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Edelweiss Helps to Protect Against Glycation-related Skin Ageing

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abstract

Glycation-induced, Advanced Glycation End Products (AGEs) make a significant contribution to signs of skin ageing. In the pro-ageing skin care market, cosmetic antioxidants are often exploited for their ability to protect against free radicals and oxidative stress, but some of these ingredients also have potential to reduce or prevent the effects of glycation. DSM has conducted two new studies to explore the anti-glycation potential in a bioactive with proven antioxidant properties, ALPAFLOR® EDELWEISS CB [INCI *Leontopodium alpinum extract*] an extract of organically grown and ethically sourced Edelweiss plants. The first study demonstrated the ingredient's ability to down-regulate accumulation of AGEs by up to 55% in skin cells *in vitro* and to activate the detoxifying enzyme named Gloxalase-1 (Glo-1), which plays a key role in protecting keratinocyte proteins against oxidative damage during skin ageing, by 65%. The second study demonstrated that the bioactive inhibited Collagen Type IV glycation by 67%.

Antioxidants in pro-ageing skincare - a burgeoning market

With a growing ageing population around the world, there is a high and increasing consumer demand for pro-ageing skin care solutions, particularly those that target the appearance of fine lines and wrinkles. The market for cosmetic antioxidants is gaining noticeable traction [1,2]. In consumers' minds, these ingredients are strongly associated both with naturals and plant extracts (perceived as being cleaner and more trustworthy and environmentally friendly) and reducing signs of skin ageing caused by external and environmental factors. When it comes to the mode of action that delivers these skin care benefits, the focus of antioxidant effects tends to be on protecting against the negative impact of free radicals. However, a process known as glycation, which is less well-known to consumers, also plays a role in the formation of fine lines and wrinkles and antioxidants can help counter this process too.

Glycation, AGEs, and their impact on skin

Glycation is a basic mechanism involved in ageing in humans. It is a non-enzymatic reaction that occurs spontaneously between sugars and proteins, leading to the formation of Advanced Glycation End Products (AGEs), a complex that impairs the function of these proteins and weakens their structure.

The glycation process and the accumulation of AGEs accelerate with lifetime, and in skin, the visible impact begins to manifest from about the age of 35 years. Here, AGEs build up in the extracellular matrix of both the dermis and epidermis. In the dermis, they bind with and alter components such as elastin and collagen, decreasing skin elasticity, increasing rigidity, and leading to signs of ageing such as stiffness and the appearance of lines and wrinkles. Accumulation of glycated proteins in the extracellular matrix is also responsible

