mibelle GROUP biochemistry

BSB Innovation Award® Cosmetics 2022
1. Prize Category Most Innovative Raw Material

in-cosmetics® global
Green Ingredient Award 2022
SILVER WINNER

MelanoGray™

The hair color restoring essence

SWISS QUALITY PRODUCT

MelanoGray™
The hair color restoring essence

Upcycled Mandarins against Hair Graying
MelanoGray™ is an anti-hair graying essence that is sustainably obtained by upcycling the peel paste from unique organic Chios mandarins. Thanks to its melanin stimulating and antioxidant activity, it markedly reduces the quantity of gray hair in both men and women.

The Chios mandarins exclusively grow under very specific conditions, which are optimally found in the beautiful historic orchards of the Greek island of Chios. MelanoGray™ was produced from the leftovers of these special mandarins that are used in the distillation process of organic fragrance production. The extract is supplemented with acetyl tyrosine, which is the substrate for melanin synthesis.

In vitro and in vivo studies have demonstrated that MelanoGray™:
- Stimulates melanin production through several mechanisms, including the increased production of eumelanin, enhanced melanocyte proliferation, and the protection of melanocytes due to its antioxidant properties.
- Reduces the negative effects of noradrenaline-induced stress in melanocytes.
- Reduces in a significant and long-lasting way (>1 month) the amount of gray hair by 5 % and increases the non-white hair / white hair ratio by 21.6 % after 120 days treatment in men and women.

MelanoGray™ is an attractive upcycling ingredient derived from a unique locally sourced mandarin that leads to a long-lasting significant reduction of gray hair, even during times of stress when hair graying can be accelerated.

MelanoGray™
- Reduces gray hair ratio by 21.6 %
- Long-lasting repigmentation effect (> 1 month)
- Stimulates melanin synthesis
- Enhances resistance of melanocytes against stress

Applications
- Anti-hair graying serum
- Intensive anti-hair graying lotion for women and men
- Protective pigment restoring elixir
- Premature graying prevention treatment

Formulating with MelanoGray™
- Recommended use level: 1–2 %
- Incorporation: For cold processes, dissolve MelanoGray™ into the aqueous phase. In hot / cold processes, add during the cooling phase below 40 °C
- Thermostability: Temperatures of up to 40 °C for a short time will not affect the stability of MelanoGray™

INCI (EU / PCPC) Declaration
Citrus Reticulata Extract / Citrus Reticulata (Tangerine) Extract (and) Acetyl Tyrosine (and) Pentylene Glycol (and) Gluconolactone (and) Sodium Benzoate (and) Aqua / Water

Additional Information
- Organic source
- Upcycling ingredient
- Unique source from a historical place in Greece

