Activoil Echnidium ZRO for skin barrier function

Dr Valeria Quaranta – Innovacos Corp., US

Today the importance of accurate personal hygiene is globally recognised in preventing the spread of the COVID-19 virus. However, attention must also be paid to the possible negative consequences for the skin.

The regular use of alcohol-based gels or cleansing products is usually well-tolerated, but repeated exposure to water and other chemical agents can induce several pathophysiologic changes, such as epidermal barrier disruption, keratinocyte impairment, the subsequent release of proinflammatory cytokines, the activation of the skin's immune system and delayed-type hypersensitivity reactions. Adverse dermatologic effects, such as excessive skin dryness, skin irritation or even contact dermatitis, can occur, especially in individuals with a history of atopic dermatitis (Figure 1).[1]

Alcohol-based sanitisers have a key role in mitigating the transmission of pathogens in healthcare settings. The US Center for Disease Control & Prevention (CDC) recommends using one that contains at least 60% alcohol as a reasonable alternative to handwashing, if the hands are not visibly dirty and greasy.[2] Alcohol-based sanitisers work by killing germs and viruses. Their viral targets are predominantly the viral lipid envelope and the protein capsid, which contains and protects the genetic material, and the genetic material itself.[3]

However, lipid extraction and the denaturation of proteins, due to alcohol topical application, may be relevant also for the skin, in particular for the intercellular lipid structure and stratum corneum enzymes that are involved in barrier functions, causing skin dryness.[4] In addition, topically applied ethanol acts as a skin-penetration enhancer and may facilitate the transdermal absorption of irritating agents, increasing skin sensitivity.[5]

Figure 2: Activoil Echnidium ZRO: a natural defence for the skin

Personal cleansing products may rinse away germs and dirt, but, at the same time, they also strip away the skin's natural protective lipids, causing it to dry out. Surfactants, the main ingredients of cleansing products, are known to induce skin irritation by damaging the barrier proprieties of the stratum corneum, the outer layer of the skin, and denaturing proteins in the epidermis and dermis.[6]

The skin is the outermost layer of the body and is the first line of defence against a multitude of external pathogens and environmental attacks.[7] Unfortunately, a compromised skin barrier is also a source of pathogen access. For this reason, restoring the skin barrier function is just as important as disinfecting the skin.

As part of the Activoil™ series, Innovacos has developed Activoil Echnidium ZRO (INCI: Echium Plantagineum Seed Oil, Octyldodecanol, Octyldodecyl Oleate, Octyldodecyl Stearoyl Stearate, Cnidium Monnieri Fruit Extract, Rosmarinus Officinalis (Rosemary) Leaf Extract; hereafter referred to as 'the lipophilic active').

This represents a natural defence for the skin from the aggressiveness of both alcohol-based sanitisers and strong cleansing products (Figure 2). It combines the best of Echium seed oil with *Cnidium monnieri* fruit supercritical CO_2 extract, in a lipophilic matrix, stabilised from oxidation with Rosemary extract (Figure 3).

Echium oil is obtained from *Echium plantagineum* seeds and is a powerful source of omega-3 and -6 essential fatty acids, such as α- and γ-linolenic acid (ALA, 18:3 n-3, GLA, 18:3 n-6) and stearidonic acid (SDA, 18:4 n-3). Essential fatty acids have a key role in epidermal homeostasis – their deficiency results in a characteristic scaly skin disorder and excessive trans-epidermal water loss (TEWL).[8]

