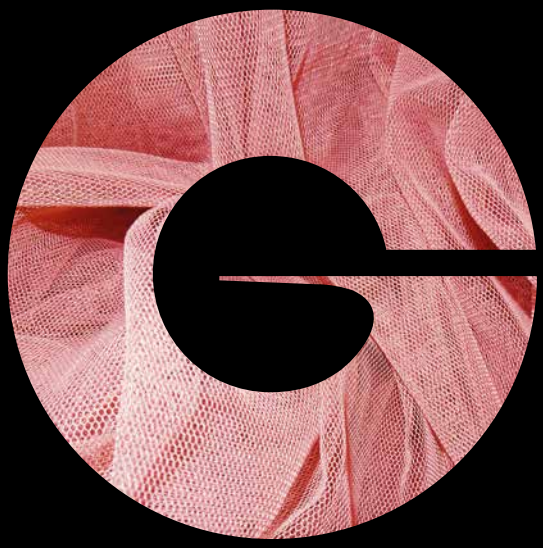
Active Beauty
Neurophroline™
Overall skin stress control

Gold
Neurophroline™
in-cosmetics Innovation Zone Best Ingredient Award 2016

Crafted by green technology



Givaudan

engage your senses

Focus on the product

Skin and brain: same origin - shared functions

Through evolution, the human body has acquired both a central nervous system (brain) and a peripheral nervous system (nerves and sensors including skin) which enable it to react to its environment and to survive.
Indeed, the brain and the skin originate from the same embryologic tissue, the ectoderm that differentiates to form neurectoderm (the future brain) and the epidermis.

The skin and the brain are constantly communicating to synchronise external and internal "danger" signals by means of cytokines, chemokines, electric impulses, and hormones.

Cortisol: a key hormone in stress response

Stress is defined as an external factor that **disrupts homeostasis**. In a stressful situation, our body responds by liberating a flush of stress hormones including adrenaline and cortisol, that will result in a fight response (positive reaction to stress and adaptative reaction). Because our body cannot keep this state for long periods of time, a feedback mechanism returns the body to normal physiological conditions (elimination of stress hormones and their consequences), thus maintaining homeostasis.[1,2]

Cortisol release is mediated by the brain through the hypothalamus pituitary adrenal gland axis in a cascade reaction. As the skin and brain share the same origin, skin cells are able to produce cortisol in response to external stress factors.

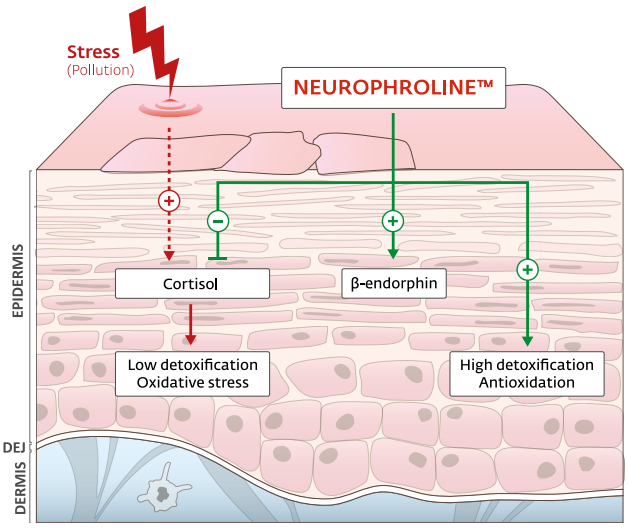
In case of prolonged exposure to stress (such as mechanical or chemical aggressions, pollution, smoke, sleep deprivation, UV exposure), skin cells do not stop producing cortisol which breaks down skin homeostasis, leading to redness (inflammation), and a visible tired look.[3]

Neurophroline™: biological skin stress inhibitor

Neurophroline™ has been discovered in the wild indigo (*Tephrosia purpurea*), a native Indian plant used in the Ayurvedic tradition for its benefits on skin. A specific extraction from the seeds of this plant has been performed to obtain a condensate enriched in specific sugars including stachyose and ciceritol.[4]

This exclusive extract has demonstrated its unique biological capabilities to:

- Break down the cortisol production by skin cells
- Activate the release of a natural calming neuropeptide acting on mood
- Improve visibly skin tone in only two weeks, proving a fast action to control stress in skin