Hair Graying
A process with many causes

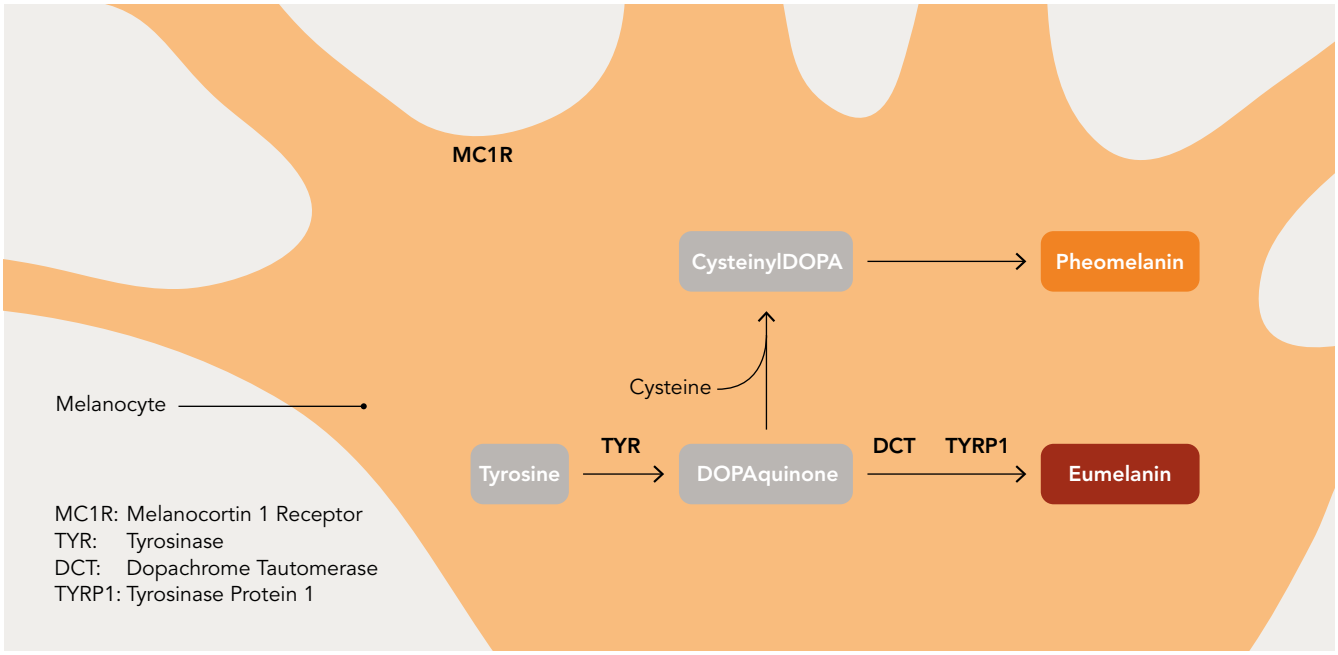
Gray Hair and its Importance
Hair has a wide-ranging impact on our lives, far beyond its natural role of protection and thermoregulation. Hair and gray hair in particular can greatly influence one's self-esteem and mood, yet the graying of hair would appear to be an inevitable process. The "50" rule of thumb says that 50% of the population will have at least 50% gray hair by the age of 50 years. However, studies have shown that this is more likely to be between 6 to 23% of the population, depending on ethnic/geographic origin and natural hair color, with men tending to gray significantly earlier than women (1).

The Follicle is the Place where Pigmentation Occurs
The pigmentation of hair is the result of a complex interaction that takes place at the hair follicle between melanocytes, matrix keratinocytes, and dermal papilla fibroblasts. Hair pigmentation occurs only during the anagen phase, which is the growing phase, and this can last from 3 to 7 years. The hair pigments, termed melanins, are produced in the cytoplasmic organelles of the melanocytes termed melanosomes, and they are later transferred via dendrites to the keratinocytes at the base of the hair. This process results in the pigmented hair shafts.

How the Hair Color is Determined
There are two types of melanin in the hair follicle that are responsible for all the different hair shades: the black-brown eumelanin and the reddish-yellow pheomelanin. Basically, the amount of eumelanins in the hair determines how dark it is. If the concentration is high, the hair is brown to black, whereas if the concentration is lower, then the hair appears lighter. Red hair is caused by higher amounts of reddish pheomelanin, while blond hair is determined by low concentrations of both pigments.

Melanin Synthesis from Tyrosine
The process of melanin synthesis, which is also called melanogenesis, can be initiated from either the hydroxylation of phenylalanine to tyrosine or directly from tyrosine. Melanogenesis is tightly regulated and it features a range of regulatory proteins and enzymes from the tyrosinase family.

The Pathway for Melanin Synthesis



The type of melanin with the resulting nuance of color is controlled by the melanocortin 1 receptor, which is encoded by the MC1R gene. The activation of MC1R results in a precise reaction cascade inside the melanocytes to produce eumelanin. MC1R is thus recognized as the key player in hair and human skin pigmentation.

Causes and Mechanisms of Canities

Hair graying, which is also called canities, is defined as the progressive loss of natural hair pigmentation. The mixture of darker, normally pigmented hair and increasingly lighter, less pigmented hair is perceived as progressive graying.

While genetic factors play the most predominant role in hair graying, psychoemotional stress, hormonal variations, and various diseases, as well as malnutrition and the use of alcohol and certain drugs have been linked to an increased likelihood of premature graying. In addition, environmental factors – such as smoking, UV radiation, and pesticides – trigger the occurrence of gray hair.

