

CLINICAL STUDY REPORT

ANTI
GREY

Neofollics Anti Grey Hair Serum



Table of contents

| | | |
|----|-------------------------------------|----|
| 1. | Introduction | 6 |
| 2. | Understanding of Grey Hair | 8 |
| 3. | Key ingredients and their functions | 9 |
| 4. | Study on active ingrediënts | 12 |
| 5. | Study on end-product | 18 |
| 6. | References | 26 |

Neofollics Hair Technology
Meander 251, 6825 MC Arnhem
The Netherlands

info@neofollics.com
www.neofollics.com

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Abstract

Background

Neofollics Hair Technology has a long history of utilising the latest scientific breakthroughs in developing effective hair care products to combat hair problems. Another key product line of Neofollics is the Anti Grey line, to reduce and slow down the appearance of greying hair. One part of this line is a targeted topical therapy. Greying of hair, especially premature greying of hair in early ages is quite common. Therefore, Neofollics Hair Technology has developed an effective, user friendly and side effect free topical serum that prevents the greying of hair and restores the natural colour of the hair.

Objective

To assess the anti greying potential of the Neofollics Anti Grey Hair Serum.

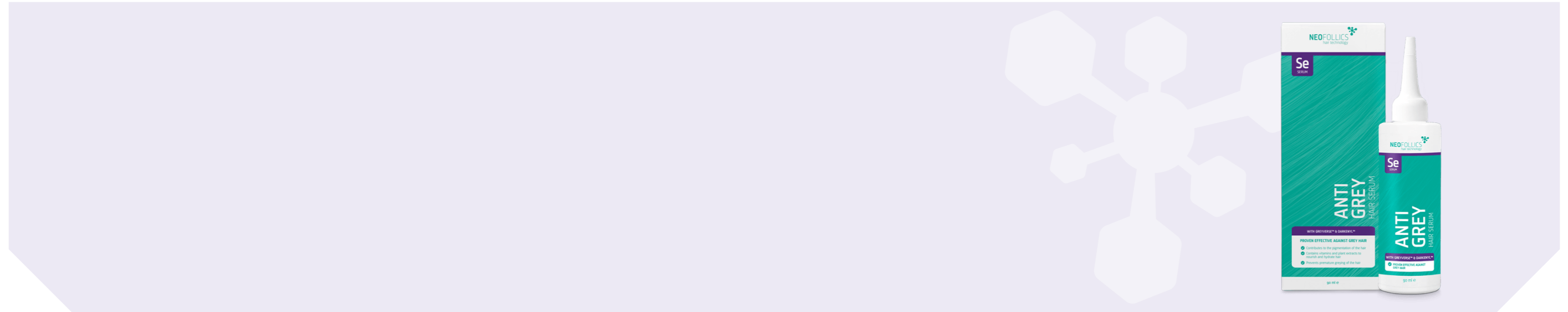
Methods

The Neofollics Anti Grey Hair Serum has been assessed by two ways:

1. Assessment based on the clinical studies on the active ingredients.
2. In vitro study on the Neofollics Anti Grey Hair Serum evaluated the anti-grey potential of the serum by determining its effectiveness on the increase of melanocytes (melanin producing cells).

Conclusion

The active ingredients used in Neofollics Anti Grey Hair Serum are clinically proven effective in increasing melanin production and reducing oxidative stress through in vitro as well as in vivo studies. In an in-vitro study Neofollics Anti Grey hair serum significantly promotes the proliferation of melanocytes (melanin producing cells) which supports the anti greying potential of it.



1. Introduction

Neofollics Anti Grey Serum has been developed to be an effective and user-friendly topical therapy to grey hair. The Serum contains a balanced combination of proven active ingredients with multiple mechanisms addressing the greying of hair.

