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The Invisible Threat for Hair and Scalp

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abstract

An urban lifestyle is exciting and brings with it a wealth of possibilities. But we often have to find a way of achieving a balance between a very fast-paced life and a more sedate form of living that does not involve stress. Hair and scalp are also daily exposed, in addition to the most common mechanical and chemical stressors, to ever-increasing air pollution and the impact of UV irradiation. Long-term exposure to particulate matter and solar irradiation is known to induce chemical damage to both hair and scalp because of the toxic and oxidising pollutants that become attached to them. RADICARE®-ECO forms a non-occlusive shield against urban pollution and protects hair and scalp against environmental stress.

Introduction

(H)air pollution and oxidative stress

A quick look at air quality measurement statistics around the world shows that the concentration of airborne particles is a global problem [1]. According to the WHO, 91% of the world's population lives in places where air pollution levels exceed the WHO guideline limits. Particulate matter causes more harm than once thought. Our integument (skin and hair) as a first line of defence experiences serious harm if exposed over the long term to the air pollutants and this is exacerbated drastically if there is simultaneous exposure to UV irradiation [2]. Luckily, the largest part of our integument is covered in clothes that block at least most of the UV irradiation. This is not the case for our hair and scalp. Daily exposure to indoor and outdoor pollutants can have detrimental effects to the entire integumentary system. Human skin, our soft, flexible but strong defence barrier against external stressors is at the same time porous enough to soak up moisture, absorb medication but also toxins and airborne pollutants (particulate matter (PM)) [3]. Many airborne pollutants are so small (< 10µm) that they can enter pores. Combustion particles (< 2.5 µm) are lipophilic and can easily pass through the fat-filled spaces between skin cells [2].

The scalp develops sensitivity, discomfort, dryness and/or oiliness when exposed to PM. Hair follicles can be weakened and there are some indications that this can even cause hair loss [4]. Hair fibres and scalp offer enough surface for the binding of PM that can then penetrate the superficial cuticle or skin layers. One of the primary mechanisms behind the adverse effects of PM is the generation of reactive oxygen species (ROS) [5]. This effect is significantly enhanced by simultaneous exposure to UV irradiation.

Despite their short lifetime, unpaired valence electrons in free radicals are able to induce radical chain reactions, which affect millions of other molecules within microseconds. This has deleterious consequences for proteins in hair and for DNA in living cells.

Powerful antioxidants - nipping the problem in the bud

The cells in our body are equipped with an extensive endogenous antioxidative defence mechanism, comprising enzymes, hydrophilic and lipophilic radical scavengers and chelating agents. Under normal conditions, cells are capable of maintaining the balance between the production of oxidants and antioxidants. Excessive concentrations of ROS and/or an impaired antioxidative defence system result in oxidative stress, which makes tissue prone to damage, cells to malfunction and can even stimulate cellular death **(Figure 1)**. This in turn

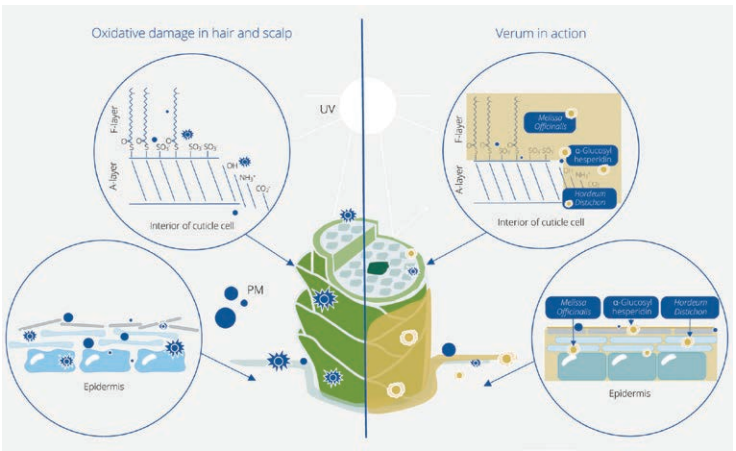

Fig. 1 Mode of action. Oxidative damage destroys proteins, lipids and impairs cellular functions. The antioxidant blend neutralises environmentally induced ROS and protects hair and scalp against adverse ROS effects.

can become apparent in the form of the development of erythema and oedema, inflammatory processes, hypersensitivity, damage to the scalp microbiome, impairment of the skin cornification and ultimately skin cancer [6]. Oxidative stress induced in hair follicles (HF) and HF stem cells may be the pivotal mechanism that eventually leads to the greying of hair and hair loss [7, 8].

