The need for night-time sun care products

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Abstract

New discoveries indicate that the skin stress mediated by sunlight continues for hours after the skin has been exposed to UV irradiation; it continues even at night-time. Sunlight induces the generation of oxygen and nitrogen radicals that bring the skin's own melanin to an excited state. Subsequently, the melanin transfers this excess energy to the DNA, which ultimately causes delayed skin damage, so-called 'dark CPDs'.[1]

HerbaProtect NOX combines plant extracts from pomegranate flower, perilla leaf and kakadu plum, in a preservative-free, standardised and concentrated glycerol-based solvent system. The COSMOS-approved anti-ageing active acts on reactive oxygen and nitrogen species as well as on peroxynitrite, making it a perfect addition for sun care, after-sun or anti-ageing formulations that aim at protecting the skin against delayed sun-induced skin stress mediated by UV radiation, visible light or even infrared radiation.

Exposure to UV radiation is the major cause of physiological skin damage, such as photoageing and erythema. Recent research, however, has shown that UV-induced skin damage can occur several hours after the radiation and even at night-time. Alarmingly, it appears that half of the damage caused by sunlight actually occurs long after exposure to the sun.[1]

It is actually not surprising that sun exposure not only induces immediate high-level skin damage, but also sustained low-level skin damage. This fact becomes obvious when considering the natural tanning reaction, which is the skin's response to damage caused by UV radiation: Immediate pigment darkening begins during UV exposure and is instantaneously visible, as it is caused by melanin already present in the skin. The delayed tanning reaction arises in the days following UV exposure and is stimulated by endogenous signalling reactions. This example shall help to understand that stepping out of the sun is not sufficient to protect against its damage. In fact, the sun can initiate a persistent challenge to the skin, referred to as delayed sun-induced skin damage (Fig 1), which needs to be additionally counteracted.

This rather disregarded, delayed part of the sun-induced skin damage illustrates that novel and innovative cosmetic concepts are required to combat sun damage entirely, for instance 'sun protection after sunset' or 'night-time sun care products'.

The generation of peroxynitrite causes damage long after UV exposure

UV radiation generates oxidative stress and DNA damage almost instantaneously, i.e.

Figure 1: Sun protection after sunset. Sunlight induces a cascade of signals that occur even under darkness and eventually lead to delayed cellular damage. This implies that mere sun protection is not enough and that 'night-time sun care products' that provide more than moisturising properties are required after sun exposure, in order to prevent the ongoing damage of UV radiation.

within picoseconds upon sun exposure. However, current research suggests that sun-induced damage continues for several hours after UV exposure.[1] The explanation for this intriguing discovery is as follows:

- Sun exposure induces the enzymes NADPH oxidase (NOX) and nitric oxide synthase (NOS), which generate reactive oxygen and nitrogen species (ROS and NO, respectively) in the skin. The reaction of these molecules with each other leads to the formation of peroxynitrite ($ONOO^-$).[2]
- The highly reactive peroxynitrite, in turn, reacts with melanin resulting in excited melanin, which slowly diffuses into the nucleus. In the nucleus, the excited melanin either emits light, or transfers its excess energy to DNA, eventually creating DNA damage, such as cyclobutane pyrimidine dimers (CPDs). Since the formation of these CPDs occurs hours after the original UV exposure, they are referred to as 'dark CPDs' (Fig 2).[1,3]

This delayed reaction apparently causes the same amount of DNA damage as immediate effects.[1] The slow course of the reaction, however, gives time for intervention. Indeed, antioxidant cosmetic active ingredients that scavenge free radicals and nitric oxide production or that quench excited melanin represent a promising approach to counteract delayed UV-induced skin damage and pave the way for innovative anti-ageing, after-sun and light protection concepts, be it a sunscreen for the length of time spent sunbathing or a novel protective cream for the subsequent period.