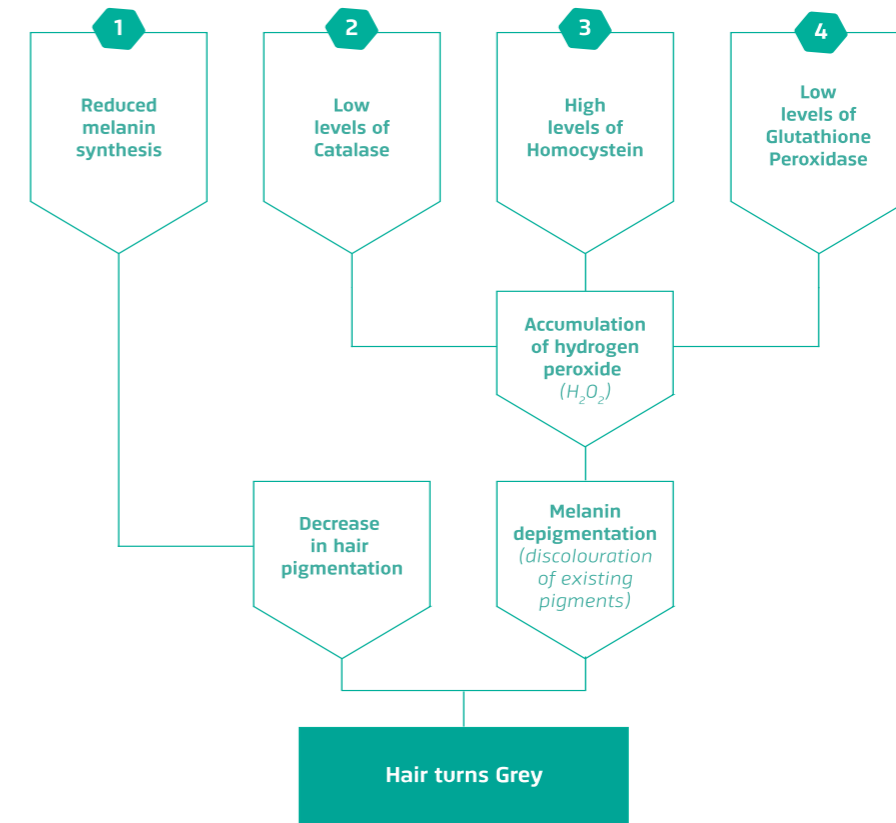
Neofollics Anti Grey serum is a powerful serum that stimulates pigmentation of hair and prevents the premature greying of hair. At the same time, it is rich in nutritional ingredients which will strengthen the scalp and hair.

2. Understanding of Greying Hair

Greying hair is a complex biochemical process. In recent years, several studies indicated the contributing factors and processes for greying hair. The following main causes on a biological level are as follows:

1. When Melanin is not produced in adequate quantities by melanocytes within the hair follicle, leading to a decrease in hair pigmentation.
2. Reduction of the enzyme Catalase, which leads to higher levels of Hydrogen Peroxide. Hydrogen Peroxide bleaches the hair pigments, leading to discoloration. This is called Oxidative Stress.
3. Elevated levels of Homocysteine increase the amount of Hydrogen Peroxide.
4. As Glutathione Peroxidase scavenges hydrogen peroxide, low levels can trigger the onset of greying hair.

Other factors of greying include genetics, poor nutrition, stress, and autoimmune disorder.