In comparison with living cells, hair shafts do not have a sophisticated antioxidative defence mechanism. For this reason, ROS are capable of inducing damage in all hair shaft layers. The outermost lipid layer of the hair shaft, the so-called F-layer (18-methyl eicosanoic acid (18-MEA) layer) can be depleted leading to impaired hair integrity. This layer can also be destroyed by chemical treatments of the hair, such as bleaching, exposure to heat and the chlorine in water. Without this protective layer, hair cuticles become exposed to the environmental stressors, and once the hair cuticles are damaged this leads to damage of the cortex. Oxidation of keratin, cleavage of disulphide bonds, modification of amino acids and irreversibly damaged cortical cells are the major consequences. Hair shine decreases and tensile strength is weakened, and brittle, dull, dry and visibly unhealthy hair result.

One way to combat oxidative stress is to provide a constant supply of antioxidants or even better a mixture of antioxidants. Plant extracts are especially suitable for this demanding task. Their protective defence mechanisms against damaging ROS are highly effective.

The active ingredient combines the three most powerful natural antioxidant ingredients; namely a mixture of antioxidant extracts of barley grass (*Hordeum vulgare*), rosmarinic acid from *Melissa officinalis* and α-glucosyl hesperidin from the pith of oranges. This powerful triad works together to form a protective shield for skin and hair against urban pollution.

Materials and Methods

RADICARE®-ECO (INCI: Water, Glucosyl Hesperidin, Melissa Officinalis Leaf Extract, Sodium Benzoate, Hordeum Distichon (Barley) Extract, Citric Acid) is produced using extracts of a medicinal plant with well-known calming effects - lemon balm (*Melissa officinalis*) - and tender young shoots of barley grass (*Hordeum distichon*), while it also contains bio-technologically produced α-glucosyl hesperidin. This sophisticated blend of three natural and very powerful antioxidants protects scalp and hair against deleterious urban airborne particulate matter and UV. The carbon footprint of the whole supply chain has been calculated and offset.

The *ex-vivo* study was performed on virgin Caucasian hair tresses (n=3). The keratin oxidation has been induced with urban dust (PM_{10}; Particulate Matter HAP, European Reference Material; Ref. CZ100; 150 µg/cm²) and UV irradiation (LED source, emission peak at 365 nm, 84 J/cm²), which is equivalent to the exposure on one day in Paris in August. The same experimental set up was used for hair tresses, which have been

previously immersed in 0.5% active ingredient for 20 minutes. Oxidative levels were quantified and visualised in all hair layers (cuticle and cortex).

The *in-vitro* studies have been performed on the EpiDerm MatTek Reconstructed Human 3D skin model (n=8) and on NHEK cells (n=3). The 3D skin culture was treated with urban dust alone (UD; 400 µg/ml) and in combination with UV-A irradiation (urban pollution, 10 J/cm^2) with and without 1 active ingredient for 24h. Caspase-9 (CASP9), cell death- and Ki67, cell proliferation indicators have been quantified and visualised. Fluorescent probe 2,7-dichlorodihydrofluorescein diacetate and MTT assay were used to quantify the production of ROS (n=3) and keratinocytes viability (n=6). Different dosages of UV irradiation conditions were chosen UV-B (100 mJ/cm^2) /UV-A (0.7 J/cm^2) for ROS quantification and three different doses of UV for cell viability determination, namely: UV-B (275 mJ/cm^2)/UV-A (2.0 J/cm^2), UV-B (300 mJ/cm2) / UV-A (2.2 J/cm^2) and UV-B (325 mJ/cm^2)/UV-A (2.4 J/cm^2).

Results

Keratin oxidation (ex-vivo study):

In situ detection of protein oxidation (carbonylation) in cross-sections of hair and entire hair shafts (lateral view) is depicted in **Figure 2**. As expected, the stressor factors (PM_{10} + UV-A) induced an increase in carbonylated proteins in both the cortex and cuticle. The presence of the powerful triad extract prevented oxidative damage.