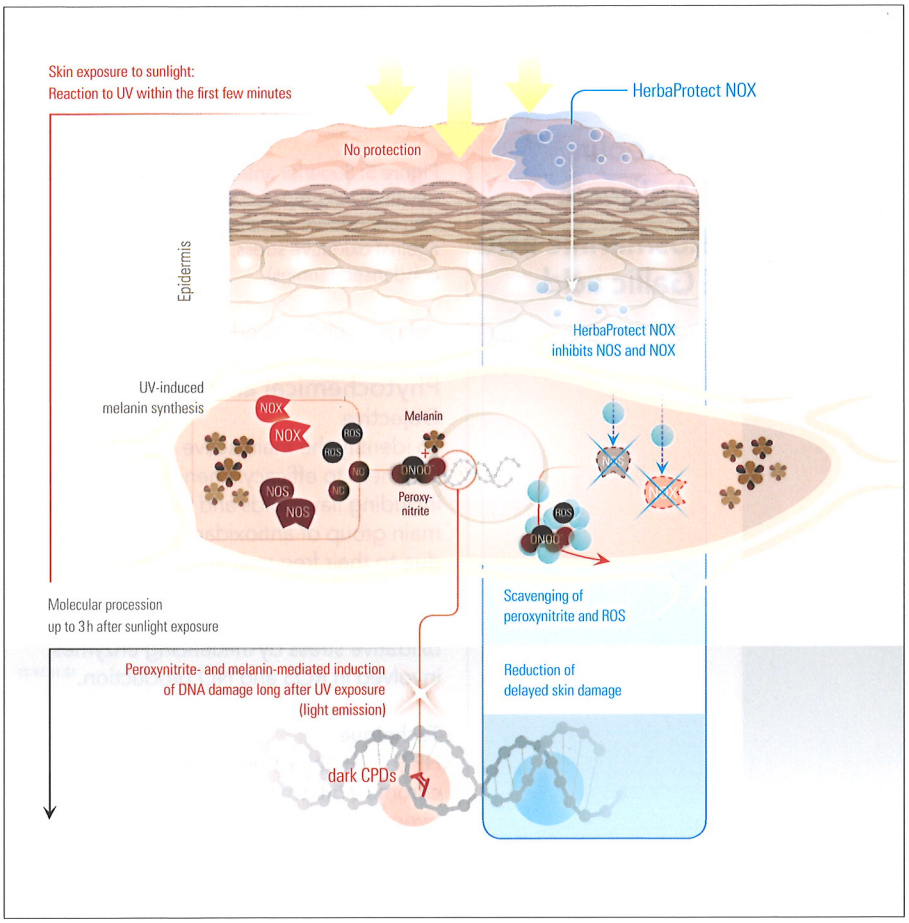


Figure 2: Formation (left) and inhibition (right) of delayed sun-induced skin damage. UV radiation induces melanin synthesis but also up-regulates NOX and NOS plausibly causing increased peroxynitrite ($ONOO^-$) formation. Peroxynitrite is a detrimental oxidant as it is capable of fragmenting and exciting melanin. Melanin eventually discharges its energy surplus to DNA where it causes 'dark CPDs' or it discharges by emitting light.[1] In light of this, interference with peroxynitrite formation and subsequent melanin excitation constitutes a promising skin protection strategy.

Counteracting delayed sun-induced skin damage

The active ingredient combines three plant extracts in a glycerine-based, self-preserving solvent system (Fig 3):

Pomegranate flower (*Punica granatum*)

The exotic fruits, which originated in Iran over 4000 years ago, have been mentioned even in Greek legends and ancient texts, including the Bible. They became famous not only as an important food source, but also because of their medicinal purposes.[4] Fruits, seeds and leaves are rich sources of valuable pomegranate phytochemicals, such as flavonoids and tannins. Moreover, these plant parts exhibit high antioxidant and photoprotective properties.[5,6] The flowers, however, have superior antioxidant activity,[7] possess the ability to scavenge reactive oxygen and nitrogen species[8] and to protect skin cells against UVA radiation.[9,10]

Perilla leaf (*Perilla frutescens*)

Leaf extracts display a range of biological activities, including anti-inflammatory and antioxidant properties. Notably, Perilla leaf extract is suggested to be a potent agent for prevention of skin ageing, as it has protective effects against UV-induced dermal matrix damage.[11] More precisely, perilla leaf extracts attenuate the expression of NOS resulting in lower levels of reactive nitrogen species.[12] Moreover, perilla leaf extracts seem to inhibit the function of NOX and reduce the levels of reactive oxygen species that are produced by this enzyme.[13] The main active ingredients were identified as polyphenols, including rosmarinic acid, a potent ROS scavenger, as well as luteolin and apigenin.[14-17]

Figure 3: Powerful protection against UV-induced, delayed skin damage due to the combination of Pomegranate flower extract (top), Perilla leaf extract (bottom left), and Kakadu plum extract (bottom right).