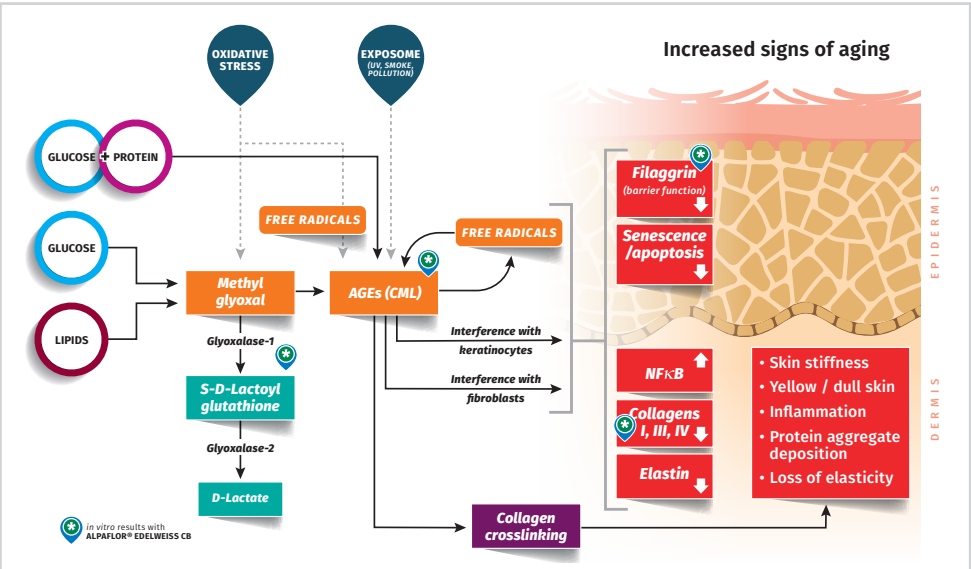

Fig. 1 Glycation processes and their impact on skin

for making the skin dull and yellowish [3]. In the epidermis, *in vitro* studies show that AGEs disrupt the proliferation of keratinocytes [4], making the skin's repair processes less efficient, and impair the skin barrier. Additionally, an accumulation of AGEs in the *stratum corneum* has a negative impact on skin texture which can cause the face to appear older.

Glycation and the formation and accumulation of AGEs are processes that accelerate with time due to an age-associated increase in oxidative stress [5]. Exposure to UV light further aggravates the formation of AGEs, weakening the skin's natural defence systems against reactive oxygen species which in turn results in signs of skin ageing **(Figure 1)**.

Could antioxidants counteract the effects of AGEs?

Given what is known about the impact of AGEs on skin, it is reasonable to assume that cosmetic ingredients that target glycation could help enhance skin appearance. To this end, at DSM, we decided to investigate the anti-glycation potential in an established skin care active already proven for its antioxidant, radical scavenging properties. *Leontopodium alpinum extract* [commercial name ALPAFLOR® EDELWEISS CB].

For our first study, we assessed, *in vitro*, the anti-glycation potential of our active on human epidermal keratinocytes exposed to glycation stress. For the second, we assessed the active's modulating effect on Collagen Type IV Glycation.

Study 1

in vitro assessment of *Leontopodium alpinum* extract's anti-glycation potential in human keratinocytes (NHEK)

Our approach in this study was to measure N^{ε}-(carboxymethyl) lysine (CML), filaggrin (FLG) expression and glyoxalase 1 (Glo1) activity in keratinocytes exposed to glycation stress.

In vitro cell cultures

For all three parts of our study, primary human epidermal keratinocyte NHEK cells from abdominal skin were grown in KGM-Gold™ medium Keratinocyte Growth Medium bullet-kit™ (Lonza).

Anti-glycation potential

The principle of the assay was to evaluate biomarkers of glycation in epidermal keratinocytes activated by glycation stress (by glyceraldehyde). To this end, N^{ε}-(carboxymethyl) lysine (CML) protein adducts, FLG expression, and Glo1 activity in human epidermal keratinocytes NHEK in response to DL-glyceraldehyde (GLA)-induced glycation stress were evaluated in untreated (control) cultures and cultures treated with the plant extract.

The dry Edelweiss extract was tested at 3 concentrations:

$$C_1 = 5\ \mu g/ml,\ C_2 = 25\ \mu g/ml \text{ and } C_3 = 50\ \mu g/ml$$

2 control groups were assessed in parallel:

- 1 unglycated control: culture medium only
- 1 glycated control: culture medium + GLA at 500µM

The incubation time under stress conditions (GLA) was 48 hours and the experiments were repeated in triplicate.

1) Intracellular accumulation of CMLs

N^{ε}-carboxymethyllysine was measured in cell extracts of untreated (control) and treated cultures using an Elisa assay kit (OXISELECT™ N^{ε}-(CARBOXYMETHYL)LYSINE (CML) COMPETITIVE ELISA KIT (CELL BIOLABS INC®). Absorbance was recorded at 450nm and calibrated to a CML standard curve. Total protein levels were also determined with a BCA Protein Assay kit (PIERCE™). CML levels were standardized to the protein content of NHEK cultures.

Findings - Control cultures

Glyceraldehyde (GLA) exposure caused a pronounced increase in CML protein adducts. A statistically significant ($p \leq 0.01$, Student's t test) 8-fold induction in basal glycation level was recorded in glyceraldehyde- exposed, untreated control (+)GLA cells.

