Extremolytes: fighting urban stress by the model of nature

Eva Galik, Janina Kienemund – bitop AG, Germany

In the past years, extremolytes became well-established active ingredients thanks to their special efficacy and characteristics. These natural molecules, which are found in impressive microorganisms and plants, are used wherever skin and hair needs regeneration and repair or has to be protected from damaging stress factors such as dryness, pollution particles or visible light.

Extremophilic microorganisms are a pretty wild bunch, boldly living on the edge. Extremophiles are so-called because they thrive in the most hostile environments one can imagine - so intensely hot, cold, salty, acidic, alkaline, pressurised, dry, radioactive, or barren that other creatures could not survive for a second.

Belonging to the smallest and most ancient forms of life on earth, these primeval creatures have developed survival and protection strategies tried and tested over millions of years. Extremophiles accumulate osmolytes, special protection molecules, in their cells to adapt to their extreme habitats. These organic osmolytes from extremophiles are called 'extremolytes'.

Extremolytes stabilise and protect the biological structures of the microorganisms such as cell membranes, proteins or DNA, thus cellular stress reactions are significantly reduced.[1] These protection molecules are a group of heterogenic substances including sugars, polyols, amino acids and their derivatives. However, as different as the individual extremolytes are, they have some basic commonalities: they are polar and highly soluble in water, which consequently leads to protection benefits when applied on human cells. Due to this efficacy, the use of extremolytes in cosmetics and personal care products has various benefits. In skin care formulations, the amino acid proline, the sugar alcohol mannitol and the amino acid derivatives betaine, ectoine and hydroxyectoine[2,3] are of particular interest.

Proline is a major component of the protein collagen. It essentially determines the mechanical properties of collagen and supports its renewal. Applied on the skin, proline leads to tightening of the upper skin layers.

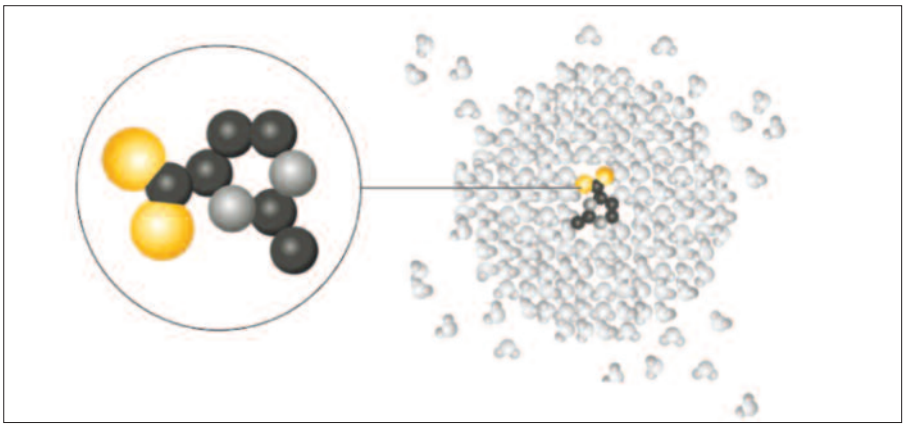

Figure 1: Ectoine hydro complex.

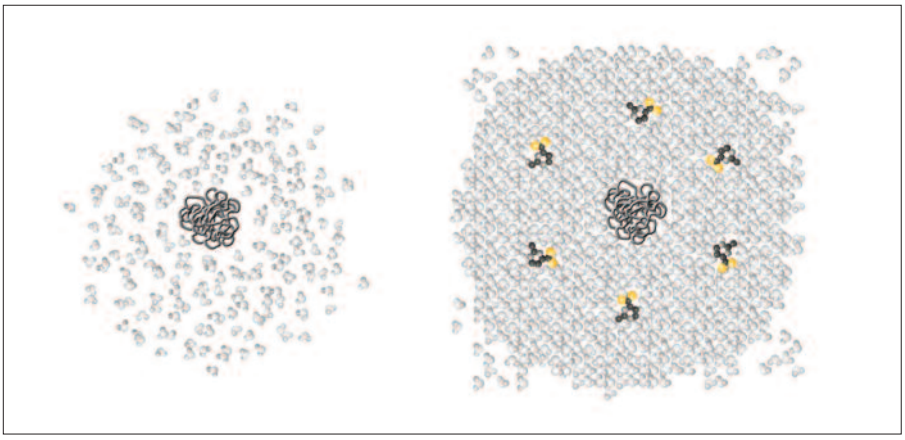

Figure 2: Protection shell around protein formed by ectoin hydro complexes.

