

A Natural Approach to Protect Thin Skin in the winter:

Key Extracts, Mechanisms and Testing Protocols

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Introduction

Winter is one of the hardest seasons for the skin which is particularly exposed to stressors such as low humidity, and cold temperatures, etc. These lead to dehydration and sensations of skin tightness – especially in the older members of the population whom nature has endowed with thinner skin as a consequence of the aging process.

Natural solutions have been developed to protect the skin from such aggression by improving the skin's natural defense system and restoring the intrinsic synthesis of essential proteins which enable to maintain skin homeostasis and create a healthy looking skin.

Expert in plant extraction, Alban Muller creates these solutions are based on plant extracts selected by observing plants which thrive in environments that simulate the stressor effects we, as cosmetic scientists seek to avoid. Indeed, as we continue our search we have discovered that plants are truly an amazing source of active molecules and provide a great inspiration for cosmetic actives with skin benefits.

Specific actives have been developed to improve skin protection for winter conditions and especially for those having thinner skin. They offer a global solution for a perfect skin care routine in the winter. Three such products are described in this article. All are created and available from Alban Muller Company in France.

- Preparami®: Improves the synthesis of natural self-defense proteins
- Cytokalmine® ER which soothes skin inflammation and reduces oxidative stress
- Amiporine® and Lipolami® ER which maintains skin hydration and preserve the skin barrier

From a scientific view, our approach is to look for plants that have developed genetic strengths that enable their survival in their hostile natural environments.

1. Natural Solution to Protect the Skin from Environmental Aggression: Preparami®

Description

Preparami® is a prickly pear extract dissolved in a jojoba oil carrier. Both the Prickly Pear cactus and the Jojoba bush are

known to grow in arid and dry environments. Their survival relies on their unique plant metabolism. Therefore, they are plants especially interesting natural sources to boost the protection of the skin during winter conditions.

1.1. Mode of action

Preparami® conditions skin cells in order to prepare them for environmental aggression throughout the day. These include sun rays, extreme temperatures and dry or polluted environments. This combination of two natural extracts functions by accelerating the appearance of chaperone proteins, or HSPs (Heat Shock Proteins). This mechanism is a universal cell-defense tool that helps organisms to adapt to unfavorable conditions set by the environment.

Preparami®'s action is based on the principle of conditioning the cells to reduce their latent reaction period. This optimizes the efficiency of the cell's reaction. For this to occur, Preparami® acts by stimulating the inducible chaperone proteins HSP 72 in the skin when an obvious stress factor is identified¹.

1.2. Efficacy Data

1.2.1. Cell Conditioning

Protocol:

An *in vitro* test was carried out on human keratinocyte layers by comparing a non-treated control population and a population treated with 5µg/mL of Preparami®. These populations undergo various types of stress (ethanol in the culture medium, UV rays, and heating at 43°C): Hsp72 synthesis is studied by Elisa immuno-enzymatic technique. This immuno-enzymatic technique which helps measure the amount of a given protein, based on it being sandwiched between two antibodies: one specific capture antibody (to Hsp72 in this case) and one detection antibody, itself paired with an enzyme catalysing the appearance of a coloured complex. The solution's absorbance is then measured at the specific wavelength of the final complex. The latter is proportional to the amount of proteins expressed.

Results:

The appearance of Hsp72 occurs within approximately 20 minutes in the treated population instead of 100 to 150 minutes in the control population (Fig. 1). Consequently, cell viability remains stable in the treated population while it collapses in the control population. Furthermore, the rate of Hsp72 remains high for 72 hours rather than 8. The cells are thus protected from stressors for a longer period of time than if left untreated.