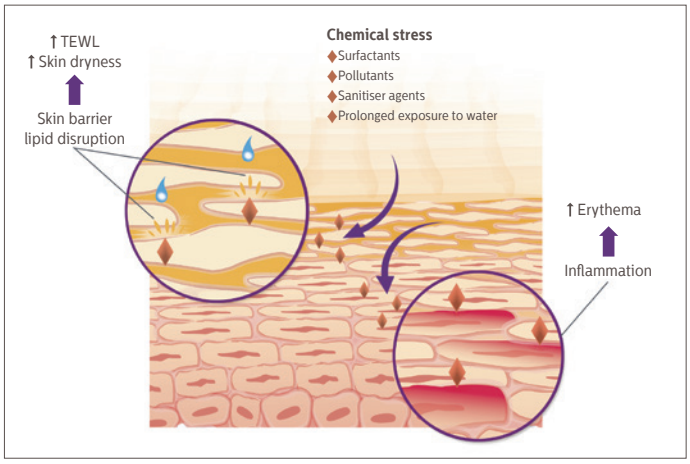

Figure 1: Cutaneous effects of chemical stress

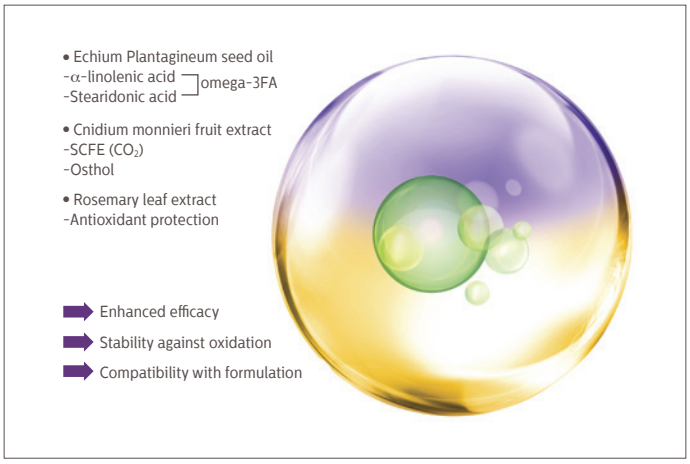

Figure 3: Composition of Activoil Echnidium ZRO

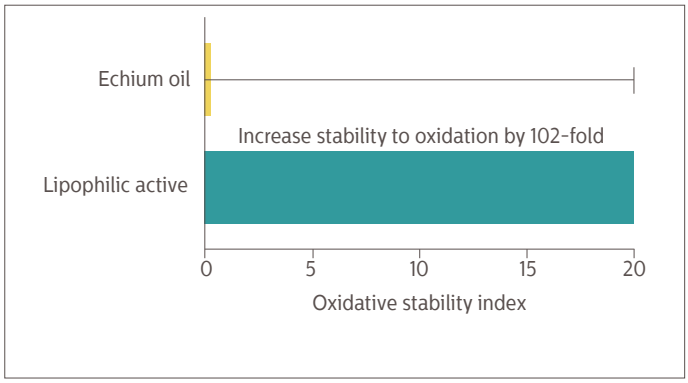

Figure 4: Oxidative stability of the lipophilic active

		Placebo %	Active %	Funtion
A	Aqua	Up to 100	Up to 100	
	Alcohol	70.00	70.00	Sanitizer
B	Acrylates/C10-30 Alkyl Acrylate Crosspolymer	0.35	0.35	Thickener
C	Activoil Echnidium ZRO	–	1.00	Lipophilic active
D	Parfum	0.20	0.20	Perfume
E	Tetrahydroxypropyl Ethylenediamine	a.n.	a.n.	Neutralising agent

Figure 5: Alcohol-based sanitiser (placebo) & same formulation containing 1% lipophilic active

The high SDS content of Echium oil makes it unique among plant seed oils. As a precursor of eicosapentaenoic acid (EPA), SDA is a powerful anti-inflammatory substance and acts also to protect the skin from environmental damage.[9]

Extracts of *Cnidium monnieri* fruit are a source of various compounds such as osthol, a lipophilic biologically active molecule used in traditional Chinese medicine.[10] Osthol is endowed with various biological properties, such as the promotion of cell viability, reduction of intracellular oxidative stress and alleviation of inflammation.[11-12] Interestingly, osthol is an activator of the PPAR- α/γ pathways involved in cell differentiation and lipid metabolism stimulation.[13]

Rosemary extract, obtained by supercritical CO_2 extraction from the leaves of *Rosmarinus officinalis*, stabilises the lipophilic matrix, preventing oxidation.[14] The lipophilic active represents an ideal stabilised complex of lipophilic ingredients to protect and repair the skin barrier function and soothe irritation upon exposure to chemical stress, such as ethanol or surfactants, the main ingredients of hand sanitisers and personal cleansing products, respectively.