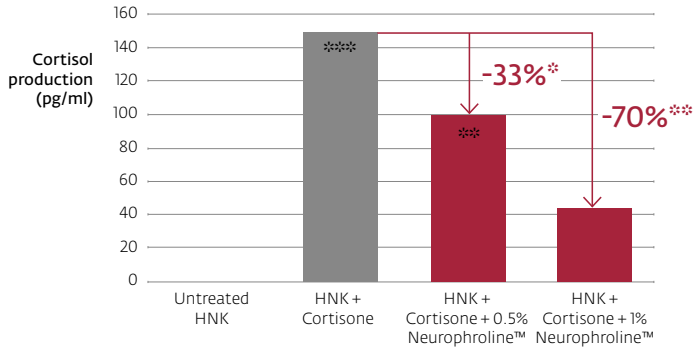
[1] J Invest Dermatol. 2015 Jun;135(6):1469-71
[2] Int J Psychophysiol. 2015 Jun;96(3):169-75
[3] J Invest Dermatol.2001 Aug;117(2):309-17
[4] J. Nat. Prod., June 23, 2015, 78 (7), pp 1609−1617

Biological activity

Regulation of cortisol production (*in vitro*)

Cortisol is produced by keratinocytes in the skin as an instantaneous answer to a stress factor. Normal human keratinocytes were treated with Neurophroline™ at 0.5% and 1% over 2 hours, and 1µM of cortisone to quantify the release of cortisol.

Results: Neurophroline™ **acts very fast (2 hours) as it inhibits up to -70% of cortisol production** by keratinocytes at 1%, and -33% at 0.5%.

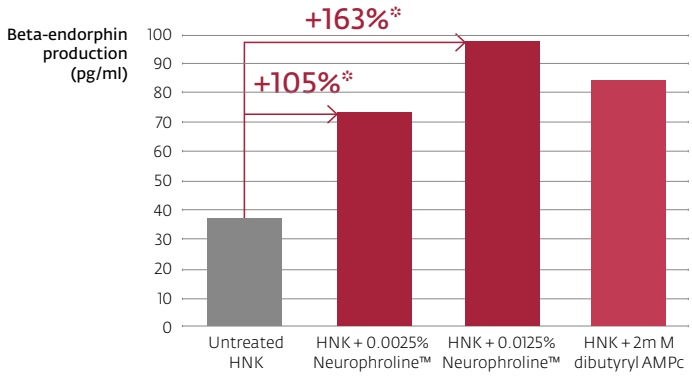


*** p<0.001 Student's t-test
** p<0.01 Student's t-test
* p<0.05 Student's t-test

Stimulation of β-endorphin production (*in vitro*)

Normal human keratinocytes were incubated for 24 hours with Neurophroline™ at 0.0025% and 0.0125%. Dibutyryl AMPc was used as a positive control to stimulate beta-endorphin release. The amount of beta-endorphin released by the cells was quantified by ELISA technic.

Results: Neurophroline™ **significantly stimulates the production of beta-endorphin by skin cells**, a natural relaxing and pain-relief peptide, **up to +163%**.



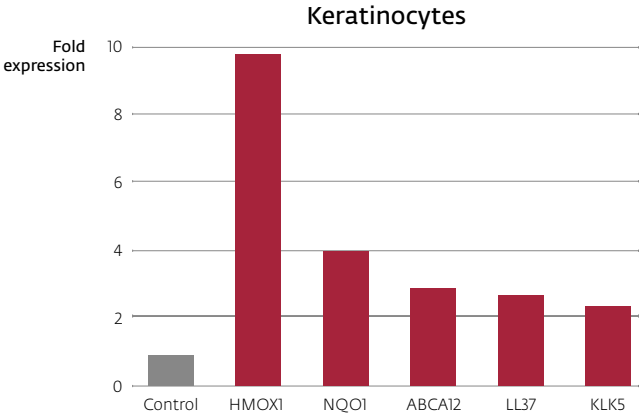
* p<0.05 Student's t-test

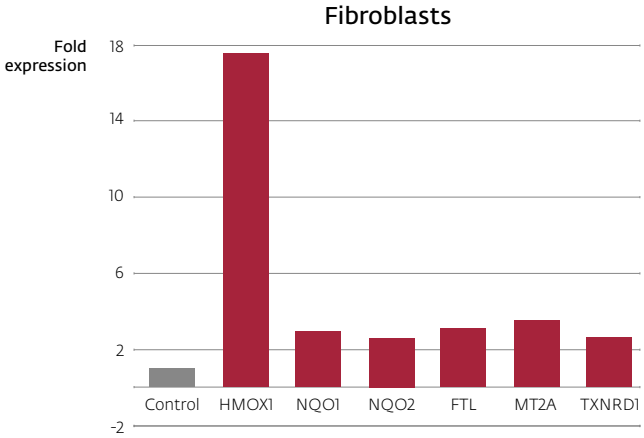
Biological activity

Instant stress regulation (*in vitro*)

1. Global activation of anti-stress genes (transcriptomic)

Normal human keratinocytes and fibroblasts were incubated with Neurophroline™ at 1% during 24 hours. The mRNA expression of genes involved in skin homeostatis was quantified by q-RT-PCR.