Betaine is chemically known as trimethylglycine or glycine betaine and named after its natural source *Beta vulgaris*, the sugar beet. This ingredient is a by-product of sugar production and a multifunctional active, showing anti-inflammatory efficacy and reduction of skin irritation. Betaine furthermore moisturises the skin by controlling the water balance.

Mannitol is found in abundance in nature, particularly in trees, in marine algae and fresh mushrooms. In cosmetics, it is used as a humectant, emulsifier, masking agent, flavouring agent and skin conditioner.

One of the best-known extremolytes is Ectoin® (chemical term: ectoine, INCI: Ectoin). It was discovered in 1985 as the self-defence substance of extremophilic bacteria found in a salt puddle in Wadi El Natrun (Egyptian desert). Today ectoine is a safe and multifunctional active ingredient, formulated worldwide in cosmetics and personal care products as well as in OTC medical devices. It offers various benefits for skin and hair, but also for oral mucosa and lung tissue due to its simple but yet effective mode of action.

Mode of action

Ectoine is a strongly kosmotropic substance. The amino acid derivate binds

with the water molecules of its surroundings and forms the so called 'ectoine hydro complex' (Fig 1). These complexes again surround cells, enzymes, proteins and other biomolecules by creating stabilising and protecting hydration shells and layers in the epidermis (Fig 2).

The presence of ectoine protection layers in the epidermis leads to a decrease and prevention of negative cell response to stress factors like intracellular inflammation, e.g. the initiation of proinflammatory signalling cascades.[4] Ectoine reduces the damaging impact on cells by stabilising and protecting the cell membrane. This protection mechanism is capable to shield the skin from various stress factors including, dryness, air pollution, UV-radiation and visible light and thus counteracts premature ageing.

Besides protecting cells and other biological structures, ectoine has a positive impact on the general condition of the cell. Cells show improved cell functions, like faster supply of protection proteins and self-defence reactions, when treated with ectoine.[5]

Skin barrier repair

Ectoine restores and stabilises the stratum corneum barrier function and consequently increases skin hydration based on its scientifically proven protection and anti-inflammatory benefits.

To demonstrate the positive impact of ectoine on the human skin barrier function, the influence of ectoine on the transepidermal water loss (TEWL) was investigated. Five independent placebo controlled studies with different ectoine concentrations (1% – 5%) and in total over 80 patients were conducted. For all concentrations and test persons, including patients with very sensitive and atopic skin, the TEWL was significantly reduced compared to placebo due to treatment with ectoine. In a placebo controlled clinical study with 20 test persons with broken skin barrier, a formulation containing 1% ectoine was outperforming a cream with 0.25% hydrocortisone ('Ebenol® 0.25%' cream) regarding the reduction of TEWL and skin barrier repair. After 7 days of twice daily application, ectoine reduced the TEWL by 24%, repairing the stratum corneum barrier function (Fig 3).