Findings - *Leontopodium alpinum extract* treated cultures

The intracellular CML accumulation at the end of the GLA stress phase in cells treated with the plant extract at 25 µg/ml (40% decrease, $p \leq 0.01$) and 50 µg/ml (55% decrease, $p \leq 0.01$) was significantly lower than in (+)GLA control cells. At 5 µg/ml it was too diluted and a slight increase in CML level was observed (+19%, $p \leq 0.01$). These results indicate that treating NHEK cells with the active decreases accumulation of GLA-induced CML protein adducts dose-dependently by up to 55% **(Figure 2)**.

2) Filaggrin expression

Filaggrin (FLG) was quantified in cell extracts using a sandwich Elisa, "Human Filaggrin ELISA Kit" (CUSABIO®) with absorbance reading at 450nm. FLG levels, calculated by interpolation from the standard curve were expressed in pg of FLG per extract. FLG levels were standardized to the protein

content of NHEK cultures in corresponding extracts. The results were expressed in pg of FLG per mg of proteins and in percentages of (+)GLA control.

Findings - Control cultures

Glyceraldehyde (GLA) exposure reduced FLG protein levels in NHEK cells. The down-regulating effect (-23%) was statistically significant ($p \leq 0.01$, Student's t test) compared to unglycated control ((-)GLA) cells.

Findings - *Leontopodium alpinum* extract treated cultures

FLG levels in NHEK cells treated with the plant extract were significantly increased compared to the (+)GLA control **(Figure 3)**. The up-regulating effect appeared dose dependent in the range of tested concentrations and at 25 µg/ml (+17%, $p \leq 0.05$) and 50 µg/ml(+39%, $p \leq 0.01$), the differences proved to be statistically significant (Student t test) compared to the (+)GLA control. Moreover, the +39% increase took FLG levels higher than they were in the non-stressed control cells without GLA.

3) Glyoxalase 1 activity

Cell extracts were assayed for Glyoxalase 1 (Glo1) activity by measuring the rate of formation of S-D-lactoylglutathione from Glo1 substrate hemithioacetal pre-formed by incubation of methylglyoxal (MGO) and glutathione (GSH) spectrophotometrically at 240nm. Glo1 activity is given in units per mg of protein where one unit is the amount of enzyme that catalyses the formation of 1.0 µmole of S-D-lactoylglutathione per minute under assay conditions. The specific activity of Glo1 is calculated and expressed as milliunits (mU) per extract.

Additionally, cell extracts were used to quantify total protein content using a BCA Protein Assay kit.

Specific activities were standardized to the protein content of NHEK cultures in corresponding extracts. The results were expressed in mU per mg of proteins and in percentages of (+) GLA control.

Findings - Control cultures

The incubation of NHEK cultures with 500µM glyceraldehyde for 48h markedly increased Glo 1 activity. A statistically significant ($p \leq 0.01$, Student's t test) 2.8-fold induction in basal Glo1 activity was recorded in glyceraldehyde-exposed untreated control (+)GLA cells.

Findings - *Leontopodium alpinum extract* treated cultures

The Glo1 activity of cells treated with the Edelweiss extract in the presence of GLA were further and markedly increased