WORKING MECHANISM

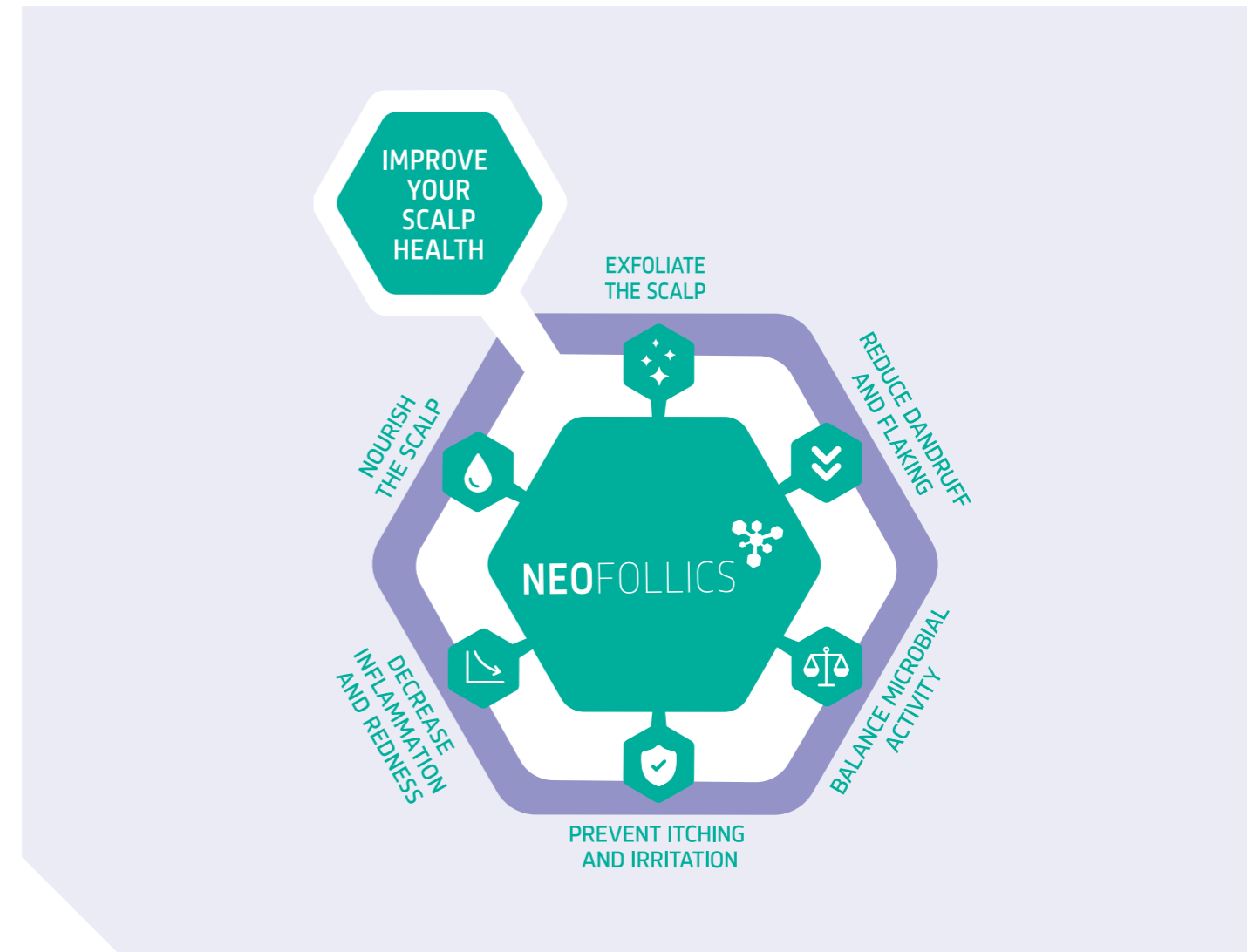
Neofollics Anti Grey Serum works on two main causes of grey hair:

1. Stimulate Melanin production.

With age number and activity of melanin producing cells in the hair bulb decreases which leads to decline in the melanogenesis (melanin production). Melanin synthesis decreases and its transfer to neighbouring skin cells also reduces. This leads to loss of pigments in hair shaft until the hair becomes grey.

2. Reduction in Oxidative Stress

Oxidative stress is an imbalance between the production of reactive oxygen species & the ability of body's natural antioxidant defence system to neutralize these harmful molecules which in turn can cause premature greying of hair. This oxidative substance is naturally degraded into water & oxygen. With age, catalase activity decreases leading to accumulation of H₂O₂. This gradually bleaches the melanin pigment. Also, decrease in the antioxidant Tyrosinase related Protein-2 over time leads to a lack of protection of melanocytes against free radicals.





3. Key Ingredients, their functions and efficacy

Neofollics Anti Grey serum contains 11 proven active ingredients. Each active ingredient has been selected based on scientific research to combat greying of hair in multiple ways:

- Greyverse effectively stimulates melanin production and decreases the oxidative stress to preserve hair's natural color.
- Darkenyl, protects hair follicles from oxidation and stimulates hair follicles stem cells to create new melanocytes.
- Cucumis melo extract, very effective natural antioxidant that contains the catalase enzyme
- Powerful plant extracts, like ECGC Extract and Carthamus Tinctorius (Safflower) Extract.
- Vitamin B5, Vitamin B3, Vitamin E, Hyaluronic acid and Quinoa extract to nourish the scalp and hair.

1. GREYVERSE

It contains an α - MSH Biomimetic Peptide which can act on the different causes of the hair greying process. Due to its complete mechanism of action, the peptide found in Greyverse™ can act simultaneously and efficiently on both factors of hair greying by stimulating MC1-R (Melanocortin 1 Receptor). The stimulation of melanogenesis enhances melanin production in the hair bulb and favors better transfer leading to hair repigmentation. The decrease in oxidative stress preserves hair pigmentation.

2. DARKENYL

It is a combination of taxifolin glucoside (a stabilized 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 melanogenesis to increase melanin production. This mode of action is completely independent from gender, hair type or hair color, making Darkenyl™ an ideal solution to universally target hair greying.