Stress is an Active Cause of Hair Graying

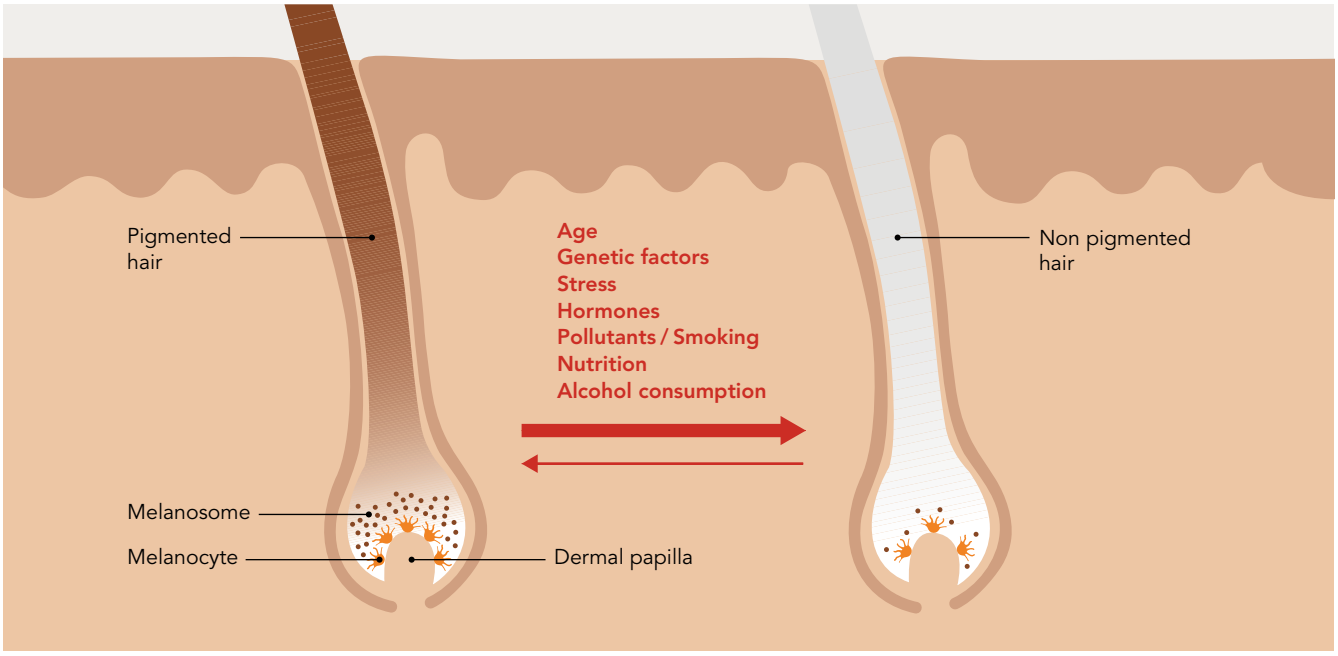
In addition to anecdotal evidence, new scientific studies have confirmed that emotional stress not only promotes biological aging but also hair graying. The hormone noradrenaline, which is released by the nervous system in response to stress, can promote hair graying by the depletion of melanocyte stem cells from their niche within the hair follicle (2).

Hair Graying is Reversible

Hair graying has always been considered to be an irreversible process. However, in 2021, new scientific data shone another bright light on the entire hair stress-induced graying process and proved that it can be reversed regardless of sex, ethnic background, or age (3).

(1) Panhard, S. et al. (2012). Greying on the human hair: a worldwide survey, revisiting the '50' rule of thumb. British Journal of Dermatology 167: 865–873.
(2) Zhang, B. et al. (2020). Hyperactivation of sympathetic nerves drives depletion of melanocyte stem cells. Nature 577: 676–681.
(3) Rosenberg, A. et al. (2021). Quantitative mapping of human hair greying and reversal in relation to life stress. eLife 10: e67437.