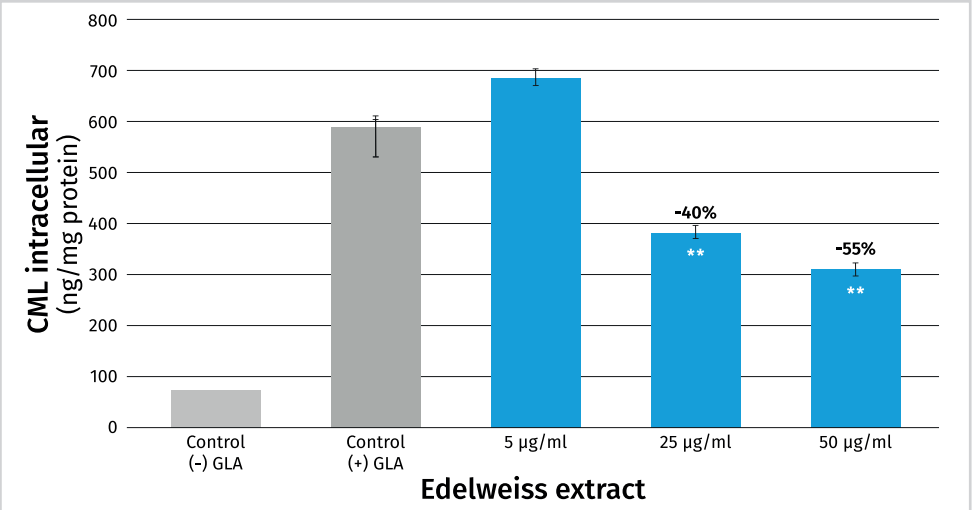

Fig. 2 Intracellular accumulation of CMLs in cultures treated with *Leontopodium alpinum extract* compared to control cultures

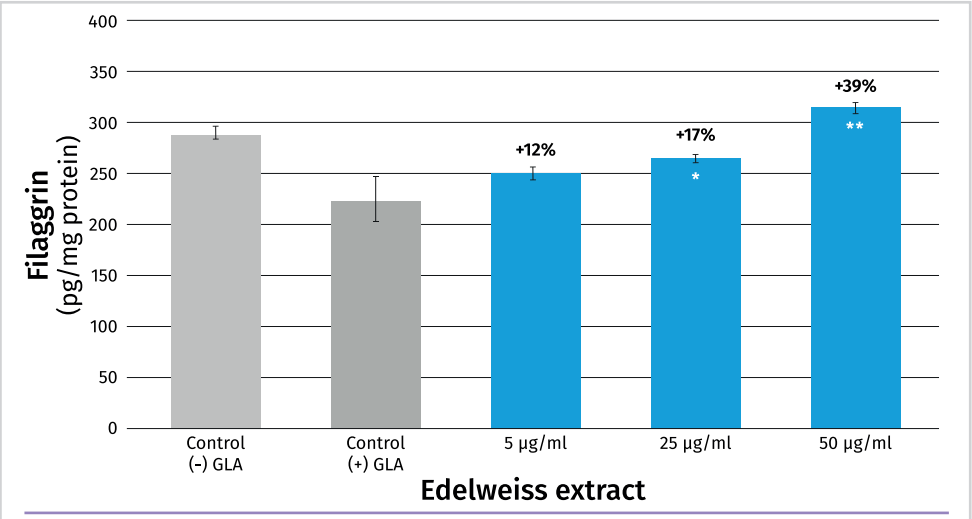

Fig. 3 Expression of Filaggrin in cultures treated with L*eontopodium alpinum extract* compared to control cultures

compared to the (+)GLA control **(Figure 4)**. The up-regulating effect was dose dependent in the range of tested concentrations. At 25 µg/ml it was +32%, ($p \leq 0.05$) and at 50 µg/ml it was +65%, ($p \leq 0.01$). Both increases are statistically significant (Student's t-test).

Study 2

Assessment of the modulating effect of *Leontopodium alpinum extract* on Collagen Type IV Glycation

For this study, we followed an in tubo experimental approach based on the time course formation of N$^{\varepsilon}$-(carboxy -methyl) lysine (CML) in collagen type IV. The assay system involved a tissue culture plate coated with collagen IV (Corning® Bio-Coat™ Collagen IV Cultureware).

Antiglycation activity

To evaluate our Edelweiss extract's anti-glycation properties, we measured the formation rate of AGEs after incubation of collagen type IV with D-ribose. The AGE level was evaluated by measuring the amount of N$^{\varepsilon}$-carboxymethyllysine (CML) in collagen.

A 0.25M D-ribose solution in phosphate-buffered saline (PBS) (pH7.4) was added to each well of a 24-well plate coated with collagen IV (Corning®) under sterile conditions. The plates were then maintained at 37°C and 5% CO_2 for 15 days.

At the end of the incubation period, the plates were washed several times with PBS to remove excess ribose. After washing, N$^{\varepsilon}$-carboxymethyllysine was measured by means of a non-competitive ELISA assay carried out directly in the wells of the plate coated with collagen IV.

