Lipoid Kosmetik

HerbaProtect NOX

- Prevention of UV-induced, delayed skin damage
- Reduction and soothing of sunburn
- Deceleration of photoaging

Introduction

HerbaProtect NOX is a novel sun care active that can protect from UV-induced skin damage, which has been shown to occur even hours after sun exposure [1]. Three active components, plant extracts from kakadu plum, pomegranate flower and perilla leafs have been combined in a preservative-free, standardized and concentrated glycerol based self-preserving solvent system. HerbaProtect NOX delivers soothing to stressed skin, reduces the UV-mediated enzymatic stress response and the formation of intracellular nitrogen and oxygen species leading to damage of cellular structures.

Based on the proposed mechanistic approach, HerbaProtect NOX was designed and tested with regards of having the ability to act on peroxynitrite, which is a key component in the formation of UV-induced delayed skin damage or dark CPD. A customized *in vitro* method was used to determine not only the antioxidative potential but also to prove the specific peroxynitrite-scavenging activity of HerbaProtect NOX. An *in vivo* study employing the innovative ICL-S technique successfully demonstrated an immediate and dose dependent effect on UV-A irradiated human skin.

Properties

- *in vitro* and *in vivo* efficacy
- Preservative-free
- Self-preserving
- Easy to formulate
- China INCI and Reach compliant

INCI

US: Glycerin, Water, Punica Granatum Flower Extract, Perilla Frutescens Leaf Extract, Terminalia Ferdinandiana Fruit Extract
EU: Glycerin, Aqua, Punica Granatum Flower Extract, Perilla Frutescens Leaf Extract, Terminalia Ferdinandiana Fruit Extract