3. CUCUMIS MELO EXTRACT

Cucumis melo, also known as melon, is a rich source of polyphenolic compounds, which are very effective antioxidants. Cucumis melo extract has a superoxide dismutase enzyme activity greater than 30 enzyme units per mg and a catalase enzyme activity greater than 45 enzyme units per mg. Therefore, it is potent antioxidant. The melon is also rich in important vitamins, such as riboflavin, thiamine, folic acid, vitamin A and vitamin C.



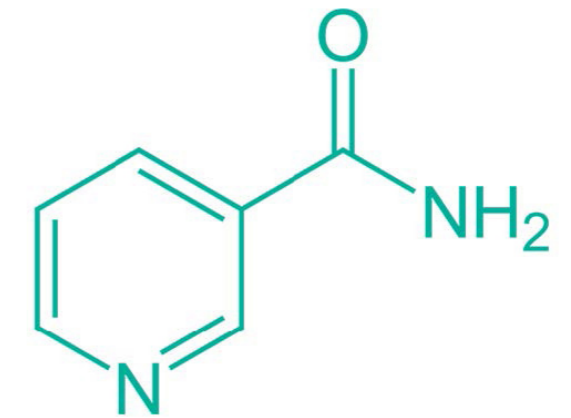
4. CARTHAMUS TINCTORIUS

Safflower (*Carthamus tinctorius*) is rich in fatty acids such as Oleic acid and linoleic acid. Therefore, it is beneficial for soothing and conditioning the scalp and hair. It also stimulates hair growth as well as its strength.



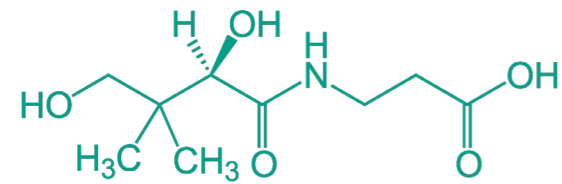
5. EGCG (EPIGALLOCATECHIN GALLATE)

Polyphenol content in green tea can help nourish the scalp. It supports healthy hair growth. It is a good antioxidant with free radical scavenging effects in response to oxidative stress.



6. NIACINAMIDE (VITAMIN B3)

Works as an anti-inflammatory. It also improves the circulation in the scalp, making it easier for nutrients to reach the hair and scalp



5. VITAMIN B5

Vitamin B5, also called pantothenic acid can noticeably increase hair follicles nourishment, has moisturizing abilities that give hair proper moisture.

6. HYDROLYZED QUINOA EXTRACT

The proteins in Hydrolysed Quinoa penetrate into the hair shaft for powerful protection and moisturization.



6. VITAMIN E

It supports the healthy scalp and hair as it has natural antioxidant properties.



6. EFFICACY BOOSTER™

The Neofollics Anti Grey Serum contains Efficacy Booster™ to improve the penetration of the active ingredients in to the scalp. Hyaluronic acid improves the condition of the skin and improves the absorption of the active ingredients into the scalp. The Liposome technology encapsulates the active ingredients and enables further penetration of the skin's upper surface. This enhances the absorption of the actives into the hair follicle significantly.

4. Clinical studies on active ingredients

In Neofollics Anti Grey Hair Serum we are using two active ingredients. These ingredients are proven effective through a clinical study as well as consumer test among users (men and women) with greying hair.

Below are the results from individual active ingredients.

GREYVERSE

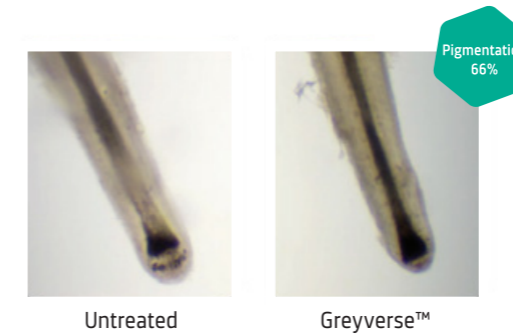
Greyverse contains an innovative α -MSH biomimetic peptide able to act on different causes of the hair greying process. It offers an efficient solution to prevent, stop, and reverse the sign of aging.

Two factors have been identified as the main causes of the hair greying process: the decrease in melanogenesis and the increase in oxidative stress in the hair bulb. Due to its complete mechanism of action, the peptide found in Greyverse™ is able to act simultaneously and efficiently on both factors by stimulating MC1-R (Melanocortin 1 Receptor).