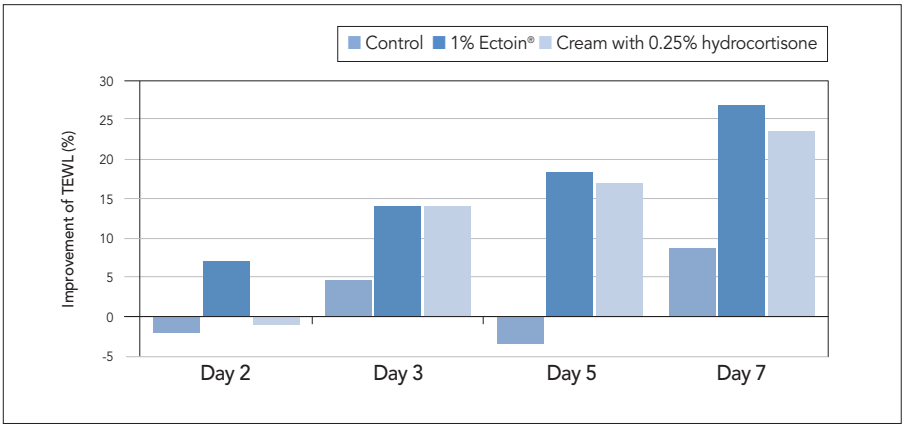

Figure 3: Average values of TEWL improvement.

The clinical study by Marini *et al.* with 65 patients with mild to moderate atopic dermatitis confirmed the efficacy of ectoine, with regard to the repair of barrier function, inflammation reduction and the consequent improvement of atopic skin conditions.[6]

Anti-pollution efficacy of ectoine

In the past years, several epidemiological and mechanistic studies proved a connection between pollutants and accelerating wrinkles as well as pigment spot formation. Air pollutants may not only induce skin ageing but are also linked to causing or worsening acne, atopic dermatitis, eczema, allergic reactions or even skin cancer.[7,8] Depending on the size and depth of penetration of pollution particles, the effects on the skin are different.

Especially for airborne particulate matter (PM) and polycyclic aromatic hydrocarbons (PAHs) negative influence on skin integrity has been shown. Mass and composition in urban environments tend to be divided into two principal groups: coarse particles and fine particles. The limit between them is fixed at $PM_{2.5}$. Whereas most of the mass is usually in the fine mode (particles between $PM_{0.1}$ and $PM_{2.5}$), the largest number of particles (more than 90%) is found in the very small sizes, less than 100 nm resp. $PM_{0.1}$. [9] These ultrafine/nanosized particles are considered the most harmful components of ambient PM, because they can penetrate the skin and even pass into our circulatory system.

Furthermore ultrafine/nanosized particles are carrier for organic compounds. Actually, the highest level of concentration of toxic organic compounds, such as PAHs and heavy metals, is related to ultrafine particles ($PM_{0.1}$ and smaller). PAHs are among the most widespread organic pollutants and are frequently bound to the surface of combustion-derived PM including cigarette smoke. PAHs are highly lipophilic and thus easily penetrate the skin.

These scientific facts clearly emphasise the need for a global approach in anti-pollution strategies. Formulation concepts, which only focus on the skin surface and do not consider the cellular level fall short to the mark. Strengthening and restoration of skin barrier seem to be an effective function in the prevention of pollution induced skin damage and is therefore also important not to neglect.

In several studies, ectoine demonstrated global and multilevel anti-pollution efficacy. The natural stress-protection molecule strengthens the skin barrier function and is capable to inhibit and reduce damage to skin and skin cells induced by various air