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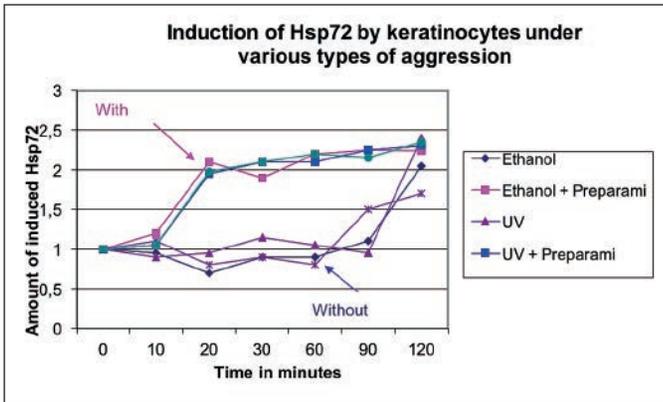


Fig. 1:

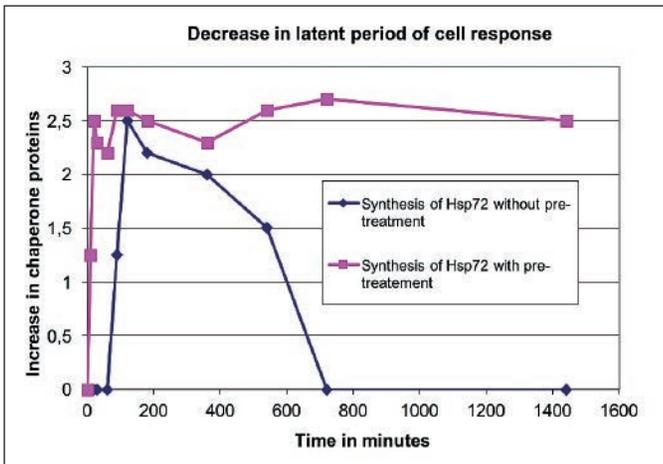


Fig. 2:

1.2.2. Resistance of Treated Cells to Sun Exposure

While this article is primarily focused on protecting thin skin in cold weather, it turns out that this approach also provides protection to sun exposure as well.

Protocol for testing Impact of Solar Exposure

The following study was carried out on 3 human keratinocyte populations: a non-treated, non-radiated negative control (Control); a non-treated, radiated positive control (UV); a population treated with 5µg/mL of Preparami® and UV radiated (UV + Preparami®)

Radiation is provided by a solar spectrum lamp (UVA + UVB) for 15 minutes. Cell viability is measured by a mitochondrial activity test (XTT* test) and the Hsp72 synthesis is quantified by an Elisa kit.

Results:

Fig.3: Compared to the control population, the Hsp72 synthesis increases by 10% in non-treated cells and by 200% in cells treated with Preparami®. Therefore Preparami® protects the cell from UV rays.

Fig 4 and 5: The photos of the marked Hsp72 reveal an acceleration of the appearance of the fluorescence level measured. This means there is an acceleration in the protection of the cells in contact with Preparami®. However, there is no difference in fluo-

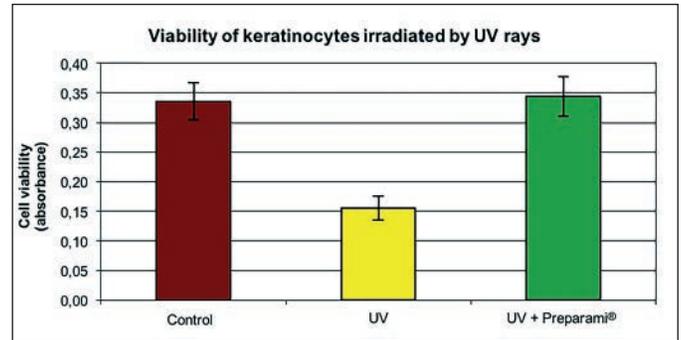


Fig. 3:

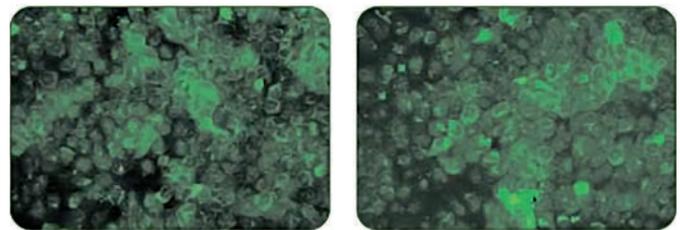


Fig. 4: Before UV radiation (left without Preparami®/right with Preparami®).