Rancimat test

The lipophilic active is a unique source of omega-3 and -6 essential fatty acids. Unsaturated fatty acids are known to have several biological actions but are also prone to oxidation, because of their double bonds. Preserving oxidative stability is mandatory to maintaining biological functionality.

In this method, a sample of oil is heated under atmospheric pressure and air is allowed to bubble through the oil at a selected temperature. Under these conditions, a lipoperoxidative reaction occurs and the short-chain volatile acids produced are recovered and measured by an increase in the conductivity of distilled water.

The time required to produce a sudden increase in conductivity, due to the formation of volatile acids, determines an induction time – also known as the Rancimat time – which can be defined as a measure of the oxidative stability of oil. A Metrohm 743 Rancimat was used in compliance with the ISO 6886-2006 method. Rancimat time was measured for the lipophilic active in comparison to standard *Echium plantagineum* seed oil. Oxidative stability was measured at 110°C.

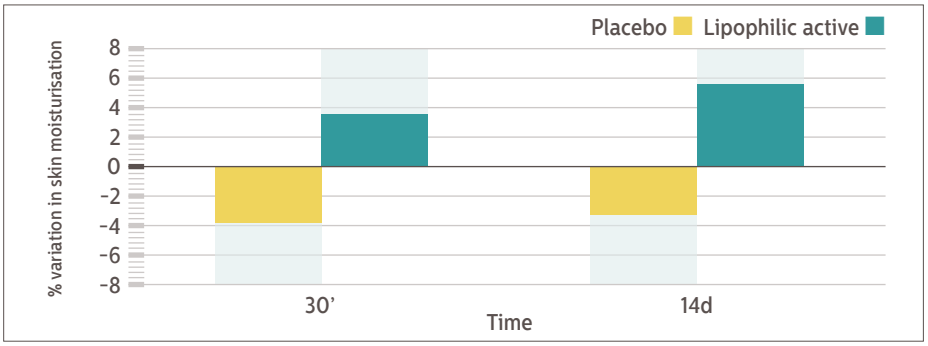

Figure 6: Variation in skin moisturisation upon alcohol-based sanitiser application

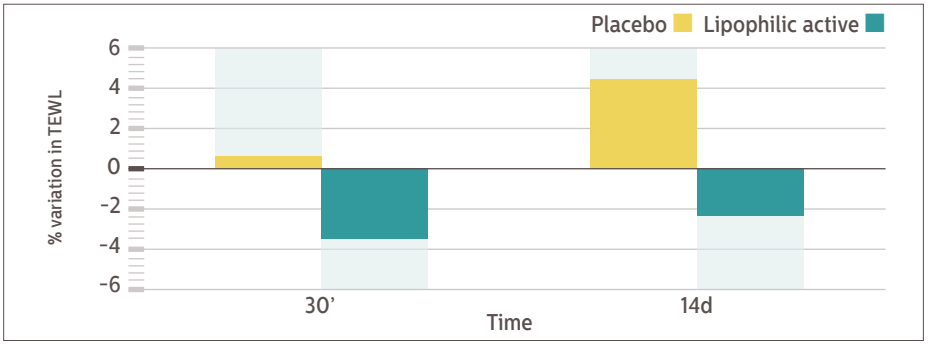

Figure 7: Variation in TEWL upon alcohol-based sanitiser application

Despite the presence of a high level of unsaturated fatty acids, a key feature of the lipophilic active is its high oxidative stability. Indeed, the incorporation of Rosemary extract together with *Echium plantagineum* seed oil and *Cnidium monnieri* fruit extract provided increased stability to oxidation 102-fold in comparison to normal Echium oil (Figure 4).