Kakadu plum (*Terminalia ferdinandiana*)

The fruits have been eaten by Aborigines on long treks or hunting trips, and were considered more valuable as a medicine rather than a food. It regained popularity and is increasingly found in innovative restaurant menus, health shops, and cosmetics as it was rated the richest source for vitamin C of any plant in the world. The content of vitamin C varies between 3.5 and 5.5 % of the wet weight of the fruit, which is 100 times the concentration in oranges.[18] In addition, Kakadu plum contains many other valuable compounds such as gallic acid, ellagic acid or luteolin, which also contribute to its high antioxidant activity and reduce oxidative stress in

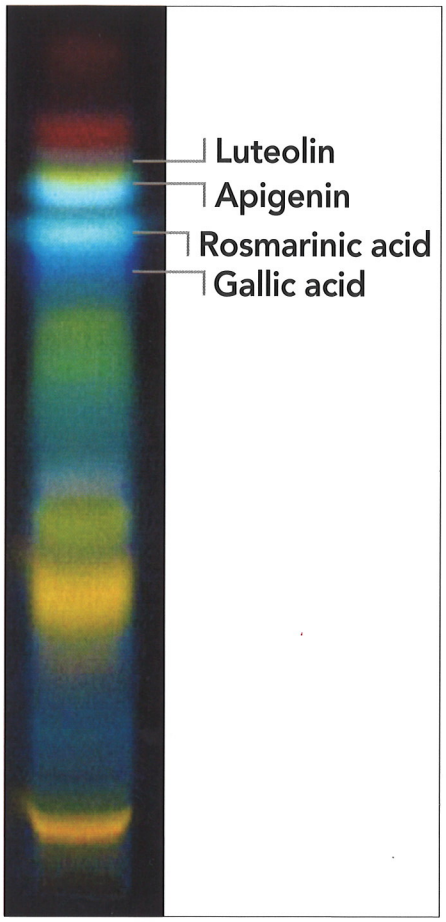


Figure 4: HPTLC fingerprint reveals potent anitoxidants and free radical scavengers such as rosmarinic acid or luteolin.

several ways: either directly by scavenging of ROS or indirectly by inhibition of the NO-producing enzyme NOS.[19-21]

In summary, these properties substantiate that the active ingredient has the potential to decrease cellular oxidative stress and counteract immediate as well as delayed sun-induced skin stress (Fig 2).

	Antioxidative Power	
	AU	Reaction Time
Active ingredient	7872	0.50
Market Reference*	148	1.00

Figure 5: Superior antioxidative capacity and reactivity as compared to a market reference (*an antioxidant complex designed to protect against oxidative stress, based on tocopherol and condensed tannins).

Phytochemical analysis

Objective

To identify the main active ingredients and link them to efficacy. Phenolic compounds, including flavonoids and tannins, are the main group of antioxidant phytochemicals due to their free radical scavenging activities. Besides the direct scavenging activity, these phytochemicals also reduce oxidative stress by influencing enzymes involved in ROS and NO production.[12,13,19-21]

Technique

HPTLC (High performance thin layer chromatography) was used to assess the overall flavonoid / polyphenol composition; Electron spin resonance (ESR) spectroscopy was used to assess the antioxidant power (AP), i.e. antioxidant capacity and reactivity. The AP method takes into account reduction potential and also reaction time (the lower the reaction time, the higher the activity). The antioxidant power is expressed as antioxidant units, where one unit corresponds to the activity of a 1 ppm solution of pure ascorbic acid as a benchmark.[22]

Results

HPTLC fingerprint analysis confirmed substantial amounts of phenolic compounds, e.g. rosmarinic and gallic acid, and flavonoids, e.g. luteolin and apigenin (Fig 4). The high proportion of free radical scavenging metabolites was reflected by an excellent antioxidative power, which was about 50-times higher compared to a reference product from the market (Fig 5).

Reduction of peroxynitrite-mediated cell stress

Objective

To assess the peroxynitrite scavenging and antioxidant activity. Peroxynitrite and ROS are key components in the formation of delayed sun-induced skin damage.

Technique

A DCF assay was used to assess peroxynitrite and H_2O_2-induced oxidative stress in primary human keratinocytes that were pre-treated with active ingredient or not.

The non-fluorescing preliminary state of a dye diffuses into the cell and gets oxidised through ROS, resulting in a fluorescing product. An enhanced fluorescence signal thus indicates enhanced oxidative stress.