Results On Key Indicators

1. Increase in melanogenesis (melanin production):

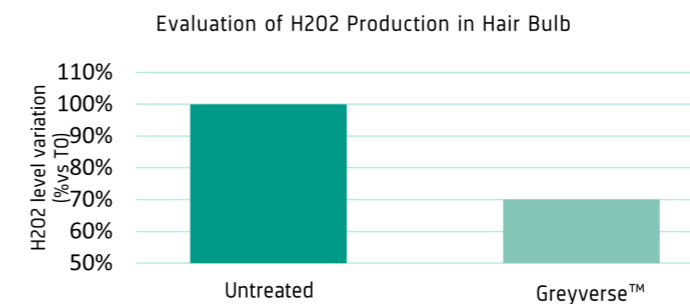
The number and activity of melanin producing cells in the hair bulb decrease over time leading to a decline in the process of melanin production. The synthesis of melanin is decreased and its transfer towards the surrounding cells is then reduced. The final quantity of pigments in hair shafts dims until the hair fibre is grey.



The stimulation of melanogenesis enhances melanin production in the hair bulb and favors better transfer leading to hair repigmentation.

2. Reduction in Oxidative Stress:

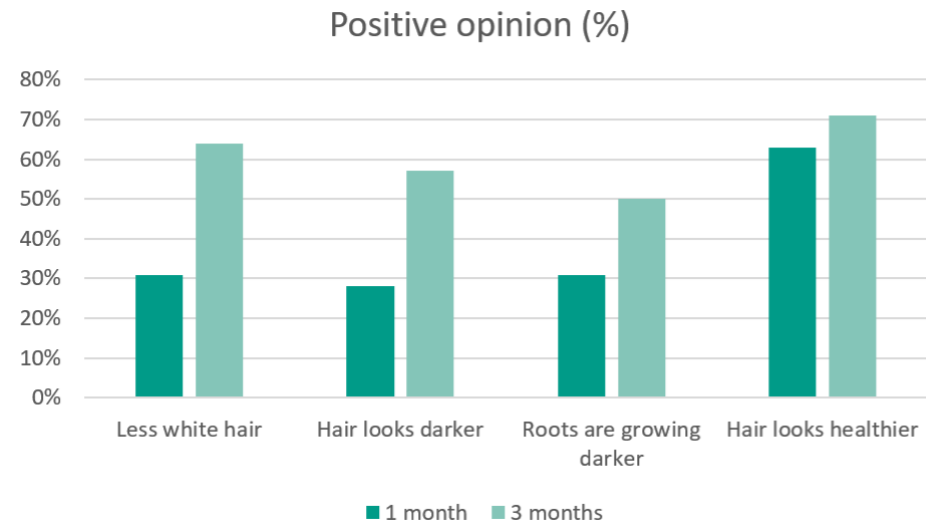
With aging, catalase activity decreases in the body leading to an accumulation of hydrogen peroxide. This then progressively bleaches the melanin pigments and the hair from the inside out. Furthermore, a decrease in the antioxidant TRP-2 (Tyrosinase Related Protein 2) over time leads to a lack of protection of melanocytes against free radicals.



This active has a proven effect on the reduction on the h₂O₂ in the hair bulb

3. Clinically Proven Hair Pigmentation

- 15 men volunteers with dark hair suffering from early canities (18-35 years old, white hair > 20%).
- Application once a day, at night of 3 mL of a lotion containing 2% Greyverse™ for 3 months on scalp.
- Evaluation of the overall hair pigmentation by chromametry (L* parameter).
- Evaluation of grey hair density by image analysis.



The study showed that Greyverse™ has similar efficacy on men and women, and on all hair colours. It also confirmed that it preferentially works on early stages of greying process: the less grey hair, the easier it is to reverse.