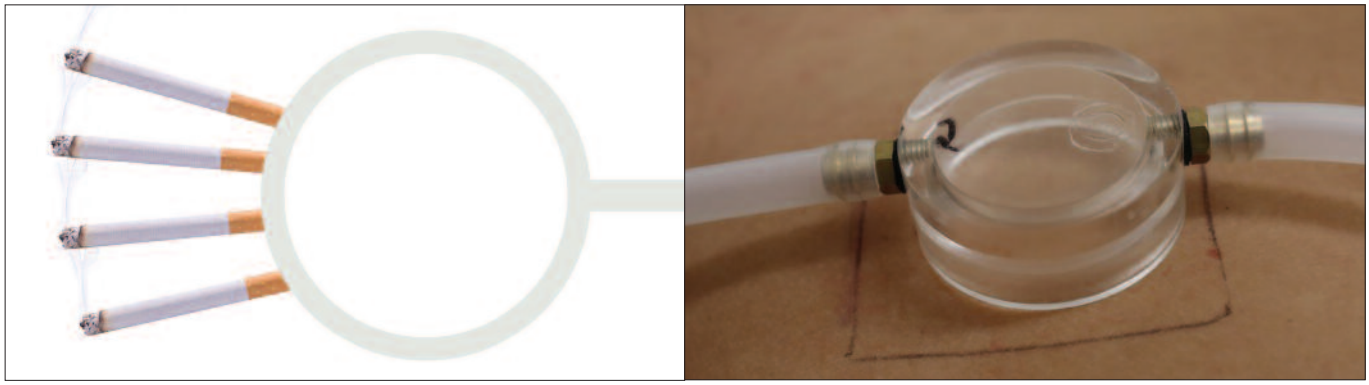

Figure 4: *In vivo* study design: smoke application on skin with standardised chamber system.

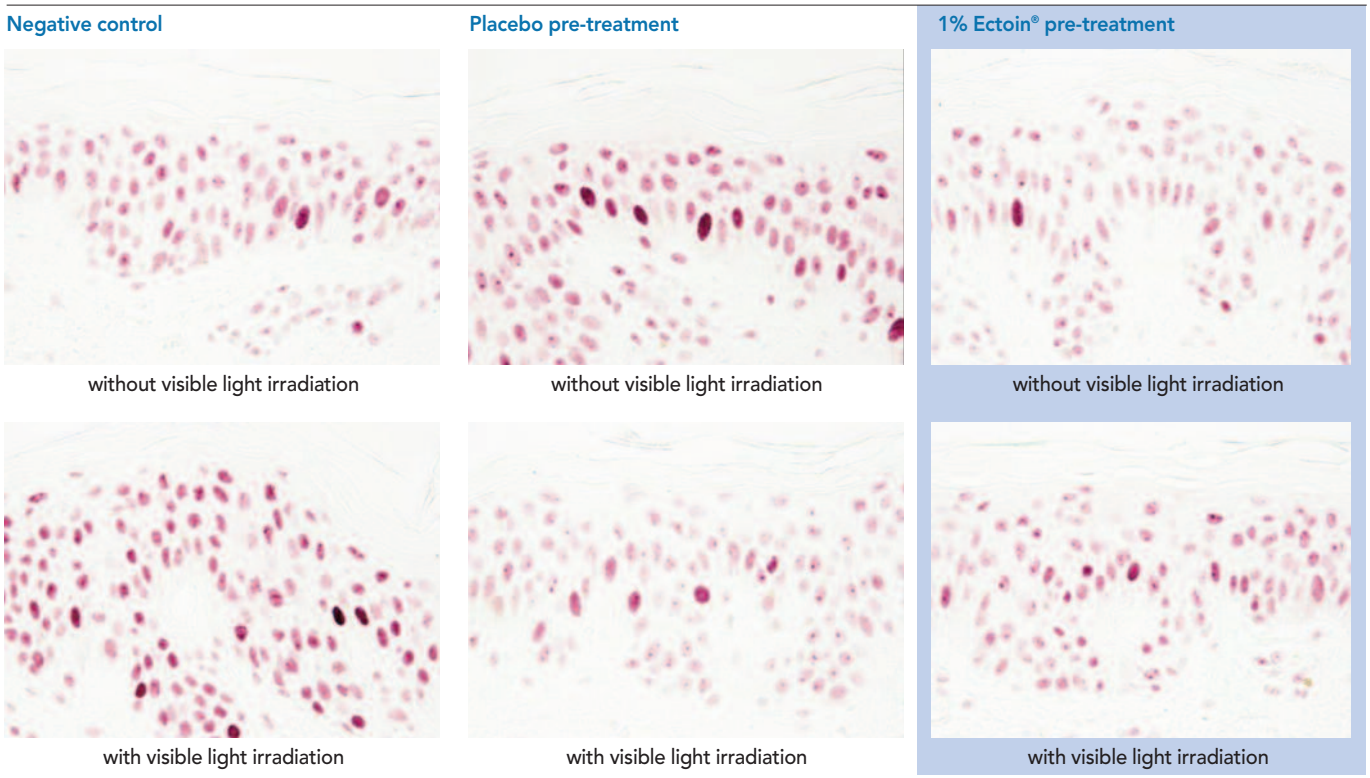

Figure 5: Nrf2 immunostaining of the negative control, placebo and 1% ectoine pre-treatment batch with and without visible light irradiation.

pollution components. This includes PAHs, heavy metals, nitrogen dioxide as well as particulate matter of all particle sizes.

