HerbaProtect NOX – Increasing the oxidative capacity of the skin after sun exposure

Dr. Peter Röthlisberger, CEO Lipoid Kosmetik AG
April 13th 2016, Innovation Seminar @in-cosmetics
Agenda

- Introduction
- UV exposure and skin aging
- Scientific background

- Herbaprotect NOX
- Efficacy tests
  - Ingredients and properties
  - *In vitro*
  - *In vivo*

- Mechanism of action

- Product Information

For more information:

Contact LIPOID KOSMETIK:

info@lipoid-kosmetik.com
UV and Skin damage

- **UV-B**: can create skin damage and DANN damage (DNA photoproducts (CPD)) almost instantaneously. Melanin act as a shield.

- **UV-A**: lower energy. No immediate skin damage. But responsible for skin aging.

- Less scattering, less absorption: deeper penetration.

Adapted from: www.molescope.com
Absorption spectrum of melanin

- The pigment melanin protects against sunlight induced burns and DNA damage (and skin cancer)

1 Kollias et al. (1991) Photobiology B9, 135-160
Assessment of **UVA** induced DNA damage in mice

UV-AB

(wild type)

immediate

DNA damage (DNA Photoproducts)

Sanjay Premi et al., Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure, Science 347:842-847, 2015
Assessment of \textbf{UVA} induced DNA damage in mice

UV-A

\begin{itemize}
\item (wild type)
\item \textbf{immediate}
\item \textbf{No DNA damage (DNA Photoproducts)}
\end{itemize}

Sanjay Premi et al., Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure, Science 347:842-847, 2015
Assessment of UVA induced DNA damage in mice

UV-A

(wild type)

3h

'delayed' DNA damage

Sanjay Premi et al., Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure, Science 347:842-847, 2015
Assessment of UVA induced DNA damage in mice

UV-A

3h
No DNA damage

3h
'delayed' DNA damage

(albino)

(wild type)
Assessment of **UVA** induced DNA damage in mice

- **UV-A**
  - (albino) after 3h: No DNA damage
  - (wild type) after 3h: 'delayed' DNA damage

Melanin plays an important role

Sanjay Premi et al., Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure, Science 347:842-847, 2015
Scientific Background

RESEARCH ARTICLE

Chemixcitation of melanin derivatives induces DNA photoproducst long after UV exposure

Sanjey Premi1, Silvia Wallisch1, Camila M. Mano1,2, Adam B. Weiner1,†, Antonella Bacchiocchi1, Kazumasa Wakamatsu4, Etelvino J. H. Bechara2,5,†, Ruth Halaban3,6, Thierry Douki7,7†, Douglas E. Brash1,6,†

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Science 20 Feb 2015:
Vol. 347, Issue 6224, pp. 842-847
DOI: 10.1126/science.1256022

Chemixcitation:
Generation of reactive molecules

Assessment of UV induced DNA damage in mice

http://news.yale.edu/2015/02/19/sunlight-continues-damage-skin-dark

CPD: Cyclobutane Pyrimidine Dimers or DNA Photoproducts
Long After Exposure DNA in Skin Cells
UV Exposure Continues to DNA in Skin Cells
A new study by Yale scientists indicates that damaging UV rays continue to wreak havoc on melanocytes, the skin cells that make melanin, for as much as three hours after sun exposure.

UV Light richer in the steepest of the sun remains.

A yet another danger ahead.

Could using sunscreen at night prevent skin cancer? Damage caused by UV light continues for hours after dark, finds study

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Proposed mechanism of action by Premi et al.

Premi et al., Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure, Science 347:842-847, 2015
HerbaProtect NOX: Mechanism of action
Approach: Mechanisms of action

UV Irradiation

UV induced enzymatic stress-reaction in cells

→ Release of ROS and NO⁻
→ Major Player: NOX family (NADPH Oxidases)
  → NO Synthase

Formation of peroxynitrite (ONOO⁻)

Reaction of peroxynitrite with eg. melanin

Chemiexcitation and Light emission and dark CPD formation
Take Home Message

• UV induced skin damage can still occur hours after sun exposure
• The mechanism of this event includes creation of reactive oxygen species, nitric oxide and peroxinitrite formation
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.