A significant increase in the carbonylation of proteins was observed in hair sections exposed to stress (PM_{10} + UV-A). The application of 0.5% active ingredient protected hair shafts against urban pollution-induced protein oxidative damage **(Figure 3)**. Quantification revealed that oxidative damage was reduced by 52% in the cuticle (left panel), by 88% in the cortex compartment (middle panel) and across the whole cross-section of hair (right panel) by up to 79%.

Oxidative stress in 3D skin model (*in-vitro* study):

The results of quantification indicated that treatment with urban dust significantly increased the levels of reactive oxygen species (ROS). Application of 1% active ingredient reduced ROS levels by 25%. Simultaneous exposure to urban dust and UV-A irradiation significantly increased ROS levels. In this case treatment with 1% active ingredient significantly reduced ROS levels by 46% (data not shown). The different conditions were selected in order to simulate the effects of urban pollution on a scalp with and without hair.

In the case of caspase-9 (CASP9) quantification, results indicated that treatment with urban dust significantly increased CASP9

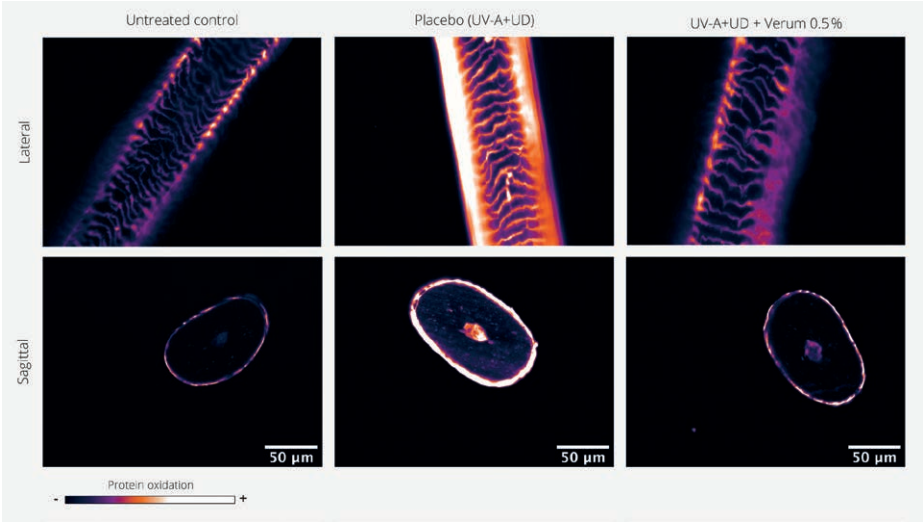

Fig. 2 Keratin protection against oxidation. *In situ* visualisation of oxidised proteins. Active ingredient significantly reduces urban pollution damage in hair. The fluorescence emission signal for carbonylated proteins was obtained using a fluorescent probe that absorbs and emits light at different wavelengths (647/650 nm).

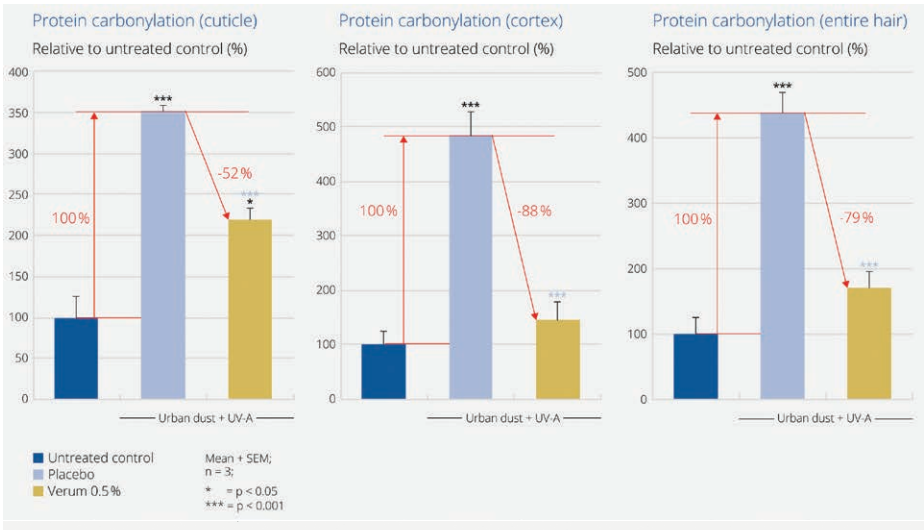

Fig. 3 Reduction against urban pollution-induced damage in all hair layers. Quantification of oxidative levels in different hair shaft compartments. Oxidative damage was evaluated in the cuticle (left panel), the cortex (middle panel) and also in whole hair (right panel). Statistical analysis to determine significant changes was performed using ANOVA variance analysis followed by Dunnett's post hoc test for multi-comparisons. The statistical values in black are the results of comparison with the untreated control and those in light blue are the results of comparison with placebo.

levels in both conditions (urban dust alone and urban dust with simultaneous UV-A irradiation).