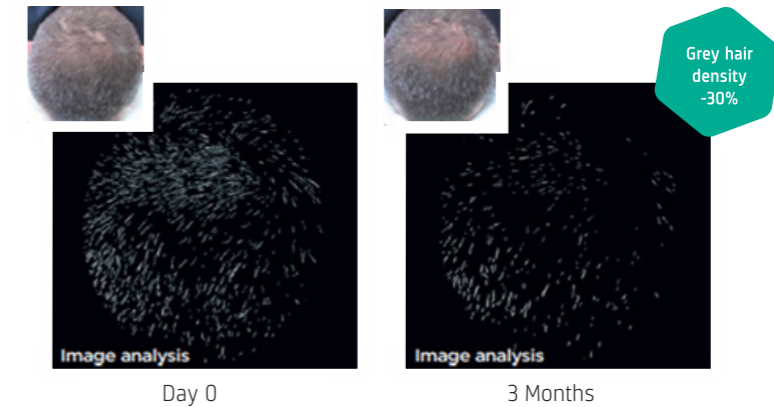
3.1 Decrease in overall grey hair:

After 3 months of treatment, the L* (measurement of luminescence) parameter has significantly decreased by 5.3% (up to 32%) confirming a darker color of hair.



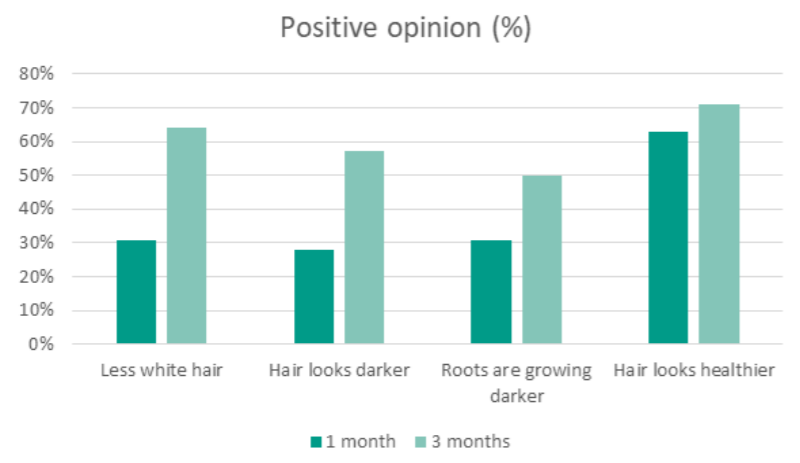
3.2 Decrease in Grey Hair Density:

The observation of the top of head clearly shows a lower quantity of grey hair after 3 months of treatment.



3.3 Consumer Test:

- 104 volunteers: 61 Women and 43 men (21 to 70 years old) with different stages of hair greying (up to 30% grey hair) & various hair colors.
- Application once a day, at night of 3ml serum containing 2% Greyverse™ on scalp and 2ml on beard and sideburns.
- Self- assessment after 1 month and 3 months by filling in a questionnaire.
- Pictures taken by volunteers after 1 month and 3 months.
- After three months, 64% volunteers reported decrease in number of white hair, 57% reported darker hair, and 50% reported darker growth of hair roots.



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DARKENYL

Darkenyl™ is a combination of taxifolin glucoside (a stabilized 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:

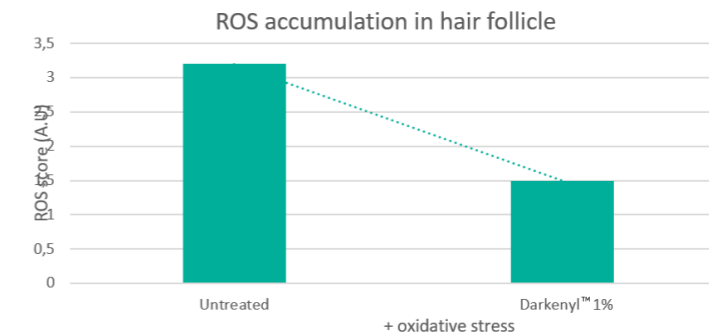
- Protects hair follicles from oxidation (ROS).
- Stimulates hair follicle stem cells to create new melanocytes.
- Activates melanogenesis to increase melanin production. This mode of action is completely independent from gender, hair type or hair color, making Darkenyl™ an ideal solution to universally target hair greying.

Results On Key Indicators

1. In vitro stimulation of melanogenesis (melanin synthesis)
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 left untreated for 72 hours. The melanin content in the cell supernatant was evaluated by colorimetric assay (405nm).