Skin barrier function & hydration evaluation on sanitiser application

The study was aimed at assessing the preventive action of the lipophilic active, included in a sanitiser gel containing 70% ethanol, in limiting the aggressiveness of the alcohol on the skin. For this placebo-controlled clinical protocol, 20 healthy subjects were enrolled. An alcohol-based sanitiser (placebo) and the same formulation containing 1% of the lipophilic active (Figure 5) were topically applied three times a day, in accordance with a randomised scheme.

Clinical evaluations of skin moisturisation (Corneometer®, Courage+Khazaka) and TEWL (Tewameter 300®, Courage+Khazaka) were measured at Day 0 (baseline), 30 minutes after the first application and after 14 days of treatment. The instrumental data were submitted to the one-way Student's t-test for paired data.

The lipophilic active, added to a formulation containing 70% ethanol, not only succeeded in preventing the dry skin caused by alcohol, but it also improved skin moisturisation and protected skin barrier function. It revealed a significant improvement in skin moisturisation (+3.7% and +5.8% after 30 minutes and 14 days respectively, Figure 6) and a reduction in TEWL from the first application (-3.5% after 30 minutes), while keeping the skin barrier conditions unchanged after 14 days of use (-2.2% after 14 days of treatment, Figure 7).

It is worth noting that the placebo caused skin dehydration (a reduction in moisturisation of -3.8% and -3.2% after 30 minutes and 14 days, respectively, Figure 6) and skin barrier disruption (an increase in TEWL of +0.7% since the first application and +4.6% after 14 days of treatment, Figure 7).

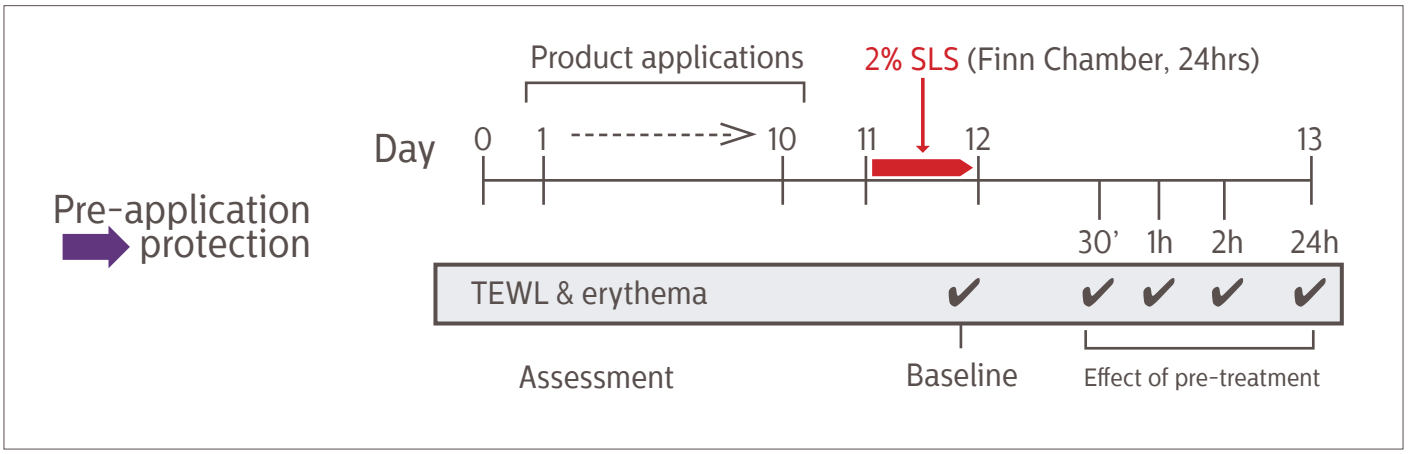

Figure 8: Protective action - clinical protocol.

Skin barrier function & irritation evaluation upon surfactants exposure

The study aimed to evaluate the protective and repairing effect of a cream cosmetic product, containing the lipophilic active, against the skin alterations caused by sodium lauryl sulfate (SLS), a typical irritant agent contained in personal cleansing products. The skin was exposed to a solution at 2% SLS, using Finn chambers, for a period of 24 hours.

