New data confirm skin revitalizing and stress protection by Glycoin® natural

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Abstract

Glycoin® natural (INCI: Glyceryl Glucoside) is an ECOCERT certified multifunctional active ingredient. In nature this strong extremolyte is produced by the desert resurrection plant Myrothamnus flabellifolia *and blue-green algae (cyanobacteria). For cosmetic use the natural glyceryl glucoside isomer (2-O-a-D-glucopyranosyl glycerol) is manufactured with high purity by an enzymatic process. Glyceryl glucosides are mentioned for increasing skin elasticity, moisturizing, and reduction of symptoms like itching, burning, tightness, tingling, and feeling of dryness. In vitro assays in cell cultures and skin models demonstrate strong activity of Glycoin® natural on aged and stressed skin cells with rejuvenating, recovering and radical scavenging properties. In vivo treatments confirm positive effects on increased skin elasticity, smoothness and thickness; the epidermis and dermis density regenerates.*

Introduction

Glycoin® natural (INCI: Glyceryl Glucoside) is used as a multifunctional anti-aging, cell-stimulating and skin moisturizing agent in cosmetics. In skin, glyceryl glucoside treatment enhanced glycerol derivatives and increased the aquaporin 3 expression in vitro and ex vivo [1)]. Glyceryl glucosides are mentioned for increasing skin elasticity, moisturizing, the reduction of symptoms like itching, burning, tightness, tingling, and feeling of dryness [2)].

Glycoin® natural belongs to the group of glyceryl glucosides and the chemical isomer is called "2-O-a-D-glucopyranosyl glycerol". This special glyceryl glucoside isomer is a strong extremolyte [3)]. In nature 2-O-a-D-glucopyranosyl glycerol is produced by the resurrection plant *Myrothamnus flabellifolia* and *Spirulina subsalsa* "marine blue-green algae" [4, 5)]. Glyceryl glucoside is an osmoregulatory and stress protecting molecule, which protects plants or bacteria under extreme conditions. The presence of glyceryl glucoside diminishes stresses and strains in the membranes at low or zero hydration level [6)].

The plant *Myrothamnus flabellifolia* for example is known for its survival strategies under extreme conditions. It could remain in the dry state under simulated field conditions for many years, without losing viability [7)]. The described glyceryl glucoside isomer is synthesized during dry season (dehydrating process) in the desert plant, to protect the plant's structure from damage due to total desiccation [5)]. In this context, Ferjani et al. study results describe glyceryl glucoside action as an osmoprotectant [8)].

As soon as water is available again the glyceryl glucoside acts as energy booster and the *Myrothamnus flabellifolia* revives and re-greens [9)].

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Picture 1:
Myrothamnus flabellifolia *during dry season (left),* Myrothamnus flabellifolia *after rain (right)*

Cyanobacteria like marine blue-green algae *(Spirulina subsalsa)* also synthesize and accumulate 2-O-a-D-glucopyranosyl glycerol [4)]. Like the *Myrothamnus flabellifolia,* blue-green algae use glyceryl glucoside as stress-protection molecule [10)]. In general, these algae favor fresh water habitats, but some strains prosper in strongly alkaline salt lakes or marine environments [11)].These blue-green algae are characterized by their impressive ability to synthesize and accumulate glyceryl glucoside to acclimate in habitats with high or fluctuating salinity [12, 10)]. Glyceryl glucosides are also naturally present in red, rosé and white wine, as well as in Japanese food brewed with koji, like sake, miso and mirin [13)].

Glycoin® natural production

bitop AG produces Glycoin® natural, the only natural glyceryl glucoside (2-O-a-D-glucopyranosyl glycerol) currently available for the cosmetics industry, in Germany with a high purity. The patented production process was developed in cooperation with the University of Graz, Austria. The Glycoin® natural production

is realized by a biocatalytical process, an enzymatic reaction, using the enzyme "succrose phosphorylase". The natural substrates succrose and glycerine are transferred into Glycoin® natural and fructose. After the downstream process, a highly pure and all-natural glyceryl glucoside remains. In this production process, the yield of Glycoin® natural is 90% [14].

Cell-protection and stabilization properties by Glycoin® natural treatment

Toxicological study results show that the use of Glycoin® natural (concentration 0.5% up to 3% w/w) as active ingredient in cosmetic/cosmeceutical formulations exhibits no aberrant risks for local toxicity or mutagenic properties. An additional in vivo sensitization study (50 volunteers) of Glycoin® natural cosmetic formulation revealed neither sensitizing properties nor any other aberrant adverse effects. Furthermore Glycoin® natural did not show any irritating effect to the skin, eyes or mucosa in different in vitro and in vivo studies.