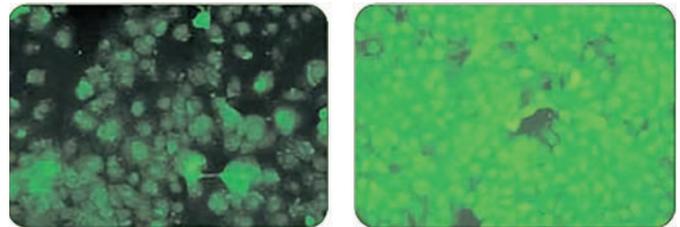


Fig. 5: After UV radiation (left without Preparami®/right with Preparami®).

rescence between the control cells and the non-stressed and Preparami® treated cells. Thus, the presence of Preparami® does not stress the cells (there is no Hsp72 synthesis).

Preparami® accelerates Hsp72 synthesis only in case of stress. Therefore the skin is well prepared for sun exposure.

2. Natural Solution to Soothe Skin Irritation: Cytokalmine® ER

Extreme conditions such as UV, heat and cold can lead to an inflammatory reaction and skin discomfort like itching and redness. The main factors responsible are an alteration of the skin barrier leading to a significant dehydration. Additionally, oxidative stress is also present and the combination leads to an abnormally high secretion of cytokines (inflammatory molecules). Extreme conditions such as UV, heat and cold can lead to an inflammatory reaction and skin discomfort like itching and redness. The main factors responsible are an alteration of the skin barrier leading to a significant dehydration. Additionally, oxidative stress is also present and the combination leads to an abnormally high secretion of cytokines (inflammatory molecules).

2.1. Description

Cytokalmine® ER is a dry extract of Carthaginian berry (*Punica granatum*). It is rich in polyphenols (particularly punicalagin and ellagic derivatives) which offers a double targeted action: anti-inflammatory and free-radical scavenging – both of which are important for dealing with thin skin in winter environments.

2.2. Mode of action

Thanks to its content in polyphenols Cytokalmine® ER acts on the cascade of cell signals appearing after an aggression such as the expression of several proteins including cytokines, mediators of inflammation triggering an over production of free radicals.

2.3. Efficacy data

2.3.1. Reduction of cytokine secretion

Protocol

Demonstration of anti-inflammatory efficacy is assessed ex vivo on an inflammatory model of reconstructed epidermis mimicking atopic dermatitis.

Human reconstructed epidermis are first cultured and then treated with a specific pro-inflammatory mixture model in order to trigger an atopic dermatitis, i.e. an allergenic inflammatory reaction. This pro-inflammatory molecule mixture is composed of IL-1 α interleukin and Poly(IC), polyinosinic-polycytidylic acid in order to obtain the specific double stimulation activating different signaling pathways, thus reproducing inflammatory conditions reflecting reality.

These “stimulated” human reconstructed epidermis are incubated (or not) with 40 $\mu\text{g/mL}$ and 75 $\mu\text{g/mL}$ of Cytokalmine® ER during 24 hours according to a systemic treatment. At the end of the incubation time, the supernatants are recovered and the cytokine secretion is quantified by ELISA technique.

Results:

Fig 6: Cytokalmine® ER reduces the secretion of cytokines IL-8 by an average of 40%, in comparison with the non-treated ones.