Two different protocols were followed: a preventive one, in which a cream formulation containing 2% lipophilic active was applied prior to the stress (protective effect), and a therapeutic one, in which the product was applied after exposure to SLS (repair action). Skin barrier function, evaluated by the measurement of the TEWL (Tewameter 300®) and skin irritation, evaluated by the measurement of skin erythema (Mexameter® MX 18, Courage+Khazaka, Electronic), were assessed on a group of ten healthy volunteers.

Protective effect: Clinical protocol

In the protective protocol (Figure 8), the effect of cosmetic topical product application prior to the skin's exposure to SLS was evaluated. Selected skin areas were kept untreated as controls. Other skin areas were treated with a placebo or the same formulation containing 2% lipophilic active (2 mg/cm^2) for a period of ten days, preceding exposure to the chemical agent.

On Day 11, a solution of SLS was applied to all the skin areas, using Finn chambers to chemically aggress the skin. The Finn chambers were kept in contact with the skin for 20±4 hours and removed on Day 12.

TEWL and skin erythema were assessed and the obtained values were set as the baseline for skin barrier disruption (high TEWL values) and skin irritation (high level of erythema), respectively. The extent of barrier repair and the disappearance of erythema were measured at intervals of 30 minutes, one hour, two hours and 24 hours after removal of the SLS patch. Parametric data were submitted to a two-way paired Student's t-test.

The results showed that the skin's exposure to SLS compromised the barrier function. Untreated skin progressively repaired itself. The placebo treatment did not affect the barrier repair compared to the untreated skin. Protecting the skin with the lipophilic active formulation allowed a faster barrier recovery, as shown by the reduction of the SLS-induced TEWL (-15.5%, -29.3%, -33.0% and -37.1% after 30 minutes, one hour, two hours and 24 hours from patch removal, respectively, Figure 9).

The measurement of skin erythema also showed that the application of the lipophilic active formulation protects the skin from chemically induced erythema.

Exposure to SLS caused clinical signs of skin irritation. An elevated level of erythema persisted on untreated skin for up to 24 hours after SLS exposure. The preventive application of the lipophilic active formulation showed a faster disappearance of skin irritation (a reduction in erythema of -7.7%, -9.5%, -12.2% and -15.6% after 30 minutes, one hour, two hours and 24 hours from patch removal, respectively) compared to the application of the placebo (Figure 10).

Repairing action – clinical protocol

In the repairing protocol (Figure 11), the restoring and soothing action of the lipophilic active versus the damage caused by surfactants was evaluated. Some skin areas were treated with cosmetic topical products only upon removal of the Finn chambers containing SLS (Day 12). Selected skin areas were kept untreated as controls.

In this case, the skin was post-treated with one single application (2 mg/cm^2) of a placebo or the same formulation containing 2% lipophilic active. The repairing effect of the treatments was assessed for skin barrier function and skin irritation at intervals of 30 minutes, one hour, two hours and 24 hours from the topical application of the formulations. The skin barrier function and irritation were assessed as described above. Parametric data were submitted using a two-way paired Student's t-test.

Results obtained in the repairing experiment revealed that applying the lipophilic active formulation after the

