HerbaProtect NOX

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

Introduction

HerbaProtect NOX is a novel sun care active that can protect against UV-induced skin damage, which has been shown to occur even hours after sun exposure. Three active components, namely plant extracts from pomegranate flower, from perilla leaf and from kakadu plum, have been combined in a preservative-free, standardized and concentrated glycerol-based self-preserving solvent system.

HerbaProtect NOX was designed based on the properties of the three plant extracts, and its activity was investigated with respect to the ability to act on peroxynitrite, a key component in the formation of UV-induced delayed skin damage. A customized in vitro method was used to determine not only the antioxidative potential, but also to prove the specificity of peroxynitrite scavenging by HerbaProtect NOX. An in vivo study employing the innovative ICL-S technique clearly demonstrated an immediate antioxidative effect of HerbaProtect NOX on UV-A irradiated human skin. Since it has been shown that an increased antioxidant capacity also protects against Infrared-A (IR-A)-induced skin aging, HerbaProtect NOX provides a perfect addition for sun care formulations.

Taken together, HerbaProtect NOX delivers soothing to stressed skin, reduces the UV-mediated enzymatic stress response as well as the formation of intracellular nitrogen and oxygen species in order to prevent damage of cellular structures.

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 Products
- Anti-Inflammatory / Antioxidant / Anti-Irritant / Soothing Products

Recommended use level: 1-3%
Exposure to ultraviolet (UV) radiation is the major cause of 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 and even in the dark[1]. Peroxynitrite (ONOO\(^-\)) is a powerful oxidant that is produced during the UV-mediated enzymatic stress response through the up-regulation of NADPH oxidase (NOX) and nitric oxide synthase (NOS). ONOO\(^-\) and intracellular melanin form highly reactive intermediates in a photon-emitting reaction, which can cause erythema, skin damage, and DNA damage in form of cyclobutane pyrimidine dimers CPD. Since the formation of CPD can also occur long after the UV exposure, 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 and in vivo). Besides inhibiting the enzymes involved in the formation of ONOO\(^-\) (namely NOX and NOS), HerbaProtect NOX also acts as an efficient scavenger of ONOO\(^-\)-precursors (nitric oxide and superoxide) as well as of ONOO\(^-\) itself.

HerbaProtect NOX is a unique combination of three plant extracts in a glycerol-based, preservative-free and self-preserving solvent system. This combination could only be realized through 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 that is associated with an inflammatory state by down-regulating 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 NOS-related release of reactive oxygen (ROS) and nitrogen species, and has additionally strong scavenging and anti-inflammatory activities[4,6,7]. These properties result in a reduction of intracellular ONOO\(^-\) concentrations[1,8].

Kakadu plum extract exhibits extremely high levels of stable ascorbic acid[9]. It has been shown to inhibit the expression of NOS, and it exhibits antioxidative, anti-inflammatory and cytoprotective activities[10,11].

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**Peroxynitrite Scavenging Activity**

Two-cell culture-based experiments were carried out in order to assess the antioxidant and peroxynitrite (ONOO⁻) 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 (ROS). After the preconditioning, cells were treated with 0.35 mM hydrogenperoxide (H₂O₂) or 41.4 mM ONOO⁻ and the generation of oxidative stress was quantified by fluorescence measurement.

**Results:**
- At a concentration of 0.06%, HerbaProtect NOX reduced H₂O₂-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 ONOO⁻, a crucial component in the formation of dark CPD.

**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 that is 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 their individually calculated minimal erythemal dose (1 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 without product application of which one was irradiated with 1 MED of UV-A; and two irradiated product test sites, which were treated with cream formulations containing 1% and 3% HerbaProtect NOX 5 min after the irradiation. The ICL signals were measured prior to the irradiation (t₀) as well as 15 min and 120 min after the irradiation. The results are shown in Fig. 3.

**Results:**
- Application of HerbaProtect NOX-containing creams caused an immediate reduction of photon emission (ICL signal).
- The reduction of the ICL signal indicates a higher antioxidant capacity of the skin and less radical-related reactions.
- 15 min after UV-A irradiation, the ICL signal in the skin treated with 1% HerbaProtect NOX was significantly reduced by 45% compared to the ICL signal at t₀; this effect remained stable for at least 2 h.
- The ICL signal in the skin treated with 3% HerbaProtect NOX was significantly reduced by 38% after 15 min compared to t₀, and 44% after 2 h.
- The results imply that 1% HerbaProtect NOX is sufficient to deliver maximum antioxidative protection to the skin.

**HerbaProtect NOX provides Infrared-A protection in sun care formulations**

Infrared-A (IR-A)-related skin aging has lately been demonstrated by several research groups around the world. Antioxidants were shown to provide a protective effect against IR-A-induced skin aging.[13]. Since HerbaProtect NOX immediately increases the antioxidant capacity of human skin *in vivo* for several hours, it is a perfect addition for sun care formulations.
**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**
HerbaProtect NOX is a raw material approved by ECOCERT GREENLIFE, conform to the COSMOS Standard.

**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 INCs 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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11. Tan et al., Antioxidant and cytoprotective activities of native Australian fruit polyphenols, Food Research International 44:2034–2040, 2011
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