Genes	Name / Function	Function
HMOX1	Heme Oxygenase	Anti stress - anti oxidation
NQO1	NADPH Quinone dehydrogenase 1	Reduction of oxidative stress, detoxification
ABCA12	ATP binding cassette transporter	Skin barrier and epidermis differentiation
LL37	LL37 cathelicidin	Antimicrobial peptide, protection against infections
KLK5	Kalikrein 5	Maturation of LL37 peptide, epidermis differentiation

Genes	Name / Function	Function
HMOX1	Heme Oxygenase	Anti stress - anti oxidation
NQO1/2	NADPH Quinone dehydrogenase 1 /2	Reduction of oxidative stress, detoxification
FTL	Ferritin	Detoxification of iron
MT2A	Metallothionein	Detoxification of heavy metals
TXNRD1	Thioredoxin reductase	Control of redox system, homeostasis

Results: Neurophroline™ **significantly stimulates the expression of the genes involved in the skin cells' homeostasis** (***p<0.001 Students' t-test, for all markers), and more precisely of:

- **HMOX1, the major cellular response to stress factors,**
- **NQO1, the main answer to oxidative stress,**
- Iron and heavy metal detoxification,
- Antimicrobial markers,
- Skin barrier marker,
- Redox homeostasis marker.

Biological activity

2. Activation of major anti-stress proteins (proteomic)

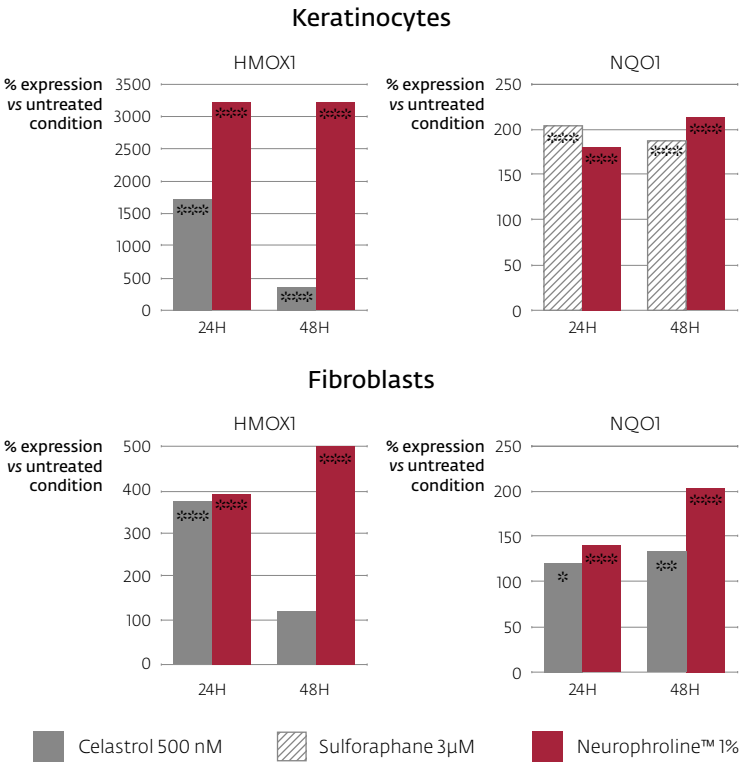
Normal human keratinocytes and fibroblasts were incubated with Neurophroline™ at 1% during 24 or 48 hours.

The level of expression of the heme oxygenase 1 and of the NADPH quinone dehydrogenase, the two main proteins involved in stress response, were quantified by ELISA assay.

Celastrol and Sulforaphane were used as positive control.

Results: Neurophroline™ at 1% **significantly stimulates the expression of the major proteins involved in cellular stress response** in both keratinocytes and fibroblasts : **up to 3000%** for **HMOX1** and **200%** for **NOQ1**.

