
[2] Kaplan PD and al. - Int J Cosmet Sci. 2011 Apr;33(2):171-82. doi: 10.1111/j.1468-2494.2010.00614.x. Epub 2011 Feb 21.
[3] Tobin DJ, and al. - J Investig Dermatol Symp Proc. 1999 Dec;4(3):323-32.
[4] Tobin DJ - J Investig Dermatol Symp Proc. December 1998 Volume 111, Issue 6, Pages 941–947
[5] Bashkatov AN and al. - Quantum Electronics 36 (12) 1111 ë 1118 (2006)
[6] Seiberg M. – int J Cosm Sc, 2013, 35, 532-538

Biological activity

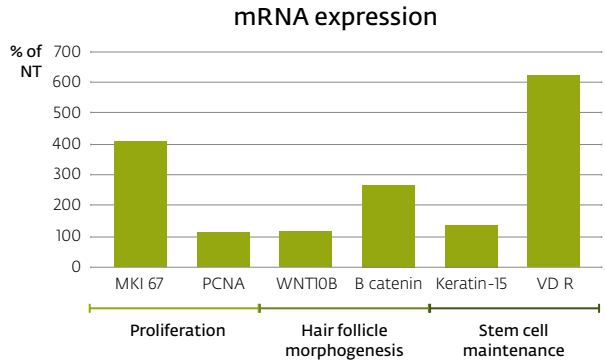
Stimulation of hair follicle stem cells (*in vitro*)

1. Stimulation of stem cells related genes

Hair follicle stem cells (ORSc) were treated with 50µM of taxifolin glucoside (one of the main component of Darkenyl™ - 50µM taxifolin glucoside=1% Darkenyl™).

The expressions of proliferation genes, morphogenesis genes and stem cell maintenance genes were evaluated by RT-qPCR.

Results: Taxifolin glucoside significantly **increases the expression of genes involved in proliferation of stem cells, hair follicle morphogenesis and maintenance of hair follicle stem cells' phenotype**.

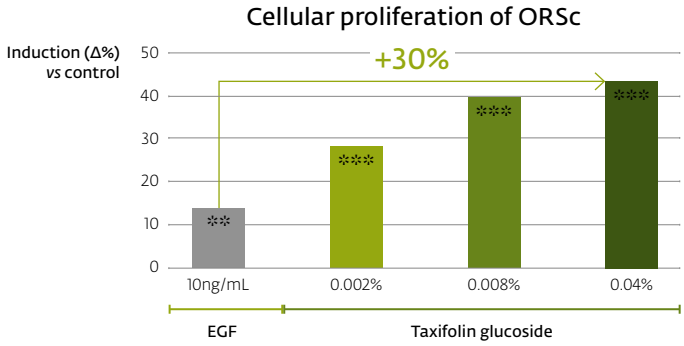


2. Stimulation of hair follicle stem cells

Hair follicle stem cells (ORSc) were treated with increasing concentration of Taxifolin glucoside (0.04% taxifolin glucoside=1% Darkenyl™).

The proliferation of ORSc was evaluated by measuring the incorporation of Bromodeoxyuridine (BrdU) into DNA of proliferating cells.

Results: Taxifolin glucoside significantly i**ncreases the proliferation of the hair follicle stem cells, up to +30%** *versus* positive control.

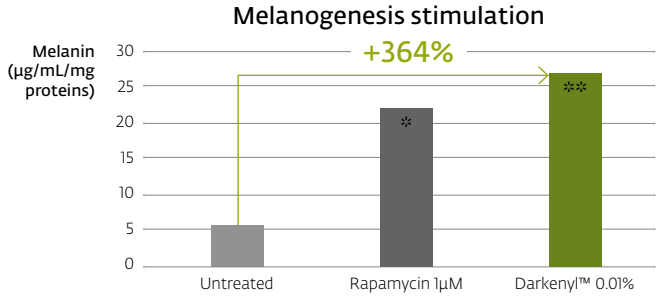


** p<0.01 Student's t-test
*** p<0.001 Student's t-test

Stimulation of melanogenesis (*in vitro*)

To mimic the natural interaction of keratinocytes and melanocytes in the hair matrix, a co-culture of normal human keratinocytes (NHK) and normal human melanocytes (NHM) were treated with Darkenyl™ at 0.01% or Rapamycin (positive control), or left untreated for 72 hours.