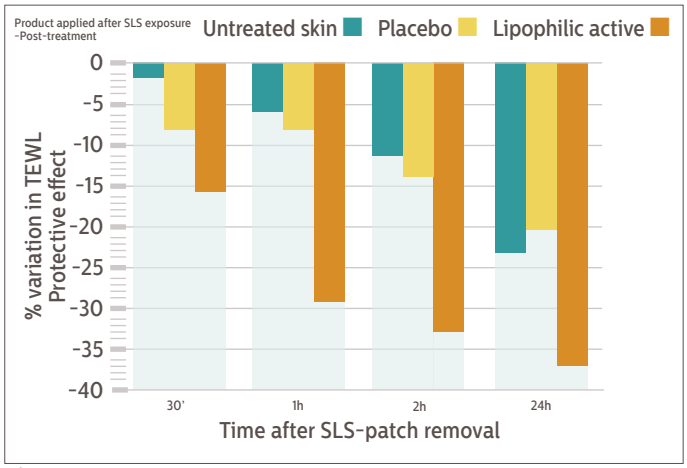

Figure 9: Variation in TEWL in the protective protocol

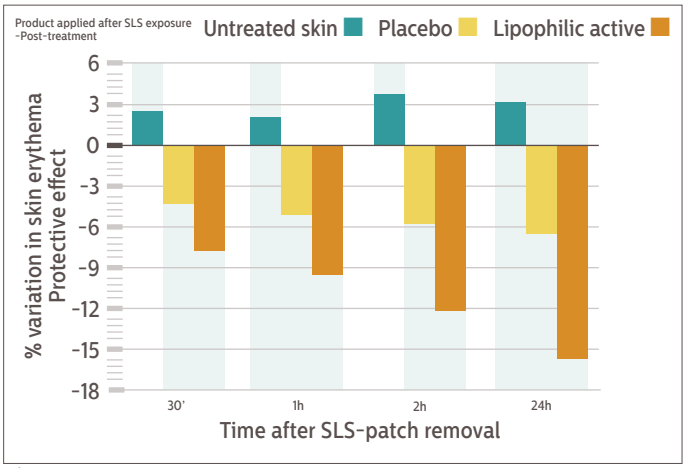

Figure 10: Variation in skin erythema in the protective protocol

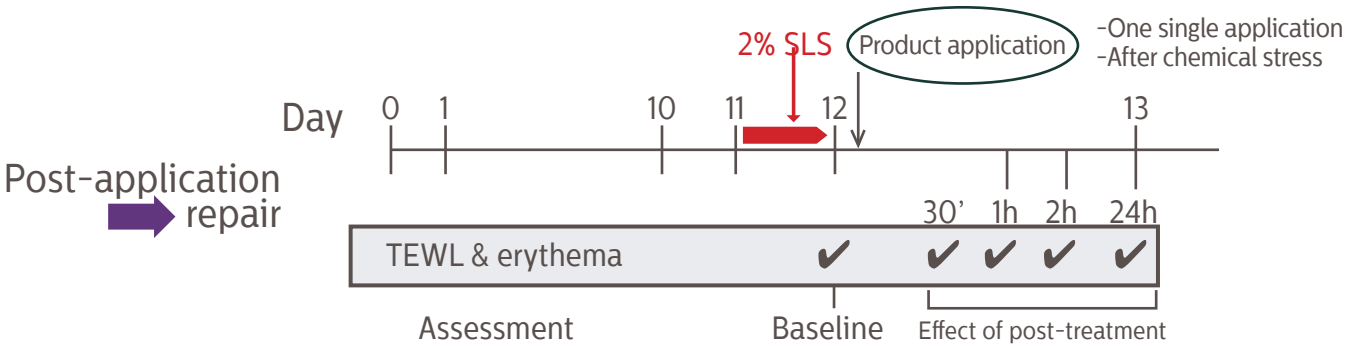

Figure 11: Repair action - clinical protocol

chemical stress with SLS significantly reduced the extent of skin barrier damage. This supports the therapeutic action of the lipophilic active in promoting skin barrier function, even after a single application.

The lipophilic active significantly accelerates the barrier function recovery when applied after stress (a reduction in TEWL of -15.1%, -28.6%, -32.2% and -37.4% after 30 minutes, one hour, two hours and 24 hours from patch removal, respectively). The placebo did not show any difference compared to untreated skin (Figure 12).

The measurement of skin erythema also demonstrated the therapeutic effectiveness of the lipophilic active formulation. Untreated skin still showed clinical signs of skin irritation after 24 hours from SLS exposure.

Post-SLS exposure application of the lipophilic active formulation hastened the recovery of skin homeostasis (a reduction in erythema -6.6%, -6.4%, - 8.4% and -10.1% after 30 minutes, one hour, two hours and 24 hours from patch removal, respectively) more than the application of the placebo (Figure 13). The lipophilic active helps to repair the skin from chemically induced erythema, showing a soothing effect after a single application.