In a placebo-controlled *in vivo* study, the application of 1% ectoine formulation for 5 days, twice daily, resulted in a significant anti-pollution effect. The ectoine treated skin showed 48% less pollution induced damage compared to placebo treated skin.

The skin was stressed with cigarette smoke as pollutant to induce oxidative stress to the skin in a standardised pollution chamber system (Fig 4). The anti-pollution efficacy was evaluated by analysis of barrier lipid oxidisation levels (measured by malondialdehyde, MDA) of samples from the skin surface.

Furthermore, the anti-pollution efficacy of ectoine was demonstrated in a clinical study on human lungs of patients with COPD (chronic obstructive pulmonary disease) and particle induced asthma. Based on this and further data, ectoine is approved for therapeutic and preventative use in health care products for the treatment and prevention of pollution induced lung diseases like COPD and asthma.[10]

Visible light and its influence on skin

Visible light has influence on our skin. Light affects the wellbeing, it can act against 'winter depression' and is able to control hormonal activities, for example those of the sleep hormone melatonin. Because of its cutaneous biologic effects, light is used for the treatment of a variety of skin diseases and aesthetic conditions, demonstrating the positive effects of light, but everything also has its drawbacks.

Although photobiologic studies of sunlight date back to 1671, most available studies focus on the negative effects of UV and the infrared radiation part of the sun spectrum. The effects of visible light have not been clearly elucidated until recently. Electromagnetic radiation is classified based on wavelength. The visible spectrum is the portion of electromagnetic radiation visible to the human eye, which responds to wavelengths from about 390 to 700 nm, and also includes blue light (450 to 495 nm). Various studies have been shown negative effects to be exerted by visible light radiation including erythema, pigmentation, thermal damage and free radical production. Visible light can also induce indirect DNA damage due to the generation of reactive oxygen species (ROS).

Ectoine: visible light protection

Most of the currently available UV-filters offer, if any, weak protection against visible light. Organic sunscreen agents usually show no protection against visible light, as their absorption spectrum is limited to UVB and UVA wavebands. Inorganic UV filters, such as iron oxide, titanium dioxide, and zinc oxide can offer some, but limited, visible light protection. However, the spectral protection of these agents varies according to their particle size. Larger particles of titanium dioxide and zinc oxide can protect in the visible range. The limited effectiveness of UV filters, organic and inorganic, for visible light protection indicates the need for further protection substances and concepts.

Ectoine could be a solution to this problem, as it has been shown to offer visible light protection properties in an independent scientific study by Botta *et al.* as well as in the latest placebo-controlled *ex vivo* study.

Botta *et al.* (2008) have demonstrated that ectoine prevents oxidative damage in the skin induced by visible light irradiation. In this study, ectoine showed a protection level of up to 90% against the damaging impact of visible light on human skin cells.[11]

In an *ex vivo* study, human living skin explants were pre-treated with 1% ectoine and exposed to visible light (65J/cm^2). Afterwards the parameters Nrf2 and MC1R were evaluated by immunostaining.

Nrf2 is a key transcription factor in the cellular response to oxidative damage induced by reactive oxygen species (ROS). Nrf2 content in the cell increases automatically, when the skin is exposed to visible light.

The study demonstrated the reduction of the cell´s stress response to oxidative stress due to ectoine pre-treatment. The presence of ectoine decreased the need of Nrf2 in the cell (Fig 5). Thereby ectoine protects the skin from visible light induced skin damage and photoageing.