When the 3D skin model was topically treated with 1% active ingredient, there was a decrease in CASP9 levels by 45% (**Figure 4**, left panel) and 90%, respectively (**Figure 4**, right panel). Visualisation of CASP9 levels for all conditions is shown in **Figure 5**.

Finally, the results of assessment of cell proliferation using Ki67 immunostaining indicated that exposure to urban dust significantly decreased cell proliferation. When the 3D skin model was topically treated with 1% active ingredient, results showed significantly increased cell proliferation levels of 95% (**Figure 6**, right panel) and 56%, respectively (**Figure 6**, left panel). Visualisation of the Ki67 immunostaining is not shown in this paper.

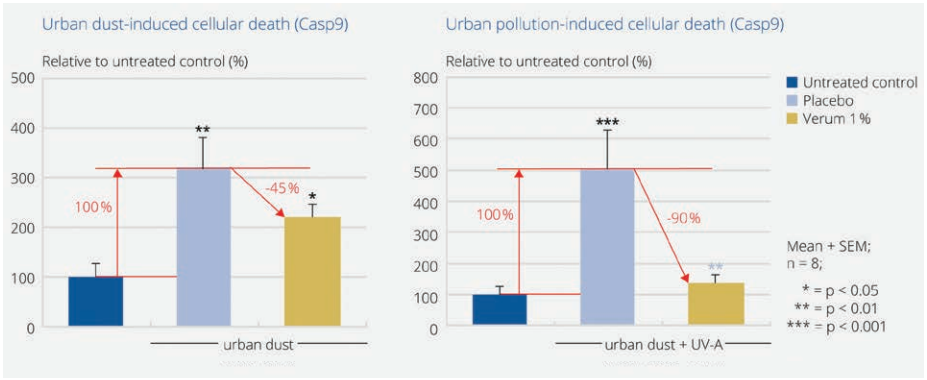

Fig. 4 Scalp protection against cell death induced by urban dust and a combination of urban dust and UV-A irradiation (urban pollution). The bar graphs represent CASP9 fluorescence on the EpiDerm MatTek Reconstructed Human (RHE) 3D skin model, treated with 1% active ingredient, and exposed to urban dust (UD) and urban dust (UD) and UV-A irradiation. Statistical analysis to determine significant changes was performed using ANOVA variance analysis followed by Tukey's test for multi-comparisons. The statistical values in black are the results of comparison with the untreated control and those in light blue are the results of comparison with placebo.