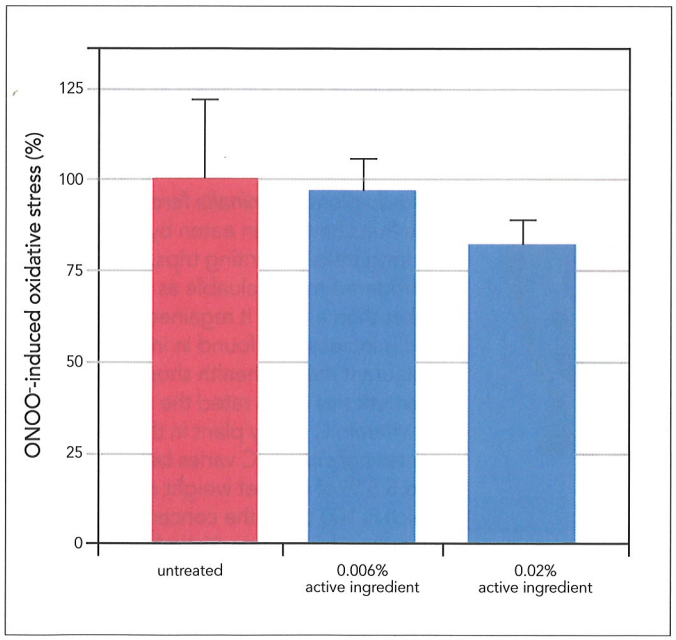
Results

The active ingredient provided specific protection against peroxynitrite-mediated cell stress and reduced the negative effect of oxidative stress in general (Fig 6). This will lead to a prevention of cellular damage, and more specifically, to reduced melanin excitation and subsequent 'dark CPD' formation.

Reduction of dark CPD formation

Objective

To show that the active ingredient prevents



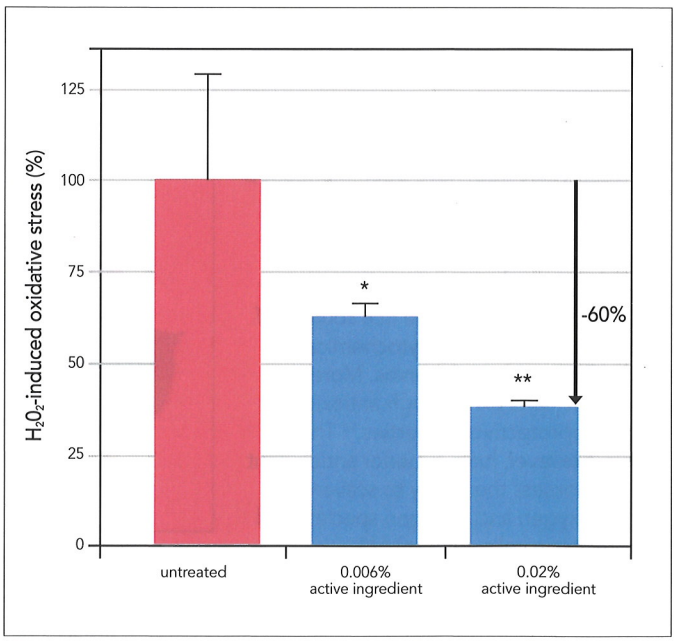


Figure 6: Reduction of peroxynitrite- (left) and H_2O_2-induced (right) skin stress. n=3; Mean±SD; Student's unpaired t-test; *=p<0.05; **=p<0.01.

the formation of DNA damage in sun-exposed skin.

Technique

Curative setting to study repair effects: Skin biopsies were irradiated with UVA followed by topical application of 0.1% active ingredient. CPDs were quantified 3 h later. A decrease in CPDs could either be explained by a reduced generation of delayed 'dark' CPDs, or by an improved repair of 'classical' CPDs. The latter are built through direct UV absorption within picoseconds and are repaired subsequently over the next few hours.

Results

UVA induced an almost 13-fold increase in CPD formation, while lacking obvious cytotoxicity. The application of 0.1% active ingredient after UVA stress significantly reduced CPD formation by 22% and thus diminished (delayed) skin damage upon sun exposure (Fig 7 and not shown).

Immediate and long-term protection against sun-induced oxidative stress

Objective

To evaluate the overall antioxidative protection upon UV exposure and to substantiate the mechanistic model of delayed sun-induced skin damage with peroxynitrite and excited melanin as active contributors.