An in vitro model was established to demonstrate that glyceryl glucoside protects cell membranes. Keratinocytes were pre-treated with Glycoin® natural or Myrothamnus plant extract and then stressed with 0.001% SDS. Only the pure Glycoin® natural protected the cell membranes of keratinocytes from damage (figure 1).

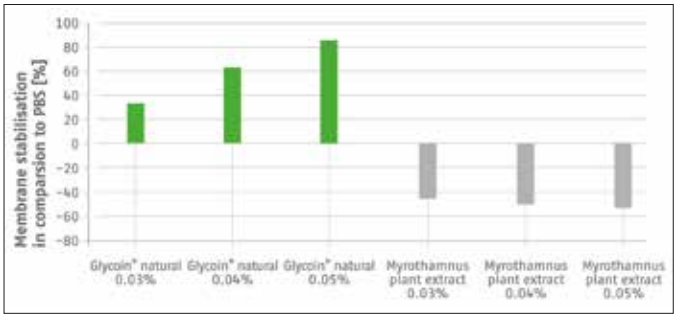

Figure 1: Keratinocytes were pre-treated with Glycoin® natural or Myrothamnus plant extract (concentration: 0.03%, 0.04% and 0.05%) Then cells were stressed with SDS and cell membrane stability was measured.

In human keratinocyte cell line (HaCaT) treatment only Glycoin® natural stimulates the ATP production in aged skin cells compared to plant extract (figure 2).

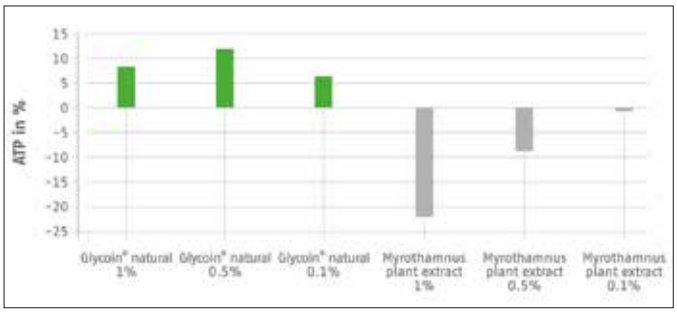

Figure 2: ATP metabolism in aged human keratinocytes after pre-treatment with Glycoin® natural and a Myrothamnus plant extract at different concentrations.

Glycoin® natural in vitro results show anti-aging effects on skin cells

Different in vitro models were used to investigate anti-aging effects of Glycoin® natural on human skin cells.

Cell vitality by glucose consumption

Cell vitality was determined by investigation of glucose consumption in human epidermal keratinocytes. Glucose plays a role in the energy metabolism of keratinocytes. It is associated with cell regeneration and renewal.

These results show the cell viability of human epidermal keratinocytes cultivated with Glycoin® natural (1%) versus untreated control. The Glycoin® natural treatment significantly increases cell vitality compared to control. The more active cells are, the more glucose they need for their metabolic activity.

Glycoin® natural increases the vitality and metabolic activity of aging skin cells by up to 170% (figure 3). These effects may be useful in reversing dried, aged skin conditions to promote healthy regenerated skin.

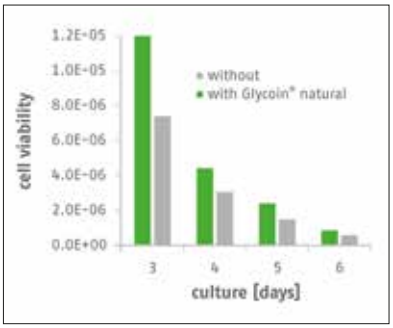

Figure 3: Measured cell viability during cell aging per treatment days (measured by glucose consumption).

Adenosine triphosphate increase by Glycoin® natural treatment – leads to stimulation of cell vitality

In two different studies, keratinocyte cell models were treated with Glycoin® natural (1%). Afterwards adenosine triphosphate expression was measured. Adenosine triphosphate (ATP) transports energy within cells for metabolism. It is responsible for cellular activity and vitality, DNA replication, regeneration and healing as well as collagen and elastin synthesis. Skin cells synthesize great amounts of ATP. During the skin aging process the amount of ATP in skin cells decreases constantly [15].