- Three plant extracts
  - Perilla leaf (*Perilla Frutescens*)
  - Pomegranata flower (*Punica Granatum*)
  - Kakadu plum (*Terminalia Ferdinandiana*)
Perilla leaf

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 activities\[^{[4,6,7]}\]. These effects lead to a reduction of intracellular peroxynitrite concentration\[^{[1,8]}\].

- **Effect on NOX (NADPH Oxidase)**\[^{[7]}\] and NOS (Nitric Oxide Synthase)\[^{[6]}\]
  - → Reduction of peroxynitrite formation

- **Direct antioxidant**
  - → Reduction of radicals and ONOO⁻

- **Anti-inflammatory**
  - → Reduction of UV induced inflammation

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1) Premi et al., Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure, Science 347:842-847, 2015
2) Huang et al., Anti-inflammatory effects of Perilla frutescens leaf extract on lipopolysaccharide-stimulated RAW 264.7 cells, Molecular Medicine Reports 10: 1077-1083, 2014
3) Osakabe et al., Rosmarinic acid inhibits epidermal inflammatory responses: Anticarcinogenic effect of Perilla frutescens extract in the murine two-stage skin model, Carcinogenesis 25/4: 549-557, 2004
5) Takahashi et al., 1,2-Di-O-a-Linolenoyl-3-O-b-galactosyl-sn-glycerol as a superoxide generation inhibitor from Perilla frutescens var. Ispa, Biosci. Biochem., 75(11): 2240-2242, 2011
6) Valencia et al., Nox1-based NADPH oxidase is the major source of UVA-induced reactive oxygen species in human keratinocytes, J Investigative Dermatology, 128:214-222, 2007
Perilla leaf

Polyphenols
Flavon-Glycosides
Phenolic acids

Perilla leaves  Rosmarinic Acid  Luteolin

Rosmarinic acid
inhibits epidermal inflammatory responses and NOS (Nitric oxide synthase)

Luteolin and its Glycosides
(Luteolin 7-O-diglucuronide and Apigenin 7-O-diglucuronide)

Antioxidative, inhibits inflammatory response. Inhibits IL-6 and Phospodiesterase.

Osakabe et al., Rosmarinic acid inhibits epidermal inflammatory responses: Anticarcinogenic effect of Perilla frutescens extract in the murine two-stage skin model, Carcinogenesis 25/4: 549-557, 2004
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\(^3\).

- **Antioxidant**
  - Upregulation of SOD and HMOX
  - Reduction of radicals and ONOO⁻
- **Anti-inflammatory**
  - Reduction of UV induced inflammation
- **Downregulation of nerve growth factor (NGF)**
  - Reduction of pain perception = Soothing
- **Downregulation of MMP-1**
  - Against photoaging effects

---
2) Klotzenburg et al., Neutralization of endogenous NGF prevents the sensitization of nociceptors supplying inflamed skin, European Journal of Neuroscience 11(5):1698-704, 1999
3) J.Smits et al., Effects of pomegranate flower complex on skin, Personal Care p. 45-50, March 2012
Pomegranate flower

Cell culture assay:
Induction of photoaging by UV-A irradiation on human fibroblasts:

Reduction of photoaging effects by 68%.
Pomegranate flower

UV-A protection in human fibroblasts NHDF
Irradiation with 12.5mJ/cm² UV-A: microscopy and XTT assay

Human fibroblasts + UV-A + PGFE 0.1%

Increase cell viability of UV irradiated fibroblasts by 50%
Kakadu Plum

Kakadu plum extract exhibits extremely high levels of stable ascorbic acid\(^8\). It was shown to have iNOS and COX-2 expression inhibition and shows antioxidative and cytoprotective activities\(^{10,11}\).