The melanin content in the cell supernatant was evaluated by colorimetric assay (405nm).



* p<0.05 Student's t-test ** p<0.01 Student's t-test

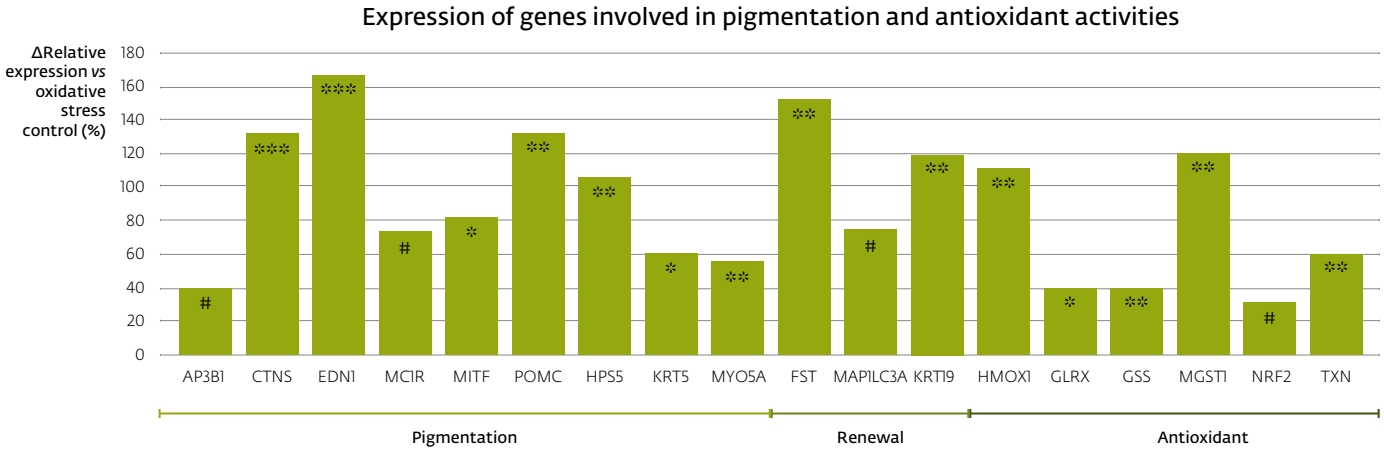
Results: Darkenyl™ significantly **increases by +364% the melanogenesis**.

Biological activity

Activation of hair follicles defences and pigmentation (*ex vivo*)

1. Antioxidant and pigmentation related genes activation

The study was conducted on face lift skin explants from 3 donors (average age 63). A mix of 9 mg of hypoxanthin + 10 units of xantin oxidase have been applied repeatedly during 1 hour in order to mimic the oxidative stress occurring in ageing hair follicle for 3 days. Then a treatment was done for 48 hours with 1% of Darkenyl™. The expression of genes involved in hair pigmentation, hair renewal and antioxidant defences were measured by RT-qPCR.

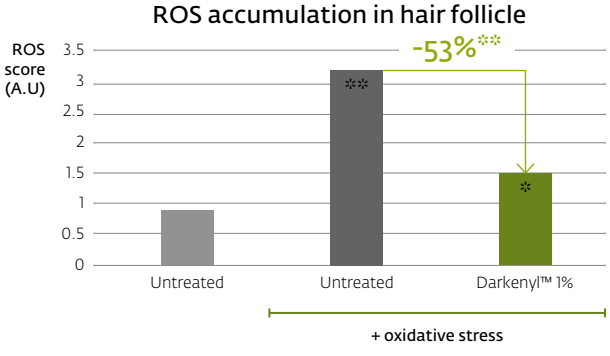


# p<0.1 ANOVA Fisher * p<0.05 ANOVA Fisher ** p<0.01 ANOVA Fisher *** p<0.001 ANOVA Fisher

Results: Under oxidative stress, Darkenyl™ significantly **stimulates the expression of genes involved in antioxidant defences**, renewal of the hair as well as pigmentation of the hair follicle.

2. Reduction of ROS production under oxidative stress

Human hair follicles from a female donor (56 years old) were treated with 1% of Darkenyl™ or left untreated. The hair follicles are then exposed to an oxidative stimulus (cumene hydroperoxide - 50µM) for 1 hour. The ROS accumulation into the hair follicle was evaluated using a green fluorescent probe.