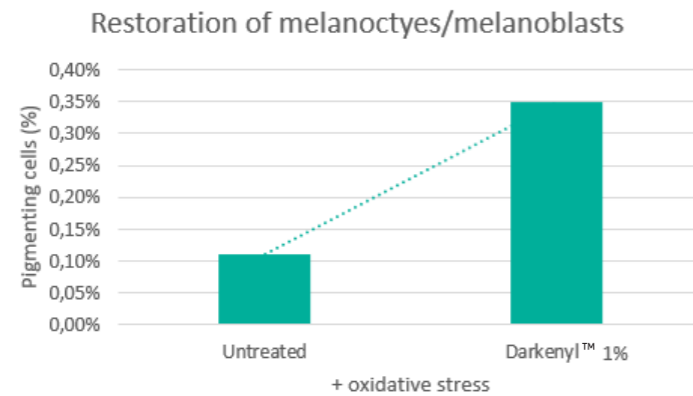
Result: Darkenyl™ significantly increases by +364% the melanogenesis.

2. Reduction of ROS production under oxidative stress (ex vivo)
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.



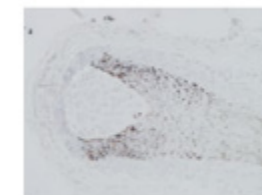
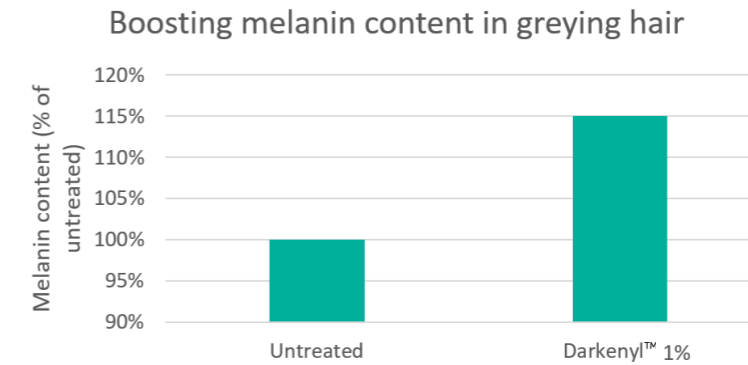
Result: Under oxidative stress, Darkenyl™ significantly decreases by -53% the ROS accumulation into the hair follicle.

3. Restoration of melanocytes after oxidative stress (ex vivo)
Human hair follicles from a female donor (56 years old) were exposed to an oxidative stimulus (cumene hydroperoxide - 50 μ M) for 1 hour. The hair follicles were then treated with 1% of Darkenyl™ or left untreated.



Result: After exposure to an oxidative stress, Darkenyl™ significantly restores the melanocytes and melanoblasts by +189% into the hair follicle.

4. 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.



Untreated



Darkenyl™ 1%

5. Reduction of the white hair proportion

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.



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. No white roots effect was observed during hair growth.

6. Illustrative macroscopic clinical results

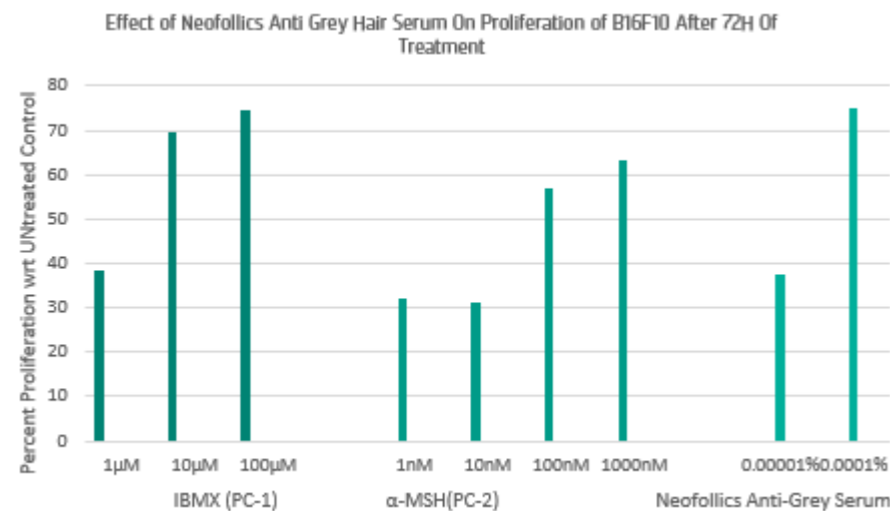
Results are illustrative in the picture.



5. Study on end product

Objective: To evaluate the anti greying potential of Neofollics Anti Grey Hair Serum by determining effect on proliferation of melanocytes (B16F10) in- vitro.