***p<0.001 ANOVA & Dunnett comparison
**p<0.01 ANOVA & Dunnett comparison
*p<0.05 ANOVA & Dunnett comparison

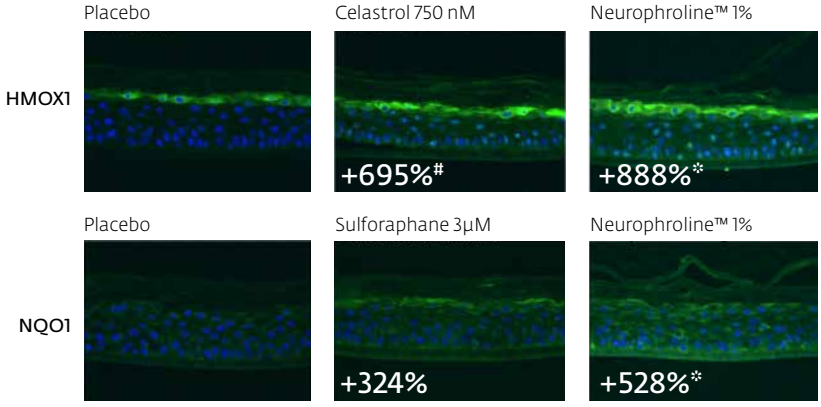


Visible expression of anti-stress markers in the epidermis (*ex vivo*)

The action of Neurophroline™ on HMOX1 and NQO1 proteins production was evaluated on human reconstructed epidermis (RHE). RHE were topically treated during 2 days with Neurophroline™ at 1% in a placebo formula, with the placebo alone, or with positive controls (celastrol or sulforaphane). RHE were sampled and analysed by immunofluorescence to quantify the amount of HMOX1 and NQO1 expressed.

Results: Neurophroline™ at 1% **significantly stimulates the expression of HMOX1 up to +888%**, and **NQO1 up to +528%** in the epidermis in only **2 days**.

*p<0.05 compared to placebo Student's t-test
#p<0.05 compared to control Student's t-test



Efficacy

Clinical efficacy on volunteers with stressed skin (polluted air)

The clinical efficacy of Neurophroline™ was evaluated in a double blind test versus placebo under dermatological control. Twenty four volunteers aged 40 to 67 years old took part in the study (average age: 53 ± 2years).

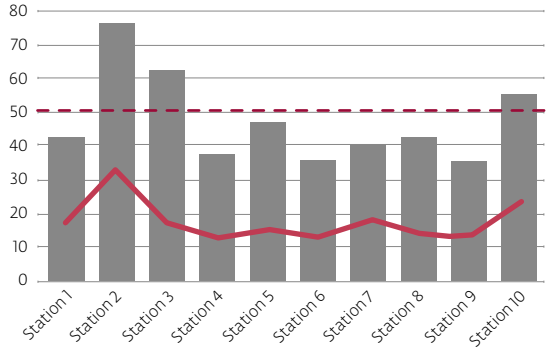
The clinical test was run during summer time, in skin-stressing conditions as the volunteers where living in an geographical area known to have significant atmospheric pollution (measured in ten air quality control stations around the location of the clinical test during the testing period).

The volunteers applied twice a day the placebo or the cream containing Neurophroline™ at 2% on the most sensitive skin part of their face: under their eyes. The clinical assessment was run for one month.

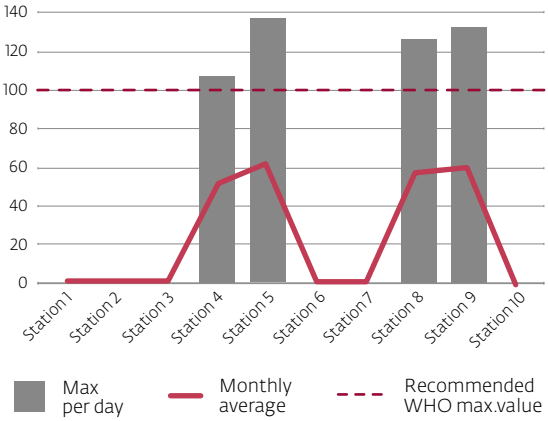
Pollution by PM10 and ozone was always very close to the recommended limits and exceeded it at some periods. Therefore the stress was very intense during the testing period.

(1) Source Airpomenia - Tricity location

Air pollution by PM10 (µg/m³) (1)



Air pollution by ozone (µg/m³) (1)



Fast improvement of skin color and luminosity

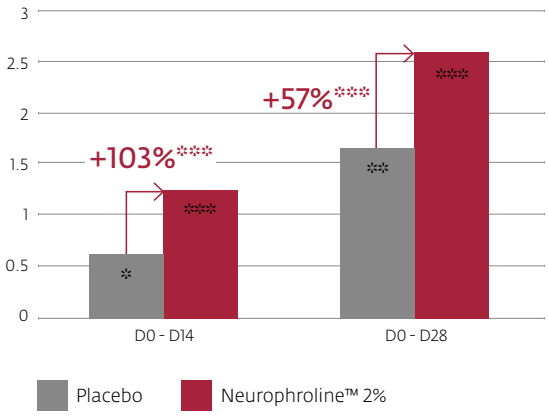
The visible efficacy of Neurophroline™ was evaluated by measuring the evolution of the L* value (skin luminosity under eyes) with a spectrocolorimeter, as well as the ITA value (skin color) and the a* value (skin redness).