* p<0.05 Student's t-test ** p<0.01 Student's t-test

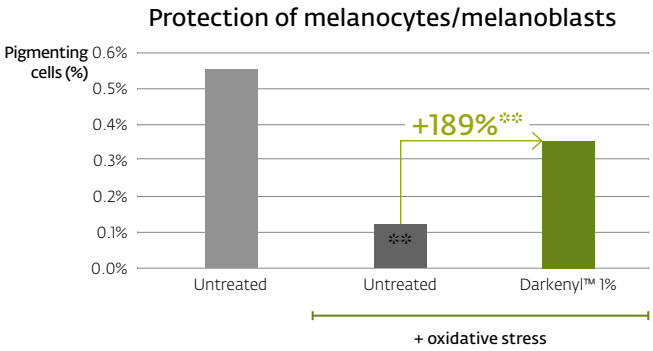
Results: Under oxidative stress, Darkenyl™ significantly **decreases by -53% the ROS accumulation** into the hair follicle.

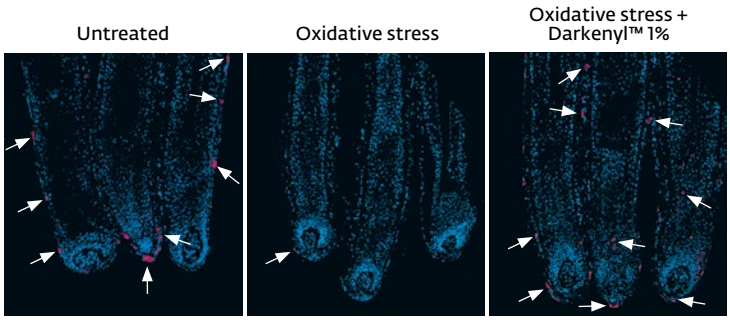
Biological activity

Protection of melanocytes against oxidative stress (*ex vivo*)

Human hair follicles from a female donor (56 years old) were exposed to an oxidative stimulus (cumene hydroperoxide - 50µM) for 1 hour. The hair follicles were then treated with 1% of Darkenyl™ or left untreated.

The pigmenting cells (melanocytes and melanoblasts) quantification was obtained following NKI/beteb-DAPI double immunostaining (in red and blue respectively on the pictures below).



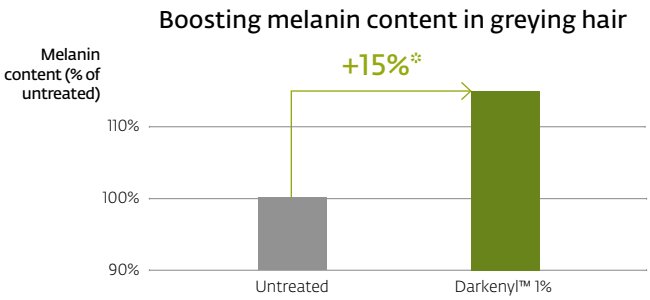


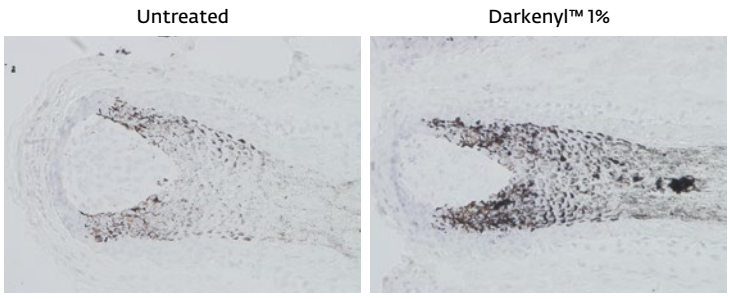
** p<0.01 Student's t-test

Results: Under oxidative stress, Darkenyl™ significantly **protects the melanocytes and melanoblasts by +189%** into the hair follicle.

Stimulation of melanin production in greying hair (*ex vivo*)

Human hair follicles in anagen phase (growing) from 2 donors (35 and 53 years old) were treated with 1% of Darkenyl™ for 72 hours or left untreated. The melanin content into the hair follicles was evaluated using melanin quantification with Fontana Masson staining (black on the pictures below) and image analysis.





* p<0.05 Student's t-test

Results: Darkenyl™ significantly **increases by +15% the production of melanin** into the greying hair follicles in 3 days.