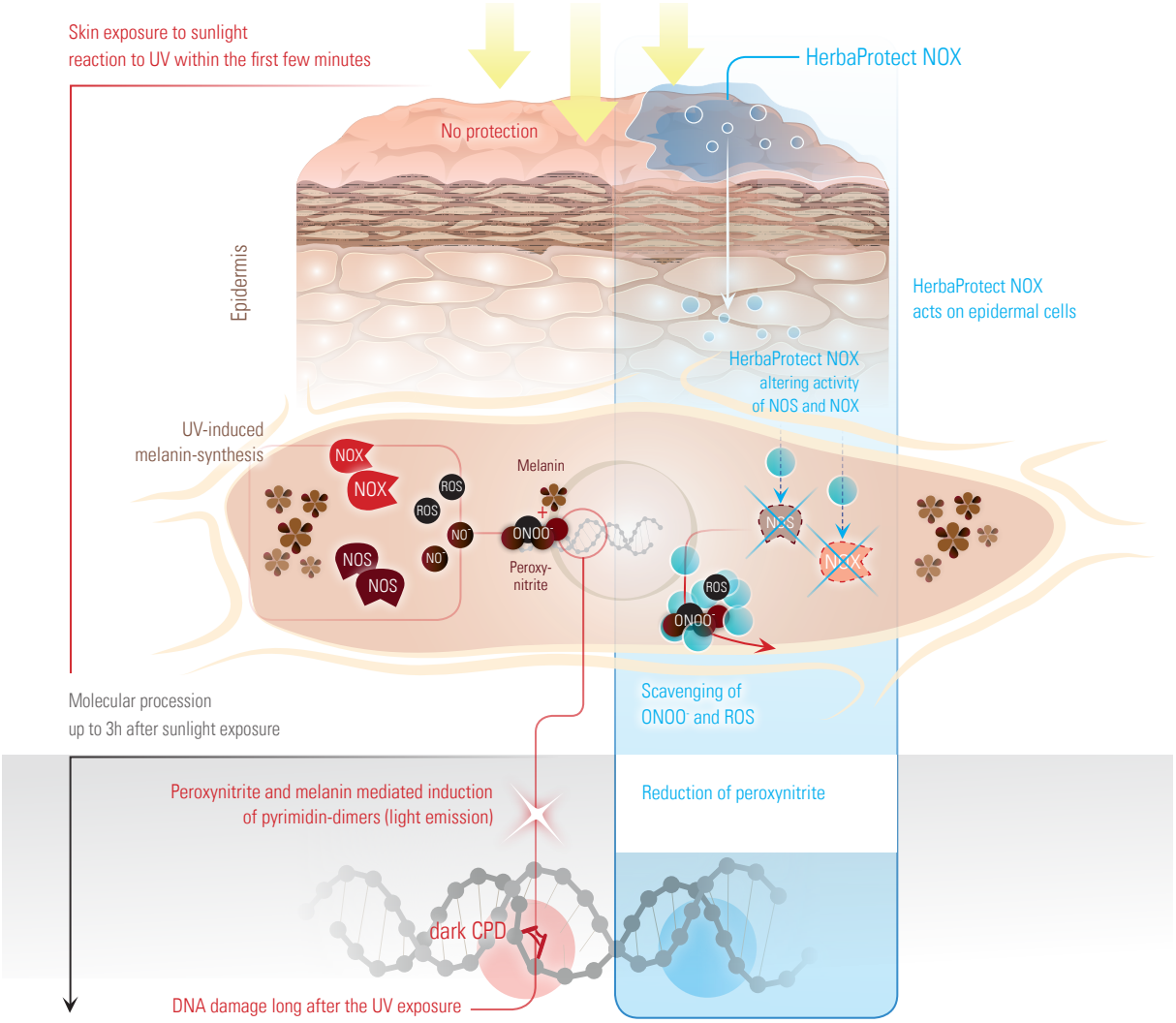
Recommended Applications & Use Levels

- After Sun Care / Sun Care / Day Care
- Anti-Aging / Anti-Wrinkle
- Anti-Inflammatory- / Antioxidant- / Anti-Irritant- / Soothing-Products

Recommended use level: 1-3%

Description

Exposure to ultraviolet (UV) radiation is the major cause for physiological skin damage such as photoaging and erythema. Recent research has shown that UV-induced DNA damage in melanin-containing cells of the epidermis can occur several hours after the radiation has ended and even in the dark [1]. Intracellular melanin and peroxynitrite ($ONOO^-$), a powerful oxidant derived from the UV mediated enzymatic stress response (upregulation of NADPH oxidase [NOX] and nitric oxide synthase [NOS]), can cause DNA damage in form of cyclobutane pyrimidine dimers (CPD). In a reaction under photon emission, melanin and $ONOO^-$ form highly reactive intermediates which can cause CPD, erythema and skin damage. Since this reaction can occur long after the UV exposure has ended these CPD are referred to as "dark CPD".



HerbaProtect NOX counteracts the formation of dark CPD by a synergistic mechanism that has been investigated in several studies (in vitro, in vivo). In addition to inhibiting enzymes involved in the formation of peroxynitrite (NOS, NOX), HerbaProtect NOX also acts as an efficient scavenger of peroxynitrite-precursors (nitric oxide, superoxide) and peroxynitrite itself.

HerbaProtect NOX is a combination of three plant extracts in a glycerol based preservative free and self-preserving solvent system. This combination is made possible by a novel production method which allows to combine and concentrate hydrophilic and hydrophobic fractions of the extracts in a standardized, stable and easy to handle product.

Pomegranate flower extract is capable of reducing the perception of pain associated with an inflammatory state by downregulation of the nerve growth factor (NGF) gene [2]. Furthermore it has anti-inflammatory and wound healing effects and shows anti-collagenase, anti-elastase and antioxidant activities [3].

Perilla leaf extract components have been shown to reduce NOX and iNOS related release of reactive oxygen and nitrogen species and also have strong scavenging and anti-inflammatory activites [4,5,6,7]. These effects lead to a reduction of intracellular peroxynitrite concentration [1,8].

Kakadu plum extract exhibits extremely high levels of stable ascorbic acid [9]. It was shown to have iNOS and COX-2 expression inhibition and shows antioxidative and cytoprotective activities [10,11].

Peroxynitrite Scavenging Activity

Two cell culture based experiments were carried out in order to assess the antioxidant and peroxynitrite scavenging activity of HerbaProtect NOX. Human keratinocytes were preconditioned with different concentrations of HerbaProtect NOX for 24 h and after a washing step, loaded with hydrophenylflourescin (HPF) which is a selective dye for the detection of highly reactive oxygen species. After this preconditioning, cells were treated with 0.35 mM hydrogenperoxide (H_2O_2) or 41.4 mM peroxynitrite ($ONOO^-$) and the generated oxidative stress was quantified by fluorescence measurement.

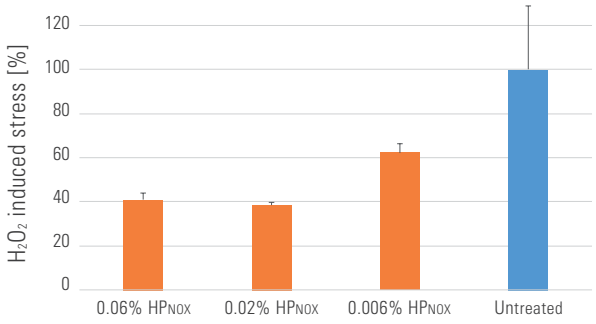


Fig. 1 Reduction of the H_2O_2 induced oxidative stress in human keratinocytes

Results:

- At a concentration of 0.06%, HerbaProtect NOX reduced H_2O_2 induced intracellular stress by 60% (Fig. 1)
- At a concentration of 0.06%, HerbaProtect NOX reduced $ONOO^-$ induced intracellular stress by 20% (Fig. 2)
- HerbaProtect NOX shows specific action against peroxynitrite, a crucial component in the formation of dark CPD