Pigment Formation in the Hair Follicle



Chios Mandarin Extract
An upcycling ingredient against hair graying

Citrus Fruits and Mandarins in History and Health
Citrus plants have accompanied civilizations for thousands of years. Due to their edible fruits, citrus plants were cultivated at a very early point of time and are today one of the world's most important fruit crops. The aromatic fruits are valued for their sweetness, antioxidant properties, and their immunomodulatory, metabolic, cardiovascular, and neuroprotective effects on human health. In fact, they are even considered as nutraceuticals. In the context of cosmetics, citrus fruits are traditionally used to treat the skin and, in some ethnic groups, even the hair.

Mandarins, or tangerines, are the most varied and largest group of citrus plants and they differ from oranges inasmuch as they are smaller and less acidic. Furthermore, the skin of mandarins is easier to peel compared to other citrus fruits. Mandarins are particularly rich in essential oils and antioxidants such as phenolic acids, flavonoids (especially hesperidin and polymethoxylated flavones), limonoids, and carotenoids.

Chios Mandarins are Greek Treasures
Mandarins are nowadays cultivated in all continents. Conversely, the variety "Chios mandarin" grows exclusively under very specific conditions that are optimally found on the island of Chios where mandarins were introduced in the 13th century following the Genoese colonization.

The island of Chios, which is also known for the mastic tree, has calcium-rich soil that is based on limestone. The stable temperatures and the Meltemi winds give a mild microclimate that protects the fruits from frost during the winter months.

The unique mandarin variety from the Greek island of Chios, *Citrus deliciosa (Citrus reticulata)* Var. *Xio,* has been registered in the EU since 2012 as a product with Protected Geographical Indication (PGI).

The Historic Orchard of Kampos

An Old Variety that is Almost Disappearing
The Chios mandarin typically has a yellow-orange skin with soft and slightly orange flesh. However, in contrast to the modern, industrially produced fruits, this old mandarin variety contains a lot of seeds and this does not correspond to today's market standards. Therefore, the fruits no longer gain a high degree of interest. Besides being processed into juice, jam, and liqueur by the locals, only one fruit juice factory continues to purchase these mandarins. As a result, only a few of the former 200 traditional citrus orchards are still maintained and cultivated today – and even these are in danger of falling into states of disrepair.

MelanoGray™ – Mandarins from a Historic Place
However, Chios mandarins are highly valued for their excellent and intense aromatic properties and delicate fragrance as exquisite perfume essences. In one of the few remaining beautiful historic orchards of an old mansion in the area of Kampos, Chios mandarins are still cultivated in a traditional way and they are then processed into perfume essences. As is typical of these secluded historic places, this orchard is surrounded by high walls made of natural stone. This mansion was also one of the first organic farms on the island.

Upcycled from Aromatic Waste
To obtain the active ingredient MelanoGray™, Mibelle Biochemistry exclusively uses the organic Chios mandarins from this precise mansion where they are carefully and sustainably harvested by hand. In the traditional small distillery at the same place, both the juice and the peel of the fruit are used for the gentle distillation process. What remains – a rich and thick peel paste (pomace) – is the starting material that is used to produce our MelanoGray™ in an upcycling-inspired sustainable process.

By using Chios mandarins for MelanoGray™, Mibelle Biochemistry therefore not only refines a high-quality waste product with an upcycling process but also helps to preserve the historic orchards and original cultivation methods of Kampos.

The Final Product: MelanoGray™
In the active ingredient MelanoGray™, this unique mandarin peel extract is supplemented with acetyl tyrosine, which is the amino acid that acts as a substrate for the melanin synthesis in the melanocytes and thus also supports the product efficacy.

Processing of the Mandarins in the Fragrance Distillery

Collecting the Peel Paste

MelanoGray™
Study results

Stimulation of Melanin Synthesis
The stimulatory effect of the MelanoGray™ on the melanin synthesis was demonstrated in B16 melanocytes.