The wells were blocked with 1% BSA in washing buffer (0.05% Tween 20 in phosphate-buffered saline (PBS-T)) at room temperature (RT) for 1 hour and then incubated with anti-CML polyclonal antibody (Antibodies-online GmbH) at RT for 2 hours After 5 washes with PBS-T buffer, the wells were incubated with alkaline phosphatase-conjugated goat anti-rabbit IgG at RT for 1hour. After incubation, the wells were washed and incubated in PNPP (para-nitrophenylphosphate) substrate solution. The absorbance of the final p-nitrophenol reaction product was measured at 405 nm.

The Edelweiss extract was tested at five concentrations from 3.125 to 50µg/ml (see **Figure 5**). As a control, a blank [(-)RIB] was performed by preparing PBS without ribose.

Each experimental condition was run in triplicate (n=3).

Expression of results

The absorbance of test-solutions (OD) was corrected by subtracting the absorbance of the blank [(-)RIB] (control collagen IV well incubated without ribose and without Edelweiss

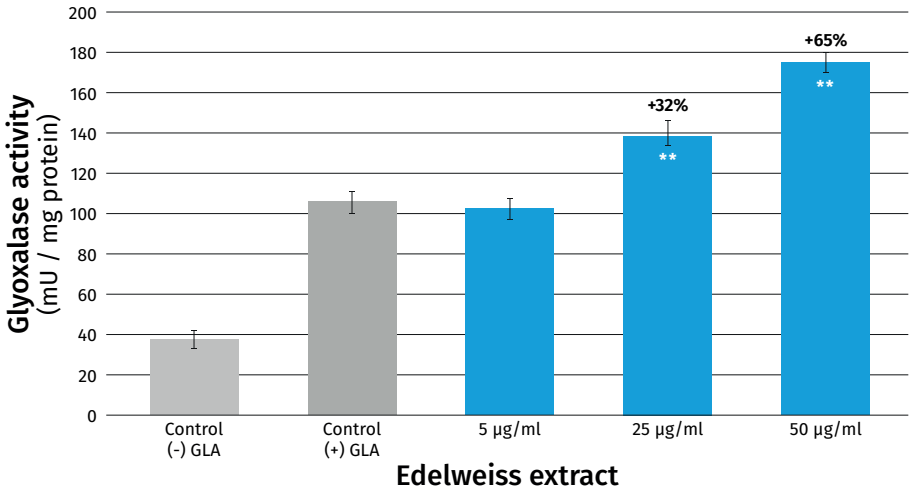

Fig. 4 Glyoxalase 1 activity in cultures treated with Leontopodium alpinum extract compared to control cultures

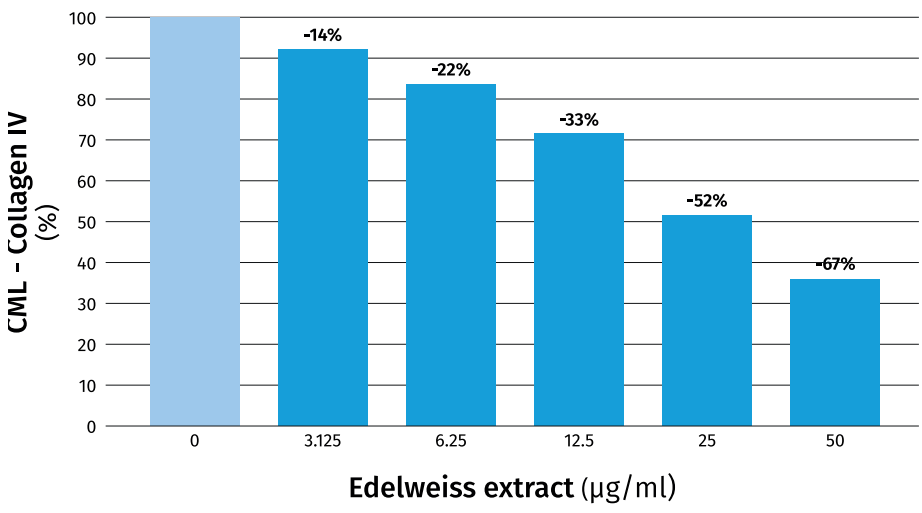

Fig. 5 Reduction of CML induction in wells treated with Leontopodium alpinum

extract). The AGE_{CML} level was calculated as follows:

$[AGE_{CML}$ %] = [OD_{cor}. Treated / OD_{cor}. Control] x 100
with OD_{cor} = $OD_{[(+)RIB]}$ - $OD_{[(-)RIB]}$.