Efficacy

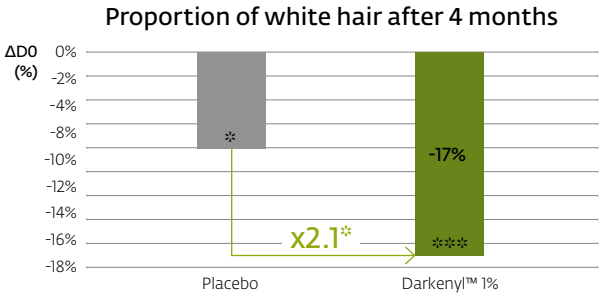
Darkenyl™ reduces white hair in 4 months (clinical test)

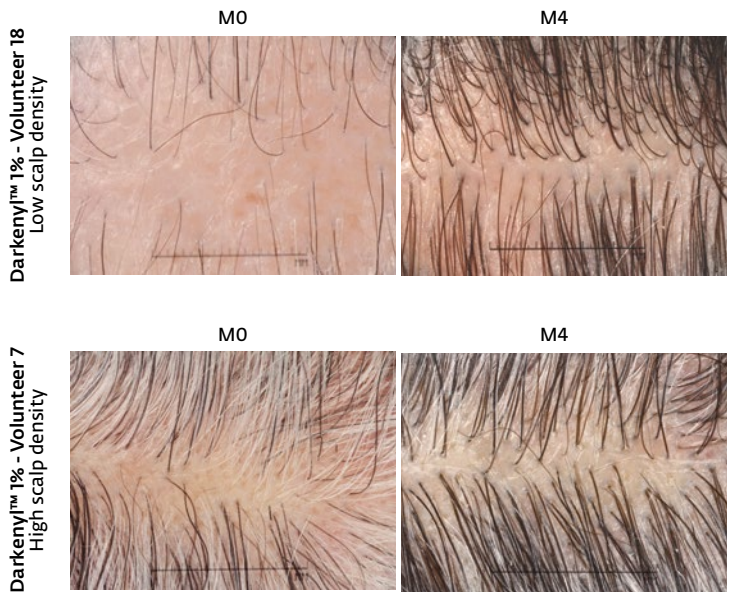
The efficacy of Darkenyl™ at 1% was evaluated in a double blind test versus placebo. Forty four Caucasian male volunteers (18 year old and more) with white hair were involved in the clinical evaluation. The panel was split into two groups of twenty two volunteers. One group was testing the placebo product the second group was testing the hair lotion containing 1% of Darkenyl™. The treatment was applied in leave-on by massage on the scalp, once a day for four months.

1. Reduction of the white hair proportion

Pictures of the scalp were taken using a NIKON D7100 in combination with the Canfield Epiflash® system, on the first day of the test and after four months of daily application of the product.

The hair parting area was defined according to the white hair localisation. A blind scoring was performed to evaluate the proportion of white hair in the picture *versus* total number of hairs.





* p<0.05 Mixed ANOVA *** p<0.001 Mixed ANOVA

Results: After four months of treatment, Darkenyl™ significantly **decreases the proportion of white hair by an average of -17%** *versus* D0 (from 59% of white hair at D0 down to 49%), **more than 2 times more efficiently than the placebo**.

The proportion of white hair is visibly reduced, with a reduction **down to -56%** for the best respondent (from 90% of white hair at D0 down to only 40% after 4 months).

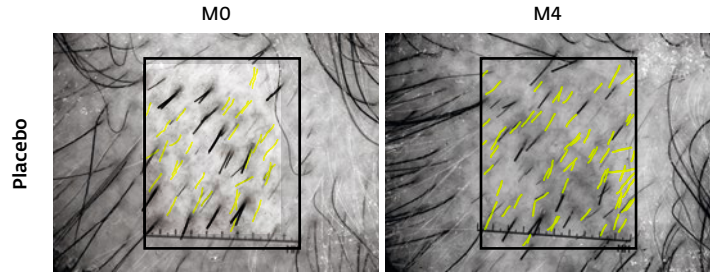
No white roots effect was observed during hair growth.