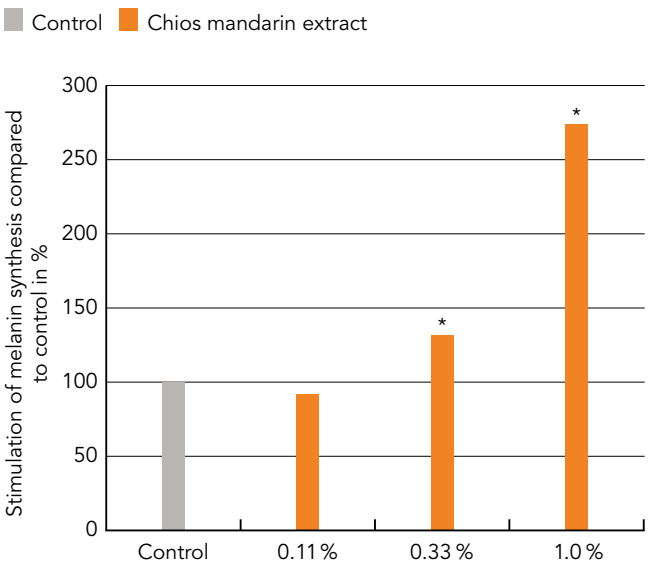
Cells were treated with different concentrations of Chios mandarin extract for 72 hours. Melanin synthesis was determined by the detection of the total melanin content in the cells and in the cell medium. For this process, absorbance at 405 nm was measured and the melanin content was calculated for each sample using a melanin standard curve. Untreated cells were used as a control.

Treatment with Chios mandarin extract significantly increased melanin production in a dose-dependent manner by 32 % and 174 % compared to untreated cells.

Synergistic Stimulation of Melanin Synthesis
The stimulatory effect of MelanoGray™ on melanin synthesis was also shown in B16 melanocytes treated with 0.11 % Chios mandarin extract in the presence of 0.1 µM NDP-MSH, an α-MSH analogue and stimulator of melanogenesis, for 72 hours. In this setup, NDP-MSH-induced melanin synthesis was synergistically enhanced by the treatment with Chios mandarin extract, which led to an additional increase of more than threefold in melanin production.

Thus, Chios mandarin extract, either alone or in combination with another melanogenesis-activator, clearly and significantly stimulates melanin synthesis.

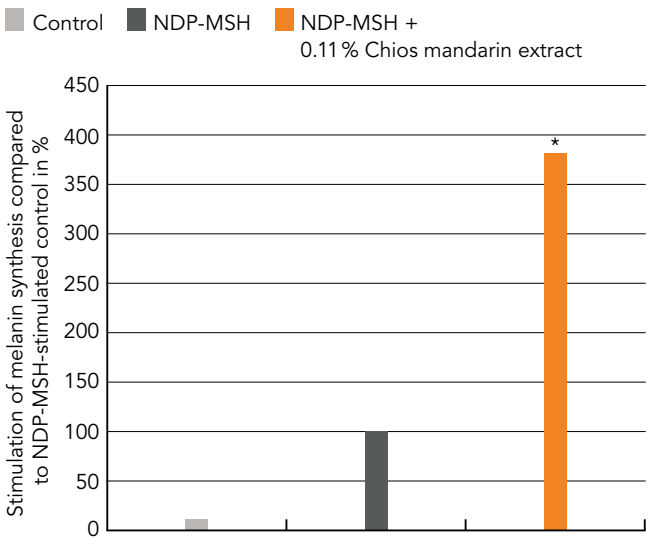
Stimulation of Melanin Synthesis



*p<0.01 versus control

S-868/ © Mibelle Biochemistry

Synergistic Stimulation of Melanin Synthesis



*p<0.001 versus NDP-MSH-stimulated control

S-849/ © Mibelle Biochemistry

Upregulation of Melanogenesis-Supporting and Antioxidant Genes

To determine the underlying mechanism of increased melanin synthesis, the effect of MelanoGray™ on the expression of melanogenesis-linked genes was investigated in lightly pigmented human epidermal melanocytes (NHEM-LP).

For this, cells were either treated or not treated (control) with 1% of the Chios mandarin extract for 24 hours before the expression analysis of genes selected in relation to their importance in melanogenesis and melanocyte function.