- **Antioxidant**
  - → Reduction of radicals and ONOO\(^-\)
- **Inhibition of NOS (NO Synthase)**
  - → Reduction of radical release
- **Inhibition of COX-2 expression**
  - → Reduction inflammatory response

---

Tan et al., Native Australian fruit polyphenols inhibit COX-2 and iNOS expression in LPS-activated murine macrophages, Food Research International 44:2362–2367, 2011
Tan et al., Antioxidant and cytoprotective activities of native Australian fruit polyphenols, Food Research International 44:2034–2040, 2011
HerbaProtect NOX: Summary of extracts’ properties

- Skin exposure to sunlight reaction to UV within the first few minutes
- HerbaProtect NOX
- No protection
- HerbaProtect NOX acts on epidermal cells
- UV-induced melanin-synthesis
- Molecular procession up to 3h after sunlight exposure
- Peroxynitrile and melanin mediated induction of pyrimidin-dimer (light emission)
- DNA damage long after the UV exposure
- Scavenging of ONOO- and ROS
- Reduction of peroxynitrile and dark CPD formation
Take Home Message

- UV induced skin damage can still occur hours after sun exposure
- The mechanism of this event includes creation of reactive oxygen, nitric oxide and finally peroxinitrite formation
- The ingredients of Herbaprotect NOX can act on the inhibition of the enzymes involved and the formation of these reactive molecules
HerbaProtect NOX: Properties

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.

- Glycerol based
- Preservative free
- Self-preserving
- Standardized
- Concentrated
- Easy to handle
- COSMOS approved

- After Sun Care / Sun Care / Day Care
- Anti-Aging / Anti-Wrinkle
- Anti-Inflammatory- / Antioxidant- / Anti-Irritant- / Soothing-Products
- Recommended use level: 1-3%
HerbaProtect NOX: Efficacy tests

- Peroxinitrite scavenging activity in human keratinocytes (*in vitro*)
- 20 Patient study of the anti-oxidative capacity of the skin by induced chemiluminescence (*in vivo*)
HerbaProtect NOX: Efficacy tests

Efficacy tests: Peroxinitrite scavenging activity (*in vitro*)

**Peroxyinitrite 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 hydrophosphylfluorescein (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 ($\text{H}_2\text{O}_2$) or 41.4 mM peroxynitrite ($\text{ONOO}^-$) and the generated oxidative stress was quantified by fluorescence measurement.

- Keratinocyte cell culture
- Preconditioned with HP$_{\text{NOX}}$ for 24h
- Loaded with ROS detection dye (HPF)
- Fluorescence measurement
- „Stress treatment“:
  - $\text{H}_2\text{O}_2$ – Hydrogenperoxide
  - $\text{ONOO}^-$ - Peroxynitrite
HerbaProtect NOX: Efficacy tests

Efficacy tests: Peroxinitrite scavenging activity (*in vitro*)

- 60% reduction in intracellular stress by 0.06% HP\textsubscript{NOX}

- 20% reduction in intracellular stress by 0.06% ONOO\textsuperscript{-}

HP\textsubscript{NOX} showed specific action against peroxynitrite, a crucial component in the formation of dark CPD.
HerbaProtect NOX: Peroxinitrite scavenging activity \textit{(in vitro)}
Take Home Message

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- The mechanism of this event includes creation of reactive oxygen, nitric oxide and finally peroxinitrite formation
- The ingredients of Herbaprotect NOX can act on the inhibition of the enzymes involved and the formation of these reactive molecules
- Herbaprotect NOX has been tested *in vitro* and *in vivo*.
- *In vitro*: Herbaprotect NOX reduced peroxide and peroxinitrite induced intracellular stress by 60% and 20% respectively
HerbaProtect NOX: induced chemiluminescence (in vivo)

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].