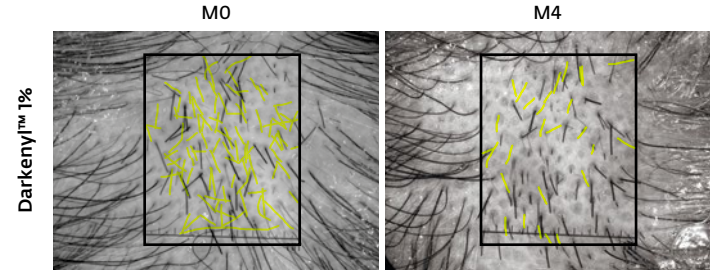
Efficacy

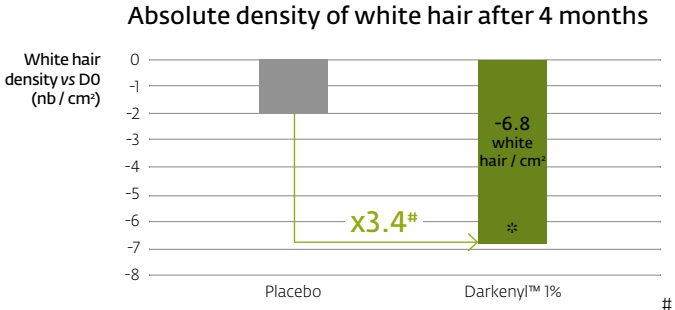
2. Reduction of the white hair density per cm²

To evaluate the efficacy of Darkenyl™ independently from the sclap density, an absolute quantification of the white hair number was also performed.

At the beginning of the test, an area of 1 cm² was shaved on every panellists. Two days after shaving, a picture was taken of the area using a NIKON D7100 in combination with the Canfield Epiflash® system. The density of white hair (number/cm²) was evaluated with a specific Photoshop® tool, on a 0.7 cm² test area (1 x 0.7 cm) defined on the image. All white hair whose root was in the analysis zone were counted.







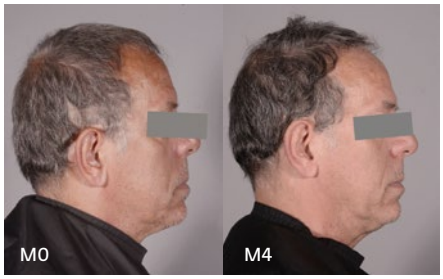
# p<0.1 Mixed ANOVA * p<0.05 Mixed ANOVA

Results: After four months of treatment, Darkenyl™ significantly **decreases the number of white hair per cm² by -6.8, more than 3 times more efficiently than the placebo**. The density of white hair is visibly reduced, with **-55.7 white hair per cm²** for the best respondent after four months, equivalent to a reduction of -33,420 white hair (the average scalp surface being 600cm²).

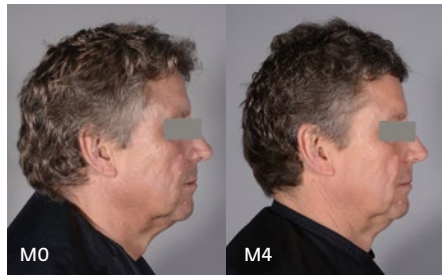
3. Illustrative macroscopic clinical results

Criteria	Volunteer #18 (51 years old)	Volunteer #7 (53 years old)	Volunteer #17 (52 years old)
% of white hair	-56%	-41%	-42%
Density of white hair per cm²	-31.4 white hair per cm²	-55.7 white hair per cm²	-35.7 white hair per cm²
Total decrease of white hair number on scalp (600 cm²)	-18.800 white hair	-33.420 white hair	-21.420 white hair

Volunteer 21
(61 years old)



Volunteer 30
(58 years old)



Volunteer 23
(62 years old)

Summary

Technical information

Suggested INCI:	Glycerin (and) Water (and) N-Acetyl-Tyrosine (and) Sodium Metabisulfite (and) Glycine (and) Larix Europaea Wood Extract (and) Zinc Chloride (and) Camellia Sinensis Leaf Extract
Origin:	Plant extracts, biotechnology and synthesis
Preservation:	Sodium Metabisulfite
Appearance:	Pale yellow liquid
Solubility:	Water soluble
Dosage:	0.1-1%
Processing:	Addition at the end of the formula below 40°C, and at pH below 5.

Claims

Claims:	Hair repigmentation action, reduce appearance of white and grey hair, decrease of grey / white hair density, prevent hair-ageing.
Applications:	Anti grey hair lotion, anti white hair shampoo, natural repigmenting hair mask, anti grey hair for beard and mustache, hair color recovery spray, gel for premature grey / white hair.

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