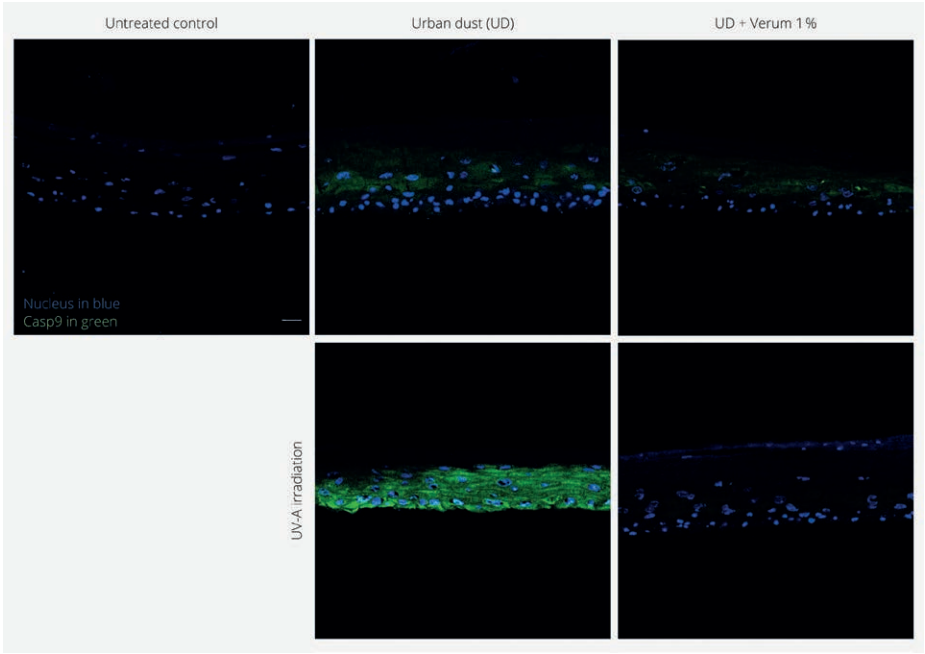

Fig. 5 Visible protection against cellular death. The images show combined nucleus (blue) and CASP9 (green) fluorescence levels under confocal microscopy in the untreated model, the model exposed to UD with and without 1 % active ingredient and with and without UV-A irradiation. Scale bar (in white) 50 µm.nm).

A 2% solution of active ingredient exhibited a protective effect against UV-irradiation-induced ROS production (73%). The reference compound had a protective effect of 45%. Active ingredient was significantly better than the reference substance and irradiated control.

Oxidative stress in keratinocytes (*in-vitro* study):

The irradiation of NHEK with 100 mJ/cm² UV-B + 0.7 J/cm² UV-A resulted in a marked increase of the basal ROS production level and this effect was significantly inhibited by the reference compound EGCG, which validated the assay. Results are depicted in **Figure 7**.

Keratinocyte's viability (*in-vitro* study):

The three tested UV-B + UV-A doses induced, as expected, a dose-dependent and linear decrease in cell viability in placebo-treated cells. Addition of 2% active ingredient with exposure to 275 mJ/cm² UV-B + 2.0 J/cm² UV-A had no measurable protective effect. Nevertheless, the morphology of the cells clearly indicated a protective effect of the powerful triad. This is because the decrease in MTT fluorescence was associated with cell metabolism rather than cell mortality. This was apparent in the cell culture (data not shown). At a UV dose of 300 mJ/cm² / 2.2 J/cm², the protective effect of active ingredient was clearly apparent, and this remained significant at the highest UV-dose (325 mJ/cm² / 2.4 J/cm²) **(Figure 8)**.

The active ingredient provided significant and consistent protection against UV-induced oxidative damage at all applied UV irradiation doses. Placebo-treated samples exhibited a continuous decrease in cell viability.

Conclusion

Ex-vivo studies of hair revealed highly significant inhibition of keratin oxidation in all hair layers. *In-vitro* studies have shown that RADICARE®-ECO significantly inhibits ROS-induced damage.
By means of effective radical scavenging, cells were able to maintain their viability and proliferation and at the same time cellular death was reduced.
RADICARE®-ECO is your personal protective shield against urban pollution for skin and hair.

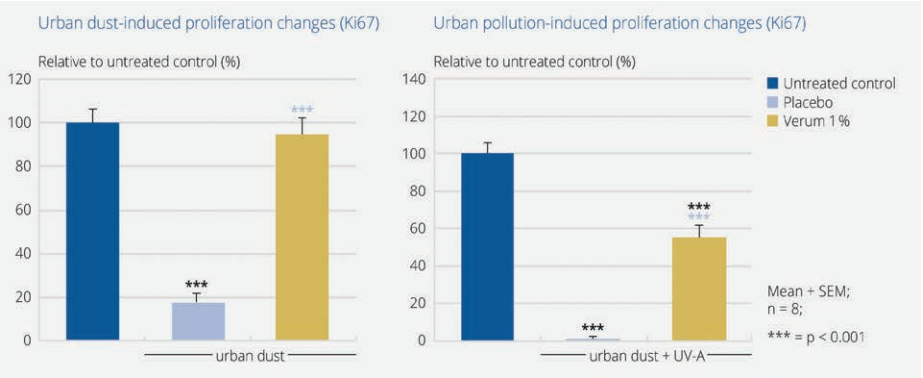

Fig. 6 Healthy and vital cells. The bar graphs represent the results of fluorescence quantification of proliferating cells using Ki67 immunostaining in an EpiDerm MatTek Reconstructed Human (RHE) 3D skin model treated with 1 % active ingredient and exposed to urban dust (UD) and UD in combination with UV-A irradiation. Statistical analysis to determine significant changes was performed using ANOVA variance analysis followed by Tukey's test for multi-comparisons. The statistical values in black are the results of comparison with the untreated control and those in light blue are the results of comparison with placebo.