- Detection of radical reactions by measurement of light emission
- After induction: ICL signal relates to „antioxidant status“ of the skin

Jain et al., Non-invasive in vivo measurement of oxidative stress in human skin, SOFW-Journal 136:9, 2010

F. Kayibri, Untersuchung oxidativer Prozesse anhand induzierter ultraschwacher Photonenemission (UPE), 2005
HerbaProtect NOX: induced chemiluminescence (*in vivo*)

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 (t₀) as well as 15 min. and 120 min. after the irradiation. Results are shown in Fig. 3.

- Modified UV-A stress ICL-S protocol
- Skin stress by application of 0.5 and 1 MED UV-A
- 20 test persons (caucasian)
- 5 min after irradiation:
  Application of HP_{NOX} formulation (1% and 3%)
- ICL-S measurement at t₀, after 15 min. and 2h
HerbaProtect NOX: Efficacy tests

Efficacy tests: induced chemiluminescence (in vivo) (0.5MED)

- Immediate and dose dependent effect
- After 15min.: 38% reduction
- After 2h: 44% red. (3% HP\textsubscript{NOX})
  45% red. (1% HP\textsubscript{NOX})

The reduction of the ICL signal indicates a higher antioxidant capacity of the skin and less radical related reactions.
HerbaProtect NOX: Efficacy tests

Efficacy tests: induced chemiluminescence (in vivo) (1 MED)

Immediate and dose dependent effect

After 15min.: 39% reduction
After 2h: 39% red. (3% HP\textsubscript{NOX})
36% red. (1% HP\textsubscript{NOX})

The reduction of the ICL signal indicates a higher antioxidant capacity of the skin and less radical related reactions.
Take Home Message

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• Herbaprotect NOX has been tested *in vitro* and *in vivo*.
  • *In vitro*: Herbaprotect NOX reduced peroxide and peroxinitrite induced intracellular stress by 60 and 20% respectively
  • *In vivo*: Herbaprotect NOX could significantly increase the anti-oxidative capacity of the skin, even hours after the application
HerbaProtect NOX: Summary

Inhibition of radical formation
Anti-inflammatory effects

Neutralization of radicals
(in vitro)

Increase of antioxidant capacity
(in vivo)
Product Informations

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

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

• 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.
Take Home Message

- UV induced skin damage can still occur hours after sun exposure
- The mechanism of this event includes creation of reactive oxygen, nitric oxide and finally peroxinitrite formation
- The ingredients of Herbaprotect NOX can act on the inhibition of the enzymes involved and the formation of these reactive molecules
- Herbaprotect NOX has been tested in vitro and in vivo.
  - In vitro: Herbaprotect NOX reduced peroxide and peroxinitrite induced intracellular stress by 60 and 20% respectively
  - In vivo: Herbaprotect NOX could significantly increase the anti-oxidative capacity of the skin, even hours after the application
- HerbaProtect NOX can prevent UV induced sun damage, can reduce and soothe the effects of sun burn and decelerate photoaging.
- Herbprotect NOX can be used in Sun Care, After Sun Care and Anti-aging products.
Thank you for your attention!

www.lipoid-kosmetik.com
# HerbaProtect NOX: Efficacy tests

## Protective After Sun Lotion
Based on HerbaProtect NOX and LIPOID P 75-3

<table>
<thead>
<tr>
<th>Phase</th>
<th>Ingredient</th>
<th>INCI</th>
<th>Function</th>
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<td>Active ingredient</td>
<td>Lipoid Kosmetik</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>NaOH 10%</td>
<td>Sodium Hydroxide</td>
<td>Neutralizing agent</td>
<td></td>
<td>q.s.</td>
</tr>
<tr>
<td></td>
<td>Perfume</td>
<td>Parfum (Fragrance)</td>
<td>Fragrance</td>
<td></td>
<td>q.s.</td>
</tr>
</tbody>
</table>