Visible light has been reported to induce

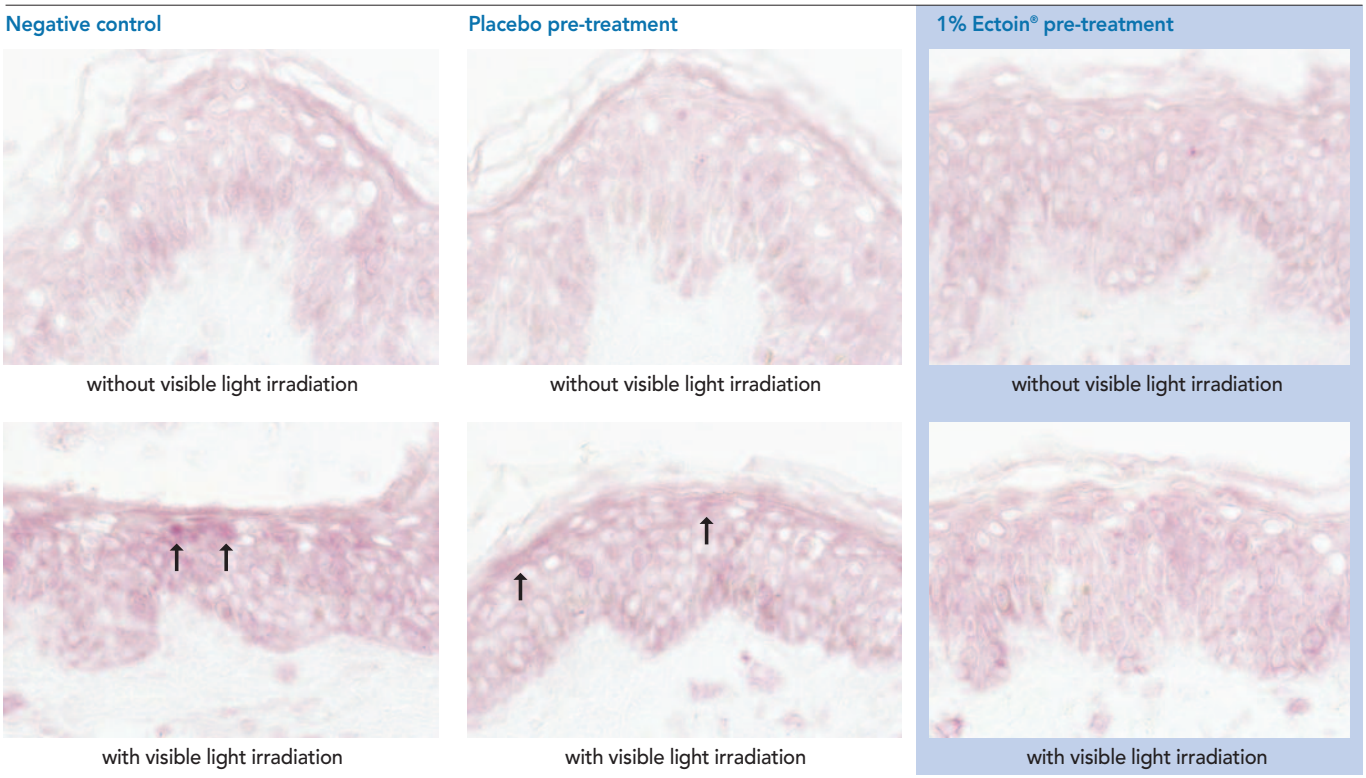

Figure 6: MC1R immunostaining of the negative control, placebo and 1% ectoine pre-treatment batch with and without visible light irradiation.

both, transient[12] as well as long lasting pigmentation in human skin[13]. MC1R is a marker for skin pigmentation and its activation leads to eumelanin production. Visible light increases MC1R content, which consequently leads to pigmentation of the skin. The results of the latest *ex vivo* study (Fig 6) indicate a reduction of visible light induced melanogenesis due to ectoine pre-treatment.

A recent study by Mahmoud *et al.* suggested that visible light exposure can increase pigmentation in patients with skin photo type IV to VI.[14] Based on the latest study results and its mode of action, it can be assumed that ectoine is well-suited to preventing this phenomenon.