1. Recovery of skin luminosity (L* value)

Results: Neurophroline™ **significantly enhances the skin luminosity** of volunteers with stressed skin **in 2 weeks**, with **up to +57% improvement in one month**.

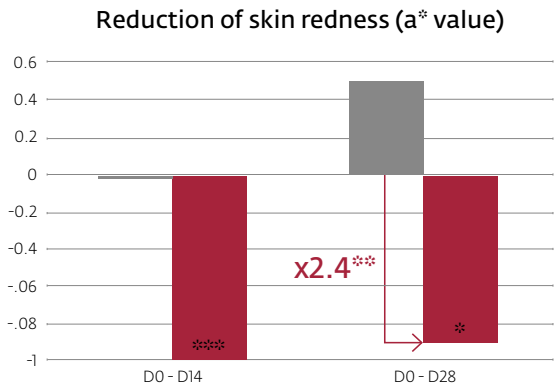
* p<0.1 ANOVA
** p<0.01 ANOVA
*** p<0.001 ANOVA

L* improvement

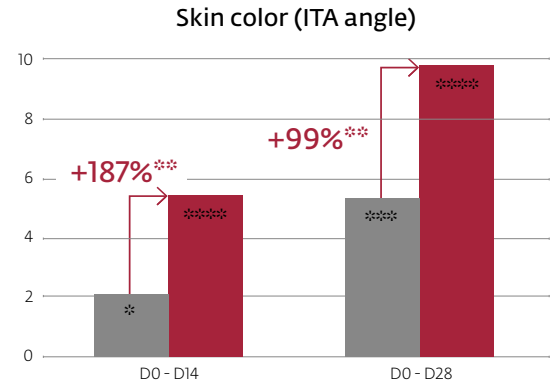


Efficacy

2. Improvement of skin color



*p<0.05 *** p<0.001 ANOVA



*p<0.05 **p<0.01 ***p<0.001 ****p<0.0001 Students' t-test

Placebo Neurophroline™ 2%

Results: Neurophroline™ **shows very rapid results at improving the global skin color** around the eye. **Skin redness is significantly reduced by 2.4 times after 28 days**, and the **skin's color is significantly improved by 99%** during the same period of time.

3. Visible efficacy after 1 month

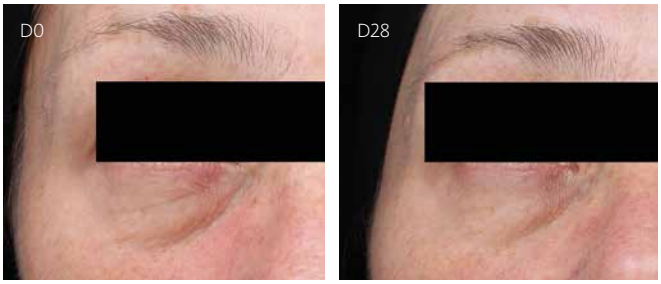
Pictures of the volunteers were taken after one month of use of Neurophroline™. The focus is done on the area under eyes since it is the most sensitive part of the skin.

Volunteer 6

Volunteer 7

Volunteer 13

Volunteer 10



Results: Neurophroline™ **visibly reduces the signs of stress/fatigue after one month** of use of the product. Dark circles are amazingly reduced, confirming the fast action of Neurophroline™ on stress relief.

Summary

Technical information

Suggested INCI:	Water (and) Propanediol (and) Tephrosia purpurea seed extract
Origin:	Vegetal extraction
Preservation:	Preservative free
Appearance:	Clear, light yellow liquid
Solubility:	Water soluble
Dosage:	0.1-2%
Processing:	Can be added at the end of the formulation process under stirring or homogenising. Can be heated for a short time with the water phase of formulation. Formulate at temperature below 50°C, and final pH below 6.0.

Claims

Claims:	cortisol release control, anti-stress, anti-ageing for sensitive skins, anti-ageing, anti-dark circles
Applications:	Anti-ageing creams, Nights and day creams fighting stress, Anti-stress / fatigue creams and serums, Dermocosmetics products, Eye care products.

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