The results were expressed as a corrected OD unit (OD_{cor}] or in percentages of the control or solvent control.

Findings – control wells

Our results showed that ribose (RIB) exposure markedly increased CML formation. A statistically significant ($p \leq 0.01$, Student's t test) 2.4-fold induction in basal glycation level was recorded in ribose-exposed (+)GLA collagen.

Findings – treated wells

The results [AGE_{CML} %] of the assay were collected in table format. The data was analysed statistically using the Student's t test.

Our results showed that in the presence of *Leontopodium alpinum* extract, there was a pronounced decrease of up to 67% in glycated Collagen IV, indicating the active's anti-glycation potential. The inhibition was dose-dependent in the range of the tested concentrations **(Figure 5)**.

The IC_{50} value was calculated by linear regression

[Inhib. (%) /(log(Conc.)]: IC_{50} = 28 µg/ml.

Conclusions

Study 1

Under the experimental conditions described above, to begin, our first study showed that exposure of NHEK cells to glyceraldehyde led to an intracellular accumulation of CMLs, a reduction in FLG and an increase in Glo 1 activity.

Next, our results indicated that in the range of the tested concentrations (5–50µg/ml), our plant extract reduced glyceraldehyde-induced formation of CML in a dose-dependent manner. Concurrently, and under the same experimental conditions, the plant extract significantly increased the Glo1 activity of cells in a dose dependent manner.

Finally, in the range of the tested concentrations (5–50µg/ml), the plant extract demonstrated the capacity to alleviate a glyceraldehyde-induced decrease in FLG in a dose dependent manner. It is also worth noting that the highest tested concentration (50µg/ml) showed the ability to restore barrier function impaired by glyceraldehyde completely.

Study 2

Under the experimental conditions described above, the results of our second study evidenced that the Edelweiss extract inhibited glycation reaction, and sustained dose dependency. The IC_{50} value was equal to 28 µg/ml.

Overall conclusion

The Edelweiss extract shows anti-glycation properties through:

- its ability to significantly down-regulate the accumulation of AGEs in skin cells which show toxic effects resulting in cell dysfunction during ageing,
- Glo 1 activation which plays a key role in the detoxification of dicarbonyls and in the protection of keratinocyte proteins against oxidative damage during skin ageing,
- its inhibiting effect on Collagen Type IV glycation.

Furthermore, it is worth noting that anti-glycation potential is associated with a protective effect on "skin barrier function" which is a key element of cellular homeostasis during skin ageing.

Therefore, in addition to its proven ability to protect skin from oxidative stress, *Leontopodium alpinum* extract also demonstrates potential to protect against glycation, further enhancing its ability to help deliver skin care benefits such as healthy-looking skin, slowing down the appearance of visible signs of skin ageing, and preserving collagen integrity for smooth, elastic and rejuvenated skin.

About the active

Leontopodium alpinum extract [commercial name ALPAFLOR® EDELWEISS CB] is produced from a unique Edelweiss variety, *Leontopodium alpinum 'Helvetia'*. It is extracted from particularly robust plants cultivated at altitudes of up to 3000 metres and that develop an exceptionally high active content of leontopodic acid and flavonoids due to the high levels of UV radiation, strong winds and temperature extremes they are exposed to.

Its antioxidant properties and protective action on the skin barrier have made it a popular choice in pro-ageing skin care formulations. Additionally, it meets growing consumer expectations for natural and ethically sourced products as it is extracted from organically grown plants sourced through a short, local, and fair supply chain. Furthermore, because the Edelweiss is a particularly resilient plant, *Leontopodium alpinum extract* is likely to have a symbolic appeal to consumers turning to sustainable ingredients as part of a drive to boost

personal resilience and contribute to a more responsible consumption as part of the rapidly growing, post-Covid 19 trend for "resilient beauty".

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References:

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