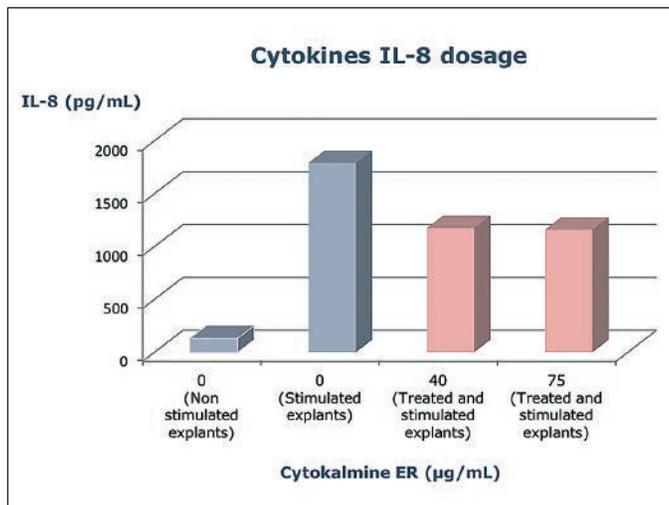


Fig. 6: dosage of cytokines IL-8 by ELISA technique.

These results demonstrate the efficacy of Cytokalmine® ER especially to reduce the complex cascade of cells signals leading to the inflammatory reaction and atopic dermatitis reproduced by the specific protocol.

2.3.2. Reduction of Free Radical Production

Protocol:

The antioxidant activity is tested on neutrophils issued from human blood samples. A cell suspension of neutrophils is isolated and incubated with increasing doses of Cytokalmine® ER or standardized ginkgo biloba extract* which is employed as an antioxidant reference. After incubation, neutrophils are activated by PMA (phorbol myristate acetate) to stimulate ROS production. Immediately after the activation of neutrophils, ROS production is measured by chemiluminescence for 30 minutes. The results are expressed as a percentage of relative chemiluminescence (CL) compared to the control AN (non treated Activated Neutrophils).

Results:

Fig 7: Cytokalmine® ER significantly reduces the fluorescence emitted by the product of the oxidation reaction. The ROS production is thus considerably reduced by Cytokalmine® ER in comparison with the ginkgo biloba extract reference.

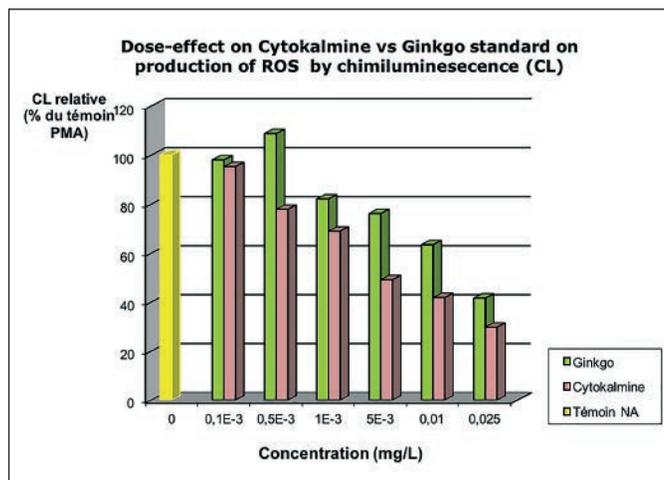


Fig. 7: dosage of antioxidant activity.

At 5 $\mu\text{g/mL}$, Cytokalmine® ER is 2 times more efficient than the reference. Moreover 10 $\mu\text{g/mL}$ of Cytokalmine® ER is enough to obtain a reduction of ROS production by 60%, while it takes 25 $\mu\text{g/mL}$ of ginkgo extract to reach the same efficacy.

Cytokalmine® ER proves to be a powerful antioxidant reducing the oxidative stress.

3. Natural Solution to Improve Skin Hydration: Amiporine® and Lipolami® ER

Cold winter conditions are often responsible for skin dryness. Therefore, preserving the epidermis barrier and maintaining skin optimal hydration is essential in a skincare routine during winter.

One of the solutions available is to improve water flow within epidermis skin cells and preserve an efficient skin barrier. Among Alban Muller natural actives, Amiporine® ER and Lipolami® ER are a perfect answer to those needs and product claims.