Technique

Induced Chemiluminescence of human skin (ICL-S) was used to assess ultra-weak light emission by the skin. The skin of 20 volunteers was stressed with 1 minimal erythemal dose (MED) of UVA light followed by an immediate, single treatment with 1% active ingredient. ICL was measured in an acclimatized light-tight darkroom before, as well as 15 and 120 minutes after treatment. ICL-S: The method evaluates oxidative stress within the skin. Free radicals damage cellular components and cause ultra-weak photon emission, which is detectable as a light signal. A decrease of the ICL signal thus indicates antioxidative potential.[23] The method may also help to substantiate the mechanistic model of delayed sun-induced skin damage: Excited melanin is a key component in delayed skin damage, as it discharges its energy to DNA, e.g. causing 'dark CPDs'. Alternatively, excited melanin discharges its energy by emitting light. A decrease of the ICL signal could thus also indicate a reduced melanin excitation.

Results

The application of the active ingredient resulted in an immediate and long-term reduction of skin chemiluminescence: 15 min after UVA irradiation, the ICL signal was reduced by 45% compared to t = 0 min. The effect remained stable for at least 2 h (Fig 8). To conclude, the active ingredient increases the skin's antioxidant capacity for several hours and is therefore a valuable cosmetic ingredient to:

- Minimise free radical-related reactions
- Reduce delayed sun-induced skin damage
- Add an anti-ageing effect to after-sun formulations
- Support sunscreens or day creams with an extra line of defence
- Protect the skin from infrared-A- or light-mediated premature ageing

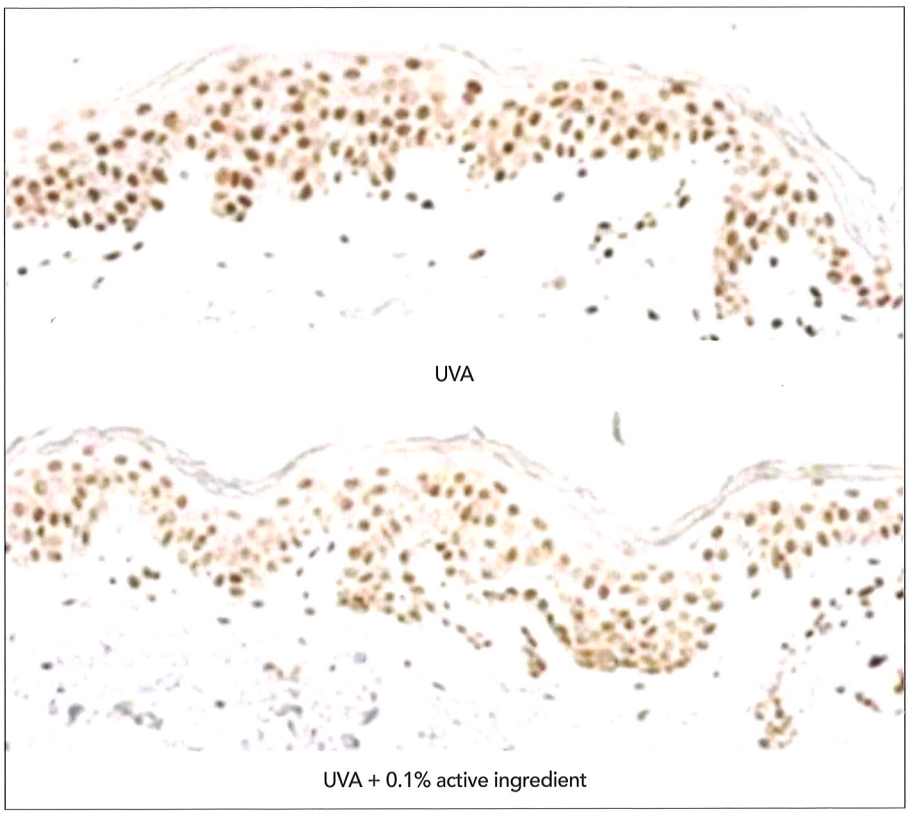


Figure 7: Reduction of delayed skin damage, such as the formation of 'dark CPDs'. Representative skin sections with specific CPD labelling (brown area). CPD formation in the epidermis was calculated by quantifying labelling intensity and area using software analysis (not shown).

Conclusion

Alarmingly, it appears that half the damage caused by sunlight actually occurs following exposure to the sun. UV-induced peroxynitrite formation and the concomitant melanin excitation trigger a process that even occurs under the cover of darkness and eventually leads to delayed cellular damage such as the formation of 'dark CPDs'.