References:

[1] Index, W.A.Q., Real-time Air Quality index. 2007: https://waqi.info/. p. non-profit project

[2] *Svoboda, E.*, When skin's defence against pollution fails. Nature, 2018. 563: p. 89-90.

[3] *E. Drakaki*, C.D.a.C.V.A., Air pollution and the skin. Frontieris in Environmental Science, 2014. 2:11.

[4] *Kwon, H.C.*, Effects of particulate matter on human dermal papilla cells. 2019: The European Academy of Dermatology and Venerology.

[5] *Vierkotter, A., et al.*, Airborne particle exposure and extrinsic skin aging. J Invest Dermatol, 2010. 130(12): p. 2719-26.

[6] *Trouba, K.J., et al.*, Oxidative stress and its role in skin disease. Antioxid Redox Signal, 2002. 4(4): p. 665-73.

[7] *Trueb, R.M.*, Oxidative stress in ageing of hair. Int J Trichology, 2009. 1(1): p. 6-14.

[8] *Trueb, R.M., et al.*, Scalp Condition Impacts Hair Growth and Retention via Oxidative Stress. Int J Trichology, 2018. 10(6): p. 262-270

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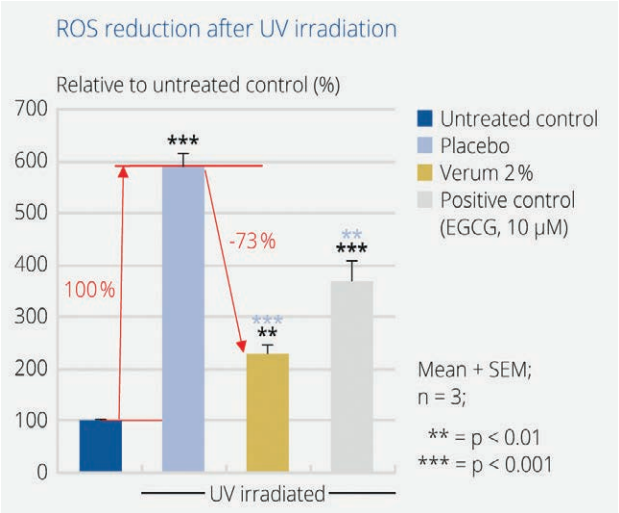

Fig. 7 Protection against UV-irradiation-induced ROS. Treatment with 2 % active ingredient had a significant protective effect against UV irradiation (UV-B and UV-A in combination). Statistical analysis to determine significant changes was performed using the unpaired Student's t-test. The statistical values in black are the results of comparison with the untreated control and those in light blue are the results of comparison with placebo.

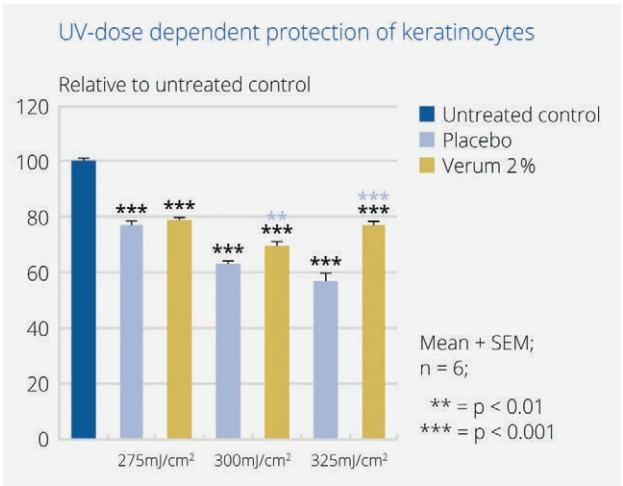

Fig. 8 Protection of cell viability. Treatment with 2% of active ingredient had significant protective effects against UV irradiation-induced keratinocyte mortality. Results above are shown only for increasing dosages of UV-B. It should be borne in mind that UV-B and UV-A were used for each condition. Statistical analysis to determine significant changes was performed using the unpaired Student's t-test. The statistical values in black are the results of comparison with the untreated control and those in light blue are the results of comparison with placebo.