The results of these in vitro models show that cell treatment with Glycoin® natural stimulates ATP expression. An increase of ATP leads to a stimulation of cell vitality and cell renewal. With chronological aging (passage of time) and extrinsic skin aging

(external stress), skin cells show a clear reduction of ATP production, followed by a reduced metabolic activity and cell vitality.

In an in vitro human keratinocytes (HaCaT cells) model, HaCaT cells were aged over 2 weeks and then treated with Glycoin® natural, glucose, fructose and glycerin (Concentration: 0.75%, 0.5% and 0.1%). After 6h incubation ATP concentration was measured with luciferase assay. Beside the increase of the cell metabolism, Glycoin® natural also significantly increased the ATP concentration in aged skin cells (figure 4).

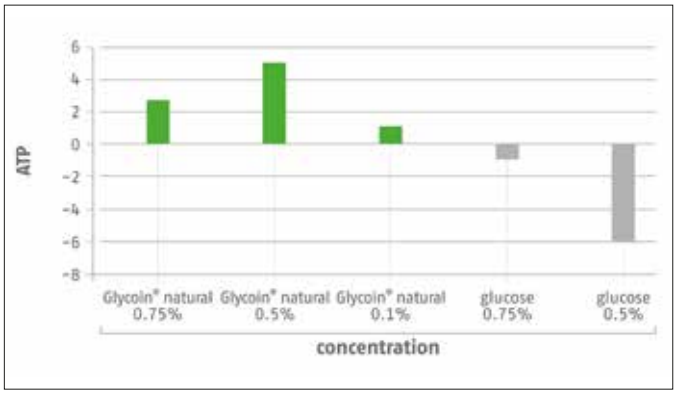

Figure 4: ATP concentration in aged human keratinocytes after pre-treatment with Glycoin® natural or glucose at different concentrations. Glucose was used as control for a potential ATP increase induced by sugar treatment.

Glycoin® natural stimulates anti-oxidant activity in aged skin cells

An in vitro study on human epidermal keratinocytes showed that the cell treatment with 1% Glycoin® natural increased production of the enzyme superoxide dismutase 1 (SOD1). SOD1 is an important anti-oxidant which occurs naturally in the human body. It plays a part in the skin's self-defense system, reduces free radical damage, and therefore prevents signs of aging, helps to heal wounds, softens scar tissue and protects against UV induced damage rays. Natural superoxide dismutase levels and activity slow down during the aging process [16].

Glycoin® natural treated aged keratinocytes produce up to 280% more SOD1 compared to untreated cells. Glycoin® natural increases the amount of SOD1, anti-oxidant activity and seems to strengthen the skin's own defense and immune system.

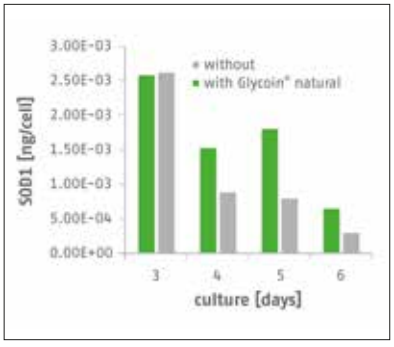

Figure 5: Amount of SOD1 per cell during the cell aging process. At day 5 Glycoin® natural treated aged keratinocytes produce up to 280% more SOD1 compared to untreated cells.

DNA array study confirms skin cell recovery

A DNA array study presented results which indicate that skin treatment with Glycoin® natural may improve aged skin, deliver anti-oxidant, anti-stress, healing and maybe whitening effects. Glycoin® natural cell treatment induces the expression of growth factors like fibroblast growth factor 7 (FGF7) and transforming growth factor beta 1 (TGF-beta 1) which are involved in the wound healing process and initiate the proliferation of new dermal cells. FGF7 is also mentioned for potent regenerative, cytoprotective effects against oxidative stress, toxic compounds and UV irradiation, and usually is up-regulated after injury and during healing.

Additionally, the expression of ROS scavenger molecules (catalase (CAT), superoxide dismutase 2 (SOD2), metallothionein-1 (MT1H)) are enhanced by Glycoin® natural [17].