This implies that mere sun protection is not enough and requires 'evening after' skin care products to be applied after exposure to the sun in order to prevent the ongoing dangers of UV radiation.

Indeed, cosmetic active ingredients that scavenge free radicals and nitric oxide production or that quench excited intermediates represent a promising approach to counteract UV-induced skin damage that continues in the night.

The natural and innovative anti-ageing active HerbaProtect NOX shields against delayed sun-induced skin damage and premature ageing. It increases the skin's antioxidant capacity, counteracts delayed sun-induced skin stress and reduces premature aeging through several mechanisms:

First, the active components are known to inhibit the enzymes NOX and NOS, and thereby reduce ROS and NO production and limit oxidative stress. Second, the active's excellent antioxidative activity helps to scavenge peroxynitrite and ROS. Taken together this leads to lower peroxynitrite and ROS levels and eventually results in a reduction of delayed UV-induced skin damage, such as 'dark CPDs' or premature ageing.

Finally, we would like to emphasise that an increased antioxidative capacity of the skin will help to protect against damage not only mediated by UV but by the entire solar spectrum including visible light and infrared (IR). Effects of IR radiation and visible light are comparable with the action of UV radiation as they also cause skin stress by initiating ROS. Because ROS constitutes the basis of any solar-induced damage, the antioxidant status of the skin is fundamental, and there is wide consensus that topical application of antioxidants represents the most effective approach to provide protection against IR- or visible

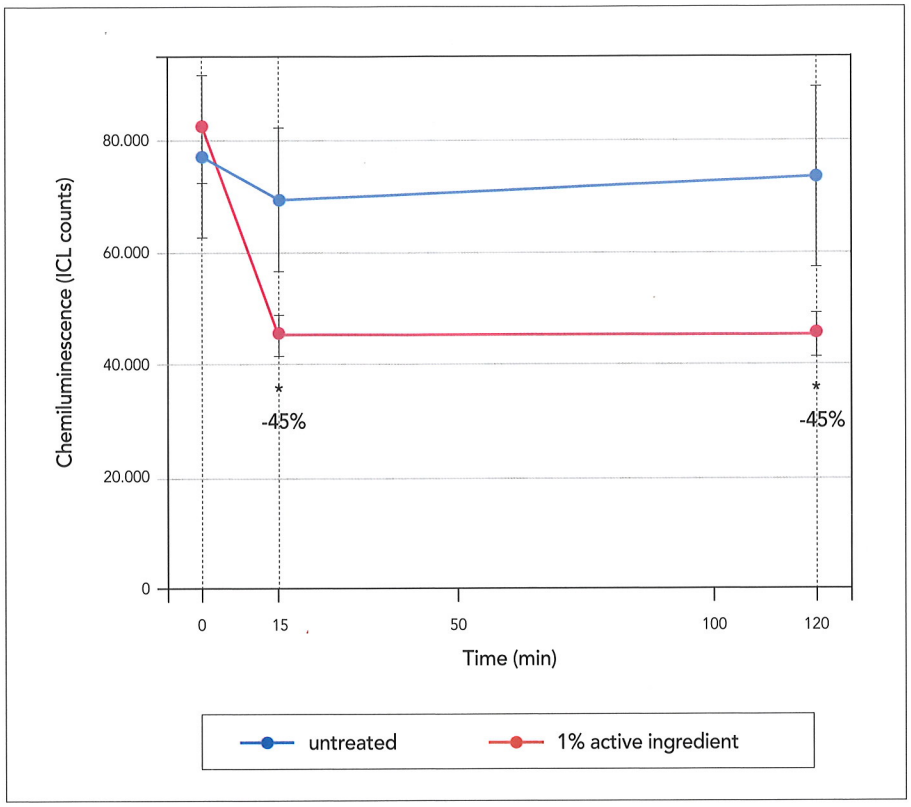


Figure 8: Increase of the skin's antioxidant capacity. Skin was stressed with UVA followed by product application or not. UV-induced stress lasted for hours on untreated skin, whereas it was immediately and steadily reduced on skin treated with active ingredient. One of two experiments is shown; n=10; Mean±SD; Student's paired t-test compared to t = 0 min; * = p<0.05.

light-induced ROS generation in the skin. Thus, powerful antioxidants such as the active presented here are considered a fundament of photoprotection products.[24-27]

To conclude, HerbaProtect NOX is a perfect addition for skin care products that aim at providing any kind of photoprotection, be it sun and day care, after-sun care, night repair or anti-ageing in general. PC

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