3.1. Description

Amiporine® ER is an extract of pomegranate fruit stabilized in glycerin that boosts the natural synthesis of aquaporins AQP3 in the epidermis and thus helps to maintain an optimal distribution of water within the skin and contributes to a healthy skin barrier.

Lipolami® ER is a designed cocktail of ethylic esters obtained from milk thistle oil and thus represents a very unique multifunctional formulation ingredient offering a silky and dry silicone-like touch while protecting the skin barrier.

3.2. Mode of action

Amiporine® ER allows an optimal management of water by stimulating the synthesis of Aquaporin 3 or AQP3 in the epidermis, a transmembrane protein forming water channels for the transportation of water throughout the tissues. Many studies show that a lack of AQP3 provokes a loss in elasticity of the epidermis, an alteration of the cutaneous barrier and a decrease in the water quantity of the stratum corneum, driving to a significant dehydration of the skin.

Thanks to its composition rich in omega 6 fatty acid ester, Lipolami® ER prevents the skin from dehydration by adding extra linoleic acid to reinforce the skin barrier. Indeed, the intercellular cement between the corneocytes is composed of lipid bilayers, which are themselves composed of free fatty acids, ceramides and cholesterol arranged in a very orderly manner. The latest research has illustrated the importance of linoleic acid in ensuring the intercellular cement strength and the efficacy of the skin barrier.

3.3. Efficacy data

3.3.1. Stimulation of the synthesis of AQP3 by Amiporine® ER

Protocol:

An Amiporine® ER efficacy study on aquaporin 3 (AQP3) synthesis was carried out on healthy skin from a 57 year-old woman having undergone plastic surgery. Three explants were prepared as follow by eliminating fat and rinsing with a sterile phosphate buffer, and then cut in slices of about 3mm in size with a sterile scalpel. The study was conducted as follow:

A first part of the explant is immediately frozen at -70°C to serve as a control at D0 and two other parts were treated with a placebo cream: one during 7 days, the other during 12 days. Following this procedure, both samples were incubated (epidermis outwards) in a culture medium to serve as a control at D7 and D12. The last two parts were treated with a 5% Amiporine® ER cream, then separately incubated in the same culture medium during 7 and 12 days. The creams were applied every two day on the epidermis surface with a sterile spatula. The culture medium was changed every two days. At the end of incubation, the explants were rinsed and frozen at -70°C for at least one night. The

explants were then cut with a cryostat in slices of about 5µm thick and frozen at -70°C until immunostaining was implemented.

Results:

On the skin explants treated with the 5% Amiporine® ER cream, we observe at 7 days (Fig. 9) and at 12 days (Fig. 10) a higher quantity of AQP 3 in the dermis and an obvious thickening of the epidermis tissue compared to the contral at day 0 (Fig 8). Thus, Amiporine® ER stimulates the synthesis of AQP3 and optimizes skin hydration.

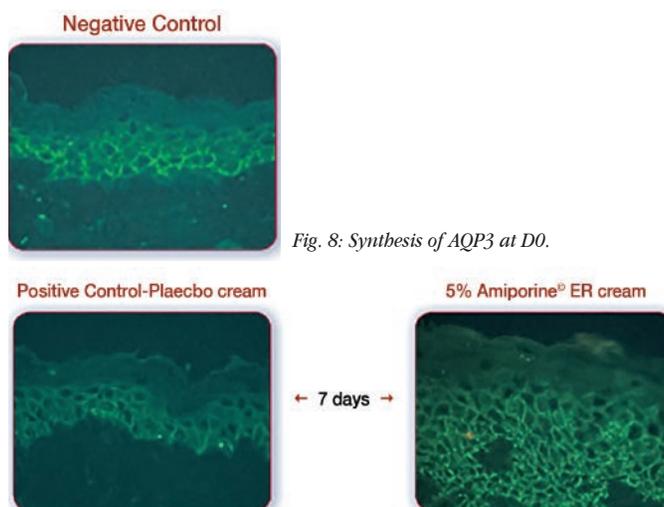


Fig. 8: Synthesis of AQP3 at D0.