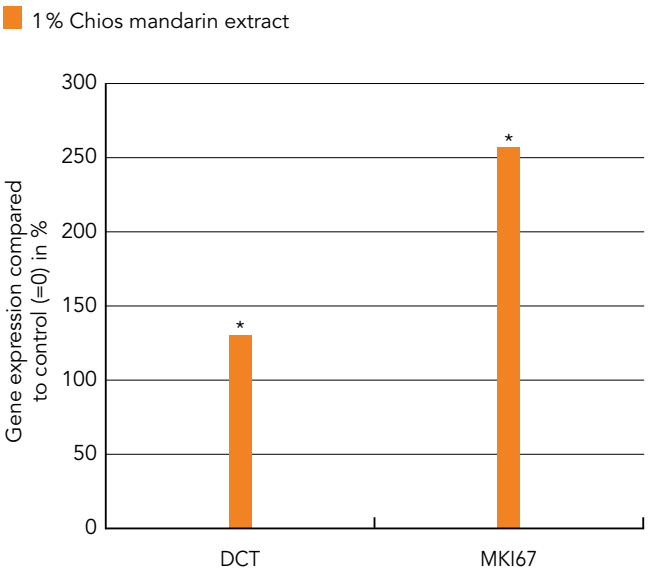
Chios mandarin extract significantly stimulated gene expression of DCT, which is the gene encoding dopachrome tautomerase, by 132%. Dopachrome tautomerase is responsible for the conversion of dopachrome into 5,6-dihydroxyindole-2-carboxylate, which is a basic step for the production of eumelanin.

In addition, treatment with 1% Chios mandarin extract strongly enhanced the expression of MKI67, which is the gene encoding marker of proliferation Ki-67, in melanocytes by 257%, indicating elevated levels of melanocyte proliferation.

Furthermore, the slightly yet statistically significant enhanced expression of HMOX1 and TXN, encoding heme oxygenase1 and thioredoxin, respectively, demonstrated antioxidant properties of Chios mandarin extract, as both enzymes are involved in the defense against oxidative stress.

In conclusion, Chios mandarin extract achieves its previously demonstrated significant stimulatory effect on melanogenesis through several mechanisms, including the increased production of eumelanin, enhanced melanocyte proliferation, and the protection of melanocytes due to antioxidant properties.

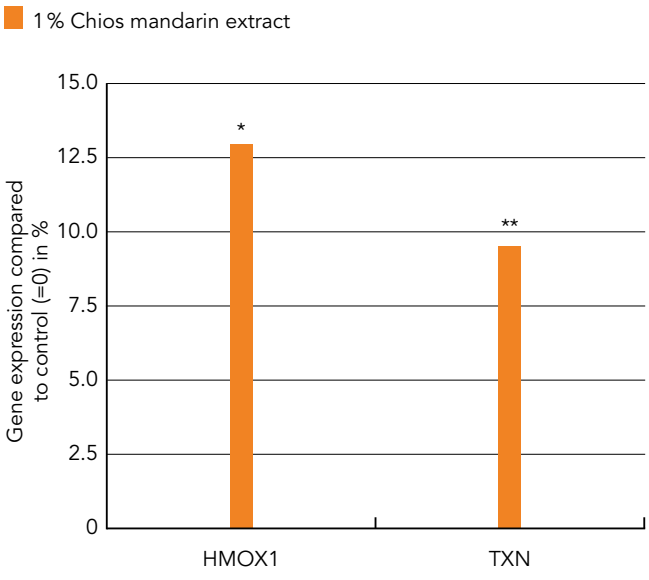
Upregulation of Melanogenesis-Promoting Genes



*p<0.001 versus control

S-976 / © Mibelle Biochemistry

Upregulation of Antioxidant Genes



*p<0.001 versus control
**p<0.05 versus control

S-976 / © Mibelle Biochemistry

MelanoGray™
Study results

Restored Gene Expression in Stressed Melanocytes

Stress is known to cause hair graying. Therefore, an additional study was implemented to assess the potential of Chios mandarin extract to prevent the stress-induced malfunction of melanocytes. Noradrenaline, which is reported to be involved in stress-induced hair graying, served as a stress inducer in this study.