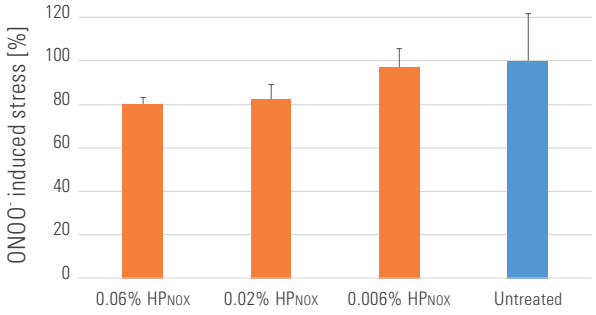


Fig. 2 Reduction of the $ONOO^-$ induced oxidative stress in human keratinocytes

in vivo induced Chemiluminescence in Human Skin

The ICL-S (induced chemiluminescence in skin) method allows an *in vivo* real time measurement of oxidative stress induced by environmental factors, such as irradiation, heat or mechanical stress. Free radicals damage cellular components and cause ultraweak photon emission, which is detectable as a chemiluminescence signal [12].

In an acclimatized light-tight darkroom, skin of the volar forearms of ten volunteers was stressed with half the minimal erythemal dose (MED) of UV-A light and the resulting ultraweak light emission (ICL signal) was measured. Skin test areas of ten volunteers were divided into 4 test fields. Two blank sites with no product application of which one was irradiated with 0.5 MED of UV-A and two product test sites, which were treated with cream formulations containing 1% and 3% Herba- Protect NOX, 5 minutes after the irradiation. The ICL signals were measured prior to the irradiation (t_0) as well as 15 min. and 120 min. after the irradiation. Results are showen in Fig. 3.

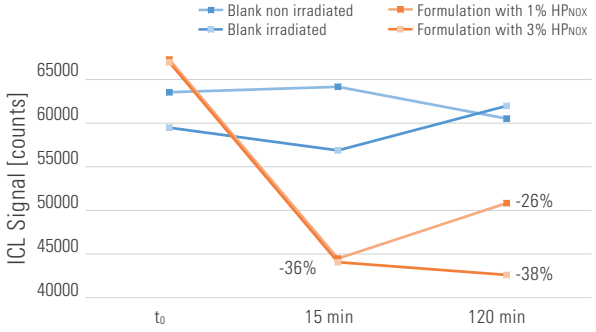


Fig. 3 Reduction of light emission (ICL signal) *in vivo* after treatment with Herba-Protect NOX containing formulations

Results:

- Application of HerbaProtect NOX containing creams caused an immediate and dose dependent reduction of the ICL signal
- The reduction of the ICL signal indicates a higher antioxidant capacity of the skin and less radical related reactions
- 15 minutes after the UV-A irradiation, the emission of photons (ICL signal) in skin treated with 3% and 1% HerbaProtect NOX was reduced by 36%
- After 2h, the emission of photons in skin treated with 3% HerbaProtect NOX was still reduced by 38%
- The reduction of the ICL signal in skin treated with 1% HerbaProtect NOX after 2 h was 26%

Regulatory and Safety

INCI of the plant material	Punica Granatum Flower Extract Perilla Frutescens Leaf Extract Terminalia Ferdinandiana Fruit Extract
Origin	Punica Granatum Flower Extract : India* Perilla Frutescens Leaf Extract: China* Terminalia Ferdinandiana Fruit Extract: Australia*
Safety	Toxicology: • Non phototoxic (OECD 432) • Non irritating for skin (SPT, single patch test, when tested at 5,5 %) • Non mutagenic (Ames test – OECD 471) • No allergens (as per current EU Cosmetic Regulation) • Moderately irritating for eyes (HET CAM), when tested at a concentration of 2.5%. Considerations should be given to include 'Avoid eye contact' or equivalent in the instruction for use of the final cosmetic product, if applicable.
EU Cosmetic Regulation	The product complies to the EU Cosmetic Regulation (EC) No 1223/2009.
Natural/organic	The product can be used in COSMOS certified formulations. Application for a 'COSMOS approved ingredient' is submitted.
ABS	The plant material used fully complies with the requirements of Access and Benefit Sharing (ABS) as derived from the Nagoya Protocol and its corresponding national legislation. Detailed information about the compliance is available.
EU Reach	The product, i.e. its substances, conforms to the Regulation (EC) No 1907/2006.
China Reach	All ingredients conform to the legislation of China Reach.
China INCI	All INCIs are listed in the current Inventory of Existing Cosmetic Ingredient China (IECIC).
Halal	All ingredients conform to HALAL requirements, considering the following: Traces of ethanol remaining in HerbaProtect NOX at the end of the manufacturing process are technically unavoidable. The ethanol is plant-derived and non-GMO. Its concentration is equal or less than 0.001% at a maximal recommended use level of max. 5 % HerbaProtect NOX in the final cosmetic product.

* Not part of the specification

References

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