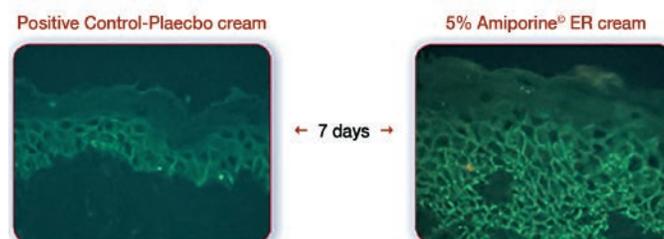


Fig. 9: Synthesis of AQP3 after 7 days.



Fig. 10: Synthesis of AQP3 after 12 days.

3.3.2. Protection of the Skin Barrier by Lipolami® ER

The evaluation of the protective mode of action of Lipolami® ER on the skin barrier is carried out ex vivo by an immunostaining study of filaggrin which is a protein marker of the state of the skin barrier and which also provides information on the degree of skin hydration.

Indeed, filaggrin is a protein participating in the formation of the stratum corneum and its barrier function and plays also a key role in the hydration of the epidermis upon its degradation into free amino acids forming a major part of the NMF (Natural Moisturizing Factor). The more the skin is dry, the greater the hydrolysis of filaggrin is in order to maintain the homeostasis of the hydration process.

Protocol:

Three batches of explants were treated as follows:

- Control explants (C) untreated explants
- Control Delipidated explants (CD), after a treatment for 2 hours with a solution of SDS at 10%

- Explants (LD) treated with Lipolami® ER and then delipidated, three hours after, by SDS (Sodium Dodecyl Sulfate) solution at 10% for 2 hours

Results:

The fluorescence of explants C (Figure 11) represents an optimal expression of the filaggrin and thus a normal state of skin hydration. On the other hand, explants CD show a decrease by half of the filaggrin expression. Indeed, filaggrin is degraded to provide compensation for losses caused by the SDS delipidation and thus provide skin hygroscopic components of the NMF. The expression of filaggrin in the explants LD, treated beforehand with the Lipolami® ER, is at 59%. This represents a significant increase of 13% compared to explants TD. Thus Lipolami® ER reinforces the lipid cement and thereby maintains its sealing capability. By this action, Lipolami® ER reduces the intense dryness of the skin caused by the SDS and reduces the need for NMF. In point of fact, the filaggrin is less hydrolyzed and its expression is higher than in explants CD.

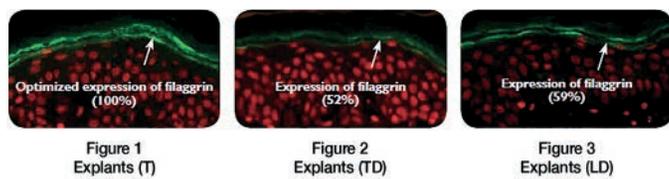


Fig. 10: Synthesis of AQP3 after 12 days.

Lipolami® ER thus preserves the skin barrier function by protecting it from dehydration. It offers an opportunity for cosmetic bases a means to reinforce the efficacy of products for dry, delipidated or atopic skin.

Conclusions

The natural plant active extracts described above are designed manufactured by Alban Muller Co. and show how nature provides natural solution to maintain healthy skin under stressful conditions. Thanks to these actives the skin and especially mature skin is reinforced and better protected against winter aggressions.

References

1 1996–Muramatsu T. and Hatoko M.–Age-related decrease in the inductability of heat shock protein 72 in normal human skin.– *Br J Dermatol.*– pp.1035-8

* A standardized ginkgo biloba extract containing 24% flavones glycosides, 6% ginkgolids and bilobalids. ■