Lightly pigmented human melanocytes (NHEM-LP) were treated with either 100 µM noradrenaline or a combination of 100 µM noradrenaline and 1 % Chios mandarin extract for 24 hours before gene expression analysis.

Results showed that the stress inducer noradrenaline affected gene expression in melanocytes and led to a significant downregulation of genes that are important for melanocyte function and melanin synthesis, including HMOX1 (encoding heme oxygenase 1), MC1R (encoding melanocortin 1 receptor), and CCN3 (encoding cellular communication network factor 3). Heme oxygenase 1 protects the cells from oxidative stress, whereas the melanocortin 1 receptor is directly involved in melanogenesis.

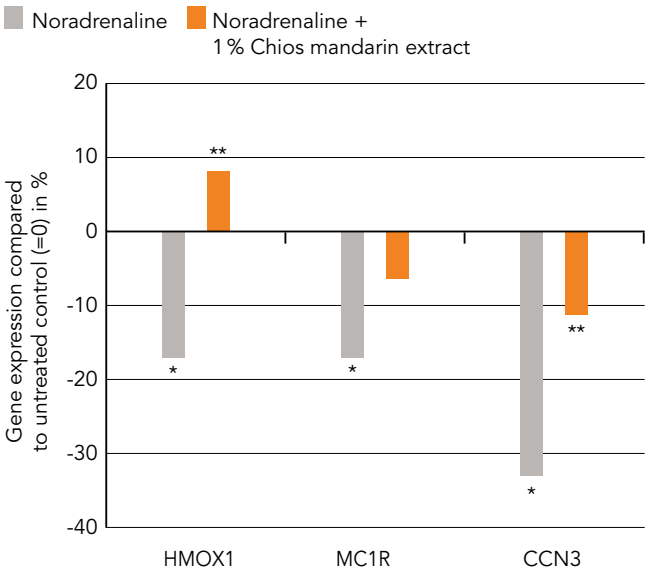
The downregulation of CCN3 can lead to reduced cell adhesion and the detachment of melanocytes from the basal membrane (4), which in turn can lead to a decreased melanin production.

Treatment with 1 % Chios mandarin extract was able to restore the expression of the genes that were downregulated due to stress induction by noradrenaline.

By reducing the negative effects of noradrenaline-induced stress, MelanoGray™ could thus help to prevent or reverse the hair graying process.

(4) Faria, A. et al. (2018). Reduced immunohistochemical expression of CCN3 in vitiligo. Indian Journal of Dermatology, Venereology and Leprology 84: 558–62.

Restored Gene Expression in Stressed Cells



*p<0.001 versus control
**p<0.001 versus noradrenaline

Long-Lasting Anti-Graying Effect

The anti-hair graying efficacy of MelanoGray™ was further investigated in a randomized, placebo-controlled clinical study. For this study, 42 male and 13 female volunteers with partially gray hair aged between 39 and 59 years (average age: 50 years) were split into two groups. One group applied a hair serum containing 2% MelanoGray™, while the other group applied the corresponding placebo hair serum on the scalp once daily in the evening for 120 days. The study was performed in Spain during the first wave of the COVID-19 pandemic in Europe in spring 2020. The timing of this study may have involved psychological stress for the volunteers due to the lockdown situation.