Viability-enhancing effect by Glycoin® natural

With an epidermis model the positive effect of Glycoin® natural treatment was investigated. The Glycoin® natural treatment on non-irradiated epidermis models (SkinEthic®) was able to achieve a viability-enhancing effect. The cells, which were in good condition and not stressed, experienced a "vitality boost" by adding Glycoin® natural.

In addition, an increased viability versus untreated control cells could also be demonstrated in an UV-irradiated epidermis model.

In both study models, a reduction of IL-1 alpha levels after Glycoin® natural treatment was achieved. This correlated with the viability results. The increase in vitality, measured by the release of lactat dehydrogenase (LDH), could be shown in both experiments.

As a result of the UV irradiation, damage to the cell membranes and the increase in the LDH values could be determined. In comparison to the untreated control, the addition of Glycoin® natural resulted in a reduction in LDH release and thus an improvement in membrane integrity and cell vitality (data not shown).

In vivo efficacy studies: Improved skin elasticity and epidermis and dermis density

In a placebo controlled, blinded in vivo study with 20 Caucasian volunteers a cream containing 1% Glycoin® natural was tested. Cream was applied twice daily for 4 weeks. Parameters for skin moisturizing, elasticity (cheek) and smoothness (lower leg) were measured. The study documented a moisturizing rise of 23% after application, an increase of skin elasticity by 93%, and of skin smoothness by 62%. The cream containing 1% Glycoin® natural was tolerated very well according to clinical dermatological criteria and led to a reduction in the roughness of the skin.

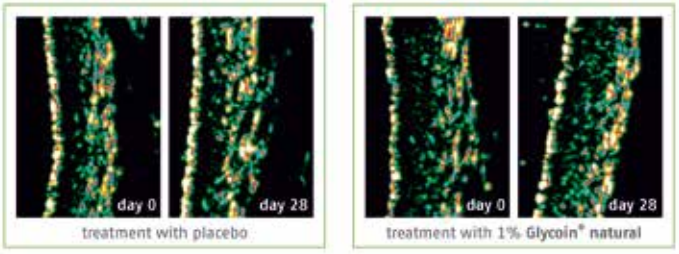

Figure 6: Subepidermal low-echogenic band measured the increase of epidermal and dermal thickness and density at the beginning and the end of the treatment period. Glycoin® natural increased significantly epidermal and dermal thickness and density compared to placebo.

An additional 4-week blinded, placebo-controlled cosmetic efficacy study evaluated the anti-aging properties of a Glycoin® natural containing cosmetic cream. Five Caucasian female volunteers were treated and assessed by ultrasonography on the inner forearms. Epidermis and dermis density increased up to 9.9% after applying Glycoin® natural containing formulation (figure 6). This increase was statistically significant and better than the placebo. All volunteers reported easy and comfortable application conditions of Glycoin® natural containing formulation. The topical Glycoin® natural containing formulation regenerates the epidermis and dermis density.

Summary and discussion

Glycoin® natural is a 100% natural active anti-aging ingredient. Glyceryl glucosides are already established in cosmetic formulations, but have only been available as a synthetically produced racemate. Glycoin® natural is the only natural alternative consisting of the molecule preferentially formed in nature. Several in vitro and in vivo studies showed the cell protecting and recovering effect of Glycoin® natural treatments. In vitro models and skin models demonstrate strong activity on aged and stressed skin cells with rejuvenating, recovering and radical scavenging properties. In vivo treatments confirm positive effects on increased skin elasticity, smoothness and thickness, the epidermis and dermis density regenerate with no irritating effects.

A number of studies confirm Glycoin® natural results. Vehicle controlled in vivo studies of two glyceryl glucoside formulations demonstrated significantly improved hydration versus controls. Furthermore significant reduction of transepidermal water loss versus untreated site and basic vehicle was shown. Both formulations (rich and light) significantly decreased visible dryness and tactile roughness. The rich formulation maintained significant moisturization and the light formulation reduced symptoms of itching, burning, tightness, tingling, and feeling of dryness [2]. In a topical study by Harada et al. a cosmetic cream with alpha-D-glucosyl glycerol was applied on 13 female volunteers. The researchers observed an increase of elasticity in cheek-skin and suggested that alpha D-glucosyl glycerol increases the production of IGF-I in the skin through sensory neuron stimulation, thereby increasing skin elasticity [17]. This elasticity results may confirm Glycoin® natural treatment observations.

In conclusion Glycoin® natural is useful for anti-aging skin care and stress protecting formulations for mature, tired and stressed skin.

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