Conclusion

Due to its global skin protection and regenerating efficacy, the extremolyte ectoine became a well-established active ingredient in the past years. Its unique and global anti-pollution activity has been demonstrated *in vivo* by analysis of barrier lipid oxidisation levels from the skin surface as well as in human lungs, where ectoine reduces inflammations induced by pollution particles. Ectoine´s visible light protection property results in the reduction of the cell`s oxidative stress response to visible light irradiation and the prevention of hyperpigmentation. In addition, the natural amino acid derivate regenerates environmentally irritated skin including the skin barrier function due to its strong anti-inflammatory benefits.

In conclusion, ectoine is a multifunctional and safe active ingredient with clinically proven efficacy - very well-suited for cosmetic formulations, which need to match various consumer demands and market trends. PC

References

1 Lentzen G, Schwarz T. Extremolytes: natural compounds from extremophiles for versatile applications, *Applied microbiology and biotechnology* 2006; **72**(4): 623-634.

2 Galinski EA, Pfeiffer HP, Trüper HG. 1,4,5,6-Tetrahydro-2-methyl-4-pyrimidinecarboxylic acid. A novel cyclic amino acid from halophilic phototrophic bacteria of the genus Ectothiorhodospira. *Eur J Biochem* 1985; 149: 135-139.

3 Da Costa MS, Santos H, Galinski EA. An overview of the role and diversity of compatible solutes in Bacteria and Archaea. Biotechnology of extremophiles (117-153). Springer Berlin Heidelberg, 1998

4 Grether-Beck S, Timmer A, Felsner I, Brenden H, Brammertz D, Krutmann J. Ultraviolet A-induced signalling involves a ceramide-mediated autocrine loop leading to ceramide de novo synthesis. *J Invest Dermatol* 2005; 125: 545–553

5 Bünger J, Degwert J, Driller H. The protective function of compatible solute Ectoin on the skin, skin cells and its biomolecules with respect to UV-radiation, immunosupression and membrane damage. *IFSCC-Magazine* 2001; **4**(2): 1-6

6 Marini A, Reinelt K, Krutmann J, Bilstein A. Ectoine-containing cream in the treatment of mild to moderate atopic dermatitis: a randomised, comparator-controlled, intra-individual double-blind, multi-center trial. *Skin Pharmacol Physiol.* 2014; **27**(2):57-65

7 Daniels C. Wie Umweltverschmutzung die Haut malträtiert. *CME* 2016; **9**(1); 55-56.

8 Vierkötter A, Schikowski T, Ranft U et al. Airborne particle exposure and extrinsic skin aging. *J Invest Dermatol* 2010; **130**(12): 2719-26

9 WHO working group. Health aspects of Air pollution with Particulate matter, Ozone and nitrogen dioxide. Bonn, Germany 2003: Regional Office for Europe.

10 Unfried K, Krämer U, Sydlik U, et al. Reduction of neutrophilic lung inflammation by inhalation of the compatible solute ectoine: a randomized trial with elderly individuals. *International Journal of Chronic Obstructive Pulmonary Disease* 2016; **11**(1): 2573—2583

11 Botta C, Di, Giorgio C, Sabatier AS, De Meo M. Genotoxicity of visible light (400-800 nm) and photoprotection assessment of ectoin, L-ergothioneine and mannitol and four sunscreens. *J. Photochem. Photobiol. B* 2008; **91**(1): 24-34

12 Pathak MA, Riley FC, Fitzpatrick TB. Melanogenesis in human skin following exposure to long-wave ultraviolet and visible light. J Invest. Dermatol. 1962; 39: 435–443.

13 Kollias N, Baqer A. An experimental study of the changes in pigmentation in human skin *in vivo* with visible and near infrared light. *Photochemistry and Photobiology* 1984; 39(5): 651–659.

14 Mahmoud BH, Hexsel CL, Hamzavi IH, Lim HW. Effects of Visible Light on the Skin. *Photochemistry and Photobiology* 2008; **84** (2): 450–462