Method: In the study, the in vitro anti-greying potential of Neofollics Anti-Grey Hair Serum was investigated by determining the effect on proliferation of Melanocytes (B16F10). In this study, B16F10 cells were treated with Neofollics Anti Grey Serum at concentrations ranging from 0.00001%v/v -0.1%v/v for 72h. After 72h of incubation with Neofollics Anti-Grey Serum, proliferation was measured by BrdU uptake method, that is a very sensitive method to estimate cell proliferation.



Result: Neofollics Anti Grey Hair Serum demonstrated the increase in proliferation by 74.9% as compared to Control cells (Untreated) after 72h of treatment which is higher than the control group.

Conclusion: Based on the results obtained, it can be concluded that Neofollics Anti Grey Hair Serum exhibited in vitro Anti Greying potential by increase in proliferation of B16F10 cells.

CONCLUSION

The formulation of the Neofollics Anti Grey serum is well researched with proven ingredients that work in different ways to prevent and reverse grey hair.

The serum already contains proven ingredients for their anti greying effects.

Studies on Greyverse™ have shown the enhancement of melanin production in the hair by 66% and decrease in overall grey hair density by 32%. Similarly, studies on Darkenyl showed the significant increase by +364% in the melanogenesis and remarkable decrease in number of grey hairs.

The Neofollics Anti Grey serum has also been evaluated in-vitro on the proliferation of melanocytes for its anti greying potential. The study showed a maximum 74.9% increase in the proliferation of melanocytes as compared to the control units. It indicated that Neofollics Anti Grey Hair Serum exhibits anti greying potential by increasing the proliferation of melanin producing cells.

Therefore, it can be concluded that on regular use, Neofollics Anti Grey Hair Serum can prevent pigmentation loss in hair fibers and stimulate the natural pigmentation process to recover a natural hair colour.

6. References

1. Greyverse

S. Almeida Scalvino, A. Chapelle, N. Hajem, E. Lati, P. Gasser, J-C. Choulot, L. Michel, M. Hocquaux, E. Loing, J. Attia, J. Wdzieczak-Bakala (2018) : Efficacy of an agonist of α -MSH, the palmitoyl tetrapeptide-20, in hair pigmentation, International Journal of Cosmetic Science, DOI: <https://doi.org/10.1111/ics.12494>

2. Darkenyl

Morgane De Tollenaere, Emilie Chapuis, Pascale Auriol, Daniel Auriol, Amandine Scandolera, and Romain Reynaud (2021): Global Repigmentation Strategy of Grey Hair Follicles by Targeting Oxidative Stress and Stem Cells Protection, Applied Sciences, DOI: <https://doi.org/10.3390/app11041533>

3. Cucumis Melo Extract

3.1 Muhammad Zulfikar, Faiza Senja Widya, Wiko Arif Wibowo, et al (2020): Antioxidant activity of melon fruit (*Cucumis melo* L. 'GMO') ethanolic extract, AIP Conferences Proceedings 2260, DOI: <https://doi.org/10.1063/5.0015748>

3.2 Ioannis Vouldoukis, Dominique Lacan, Caroline Kamate, Philippe Coste, Alphonse Calenda, Dominique Mazier, Marc Conti, Bernard Dugas: Antioxidant and anti-inflammatory properties of a *Cucumis melo* LC. Extract rich in superoxide dismutase activity; Journal of Ethnopharmacology; DOI: <https://doi.org/10.1016/j.jep.2004.04.023>

4. Carthamus Tinctorius

Junlatat, J., Sripanidkulchai, S (2013). Hair Growth-Promoting Effect of *Carthamus tinctorius* Floret Extract. *Phytotherapy Research*, 28(7), 1030-1036. Doi: 10.1002/pt. 5100.

5. EGCG

Baumann, L.S. (2007). Less-known botanical cosmeceuticals. *Dermatologic Therapy*, 20, 330-342.

6. Quinoa Extract

Priti Mudgil, Lina S. Omar, Hina Kamal, Bhanu Priya Kilari, Sajid Maqsood (2019): Multi-functional bioactive properties of intact and enzymatically hydrolysed quinoa and amaranth proteins; Department of Food Science, United Arab Emirates University, Al Ain, 15551, United Arab Emirates; DOI: 10.1016/j.lwt.2019.04.084

ANTI
GREY

The scientific approach to hair growth

The goal of Neofollics Hair Technology is to help people who are dealing with hair loss to gain control of their situation. We do this by making the latest scientific insights available to every one, developing products that stimulate hair growth effectively.

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