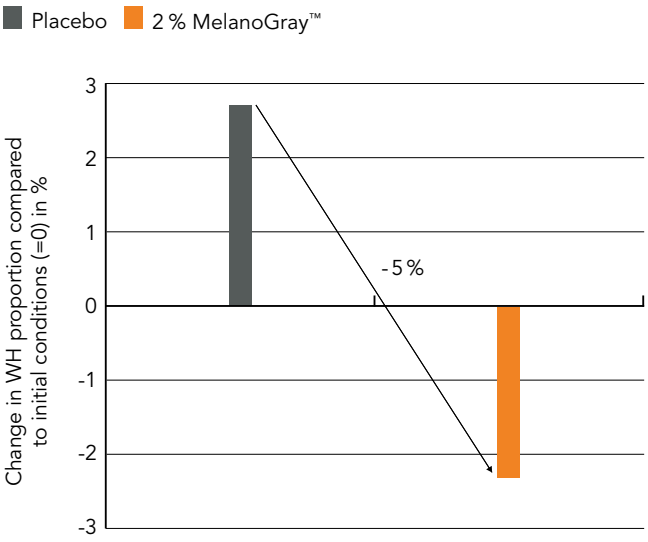
The measured parameters were the change in white hair (WH) and non-white hair (NWH) as well as the ratio of non-white to white hair (NWH/WH), which were calculated based on pictures taken with a TrichoScan® microcamera. The measurements were performed at day 0 (initial conditions), after 120 days of product application, and 30 days after the last application of the product (day 150).

Whereas the proportion of gray hair further increased in the placebo group after 120 days, the treatment with 2% MelanoGray™ led to a reduction of gray hair by 5% compared to the placebo group.

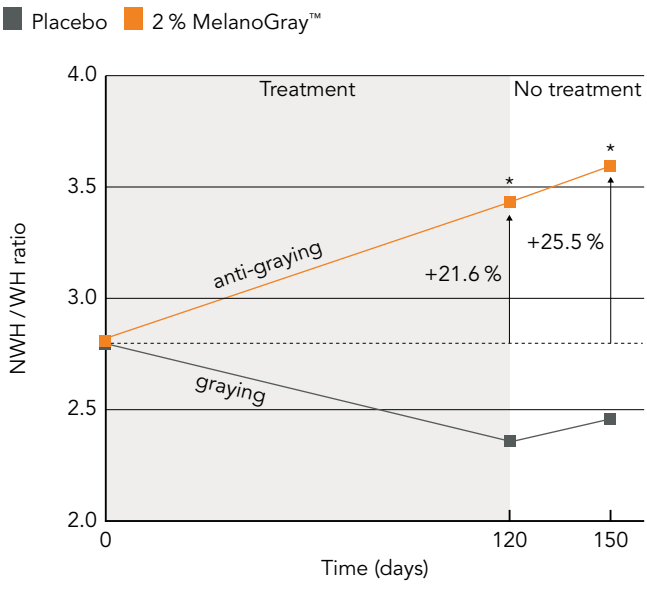
The NWH/WH ratio significantly increased by 21.6% after 120 days of treatment with MelanoGray™. In contrast, the NWH/WH ratio decreased markedly upon treatment with the placebo product. This effect was long-lasting – for a period of one month without product application – as the NWH/WH ratio was increased by 25.5% compared to initial conditions and 30 days after the last application. A positive effect was still observed in 77% of volunteers.

Thus, treatment with 2% MelanoGray™ leads to a long-lasting statistically significant reduction of gray hair, even during times of enormous stress, where hair graying could be accelerated.

Reduction of Gray Hair after 120 Days



Long-Lasting Anti-Graying Effect



*p<0.05 versus initial conditions

S-892 / © Mibelle Biochemistry

MelanoGray™
The hair color restoring essence

MelanoGray™
- Reduces gray hair ratio by 21.6 %
- Long-lasting repigmentation effect (> 1 month)
- Stimulates melanin synthesis
- Enhances resistance of melanocytes against stress

Applications
- Anti-hair graying serum
- Intensive anti-hair graying lotion for women and men
- Protective pigment restoring elixir
- Premature graying prevention treatment

Marketing Benefits
- Upcycling ingredient
- Unique, locally sourced mandarin
- Organic source
- 1st Prize at the BSB Innovation Award in the category Cosmetics – Raw Materials
- Silver Award for Green Ingredient at in-cosmetics Global 2022

Innovating for your success

Mibelle Biochemistry designs and develops innovative, high-quality actives based on naturally derived compounds and profound scientific know-how. Inspired by nature – Realized by science.

myclimate neutral Operations
myclimate.org/01-22-602304

Mibelle AG Biochemistry, 5033 Buchs / Switzerland, Phone +41 62 836 17 31, info@mibellebiochemistry.com, www.mibellebiochemistry.com