Conclusion

Public awareness of the importance of hand sanitisation during the current pandemic event is likely to have a far-reaching effect on hygiene habits across the globe. New norms of hygiene, such as the routine use of hand sanitisers and frequent handwashing, will continue beyond the COVID-19 era. It is essential to find solutions for cleaning and sanitising the skin while maintaining its main function as a barrier to external agents, including pathogens.

Chemical agents contained in hand sanitisers and personal cleansing products can affect the top layers of the stratum corneum, disrupting the lipid matrix of the skin barrier and causing excessive TEWL. They can also penetrate deeper into the skin, interacting with living cells and initiating an inflammatory reaction, ultimately leading to skin erythema.

The lipophilic active has shown a way to counteract these negative effects, thanks to the synergistic effect of its main components, *Echium plantagineum* seed oil, *Cnidium monnieri* fruit extract and *Rosmarinus officinalis* leaf extract. This combination plays a key role in the restoration of the skin lipid matrix and in anti-inflammatory processes, preventing and repairing skin barrier damages, improving skin hydration levels and decreasing irritation and skin sensitivity (Figure 14).

In a 'new normal' skincare scenario, where non-irritating products are in high demand, the lipophilic active can offer several functional solutions. It can be added directly to hygiene products, such as alcohol-based hand sanitisers and personal cleansing products, or it can be used in moisturising treatments with both protective and therapeutic functions against chemical stress.

The use of an alcohol-based hand sanitiser containing 1% lipophilic active was successful in preventing the damage caused by ethanol. An improvement in the skin barrier function was observed after its first application, accompanied by an immediate increase in skin moisturisation, which persisted through time.

The clinical action of the lipophilic active also extends to its ability to reduce the aggressiveness of surfactant agents. It acts as a protective 'mask', if applied before stress, preventing damage to the barrier function and skin sensitivity, and also as a 'destroyer' of chemical agents, if applied after the stress, accelerating skin barrier repair and reducing skin irritation. **PC**

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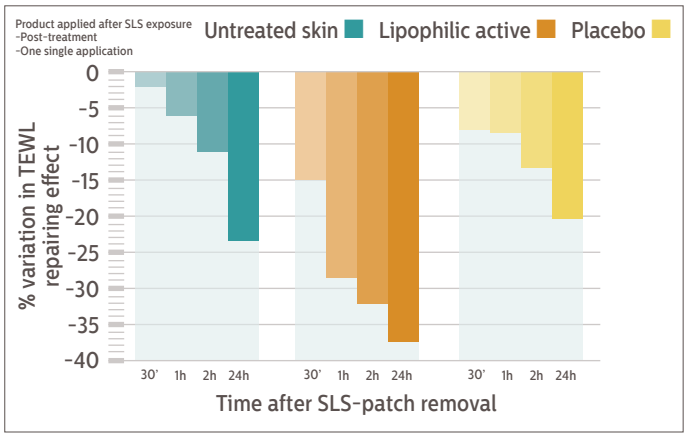

Figure 12: Variation in TEWL in the repairing protocol.

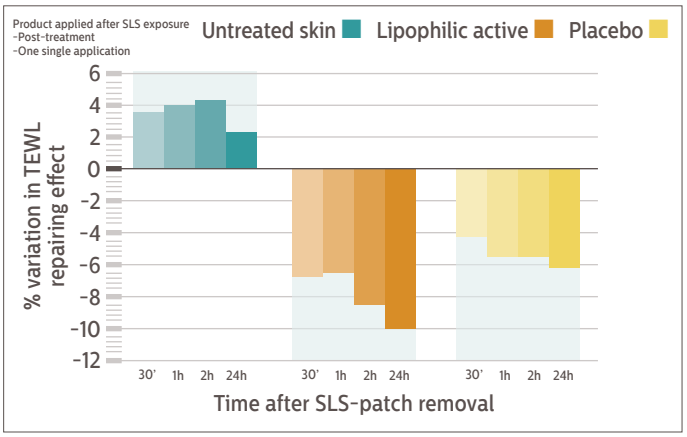

Figure 13: Variation in skin erythema in the repairing protocol

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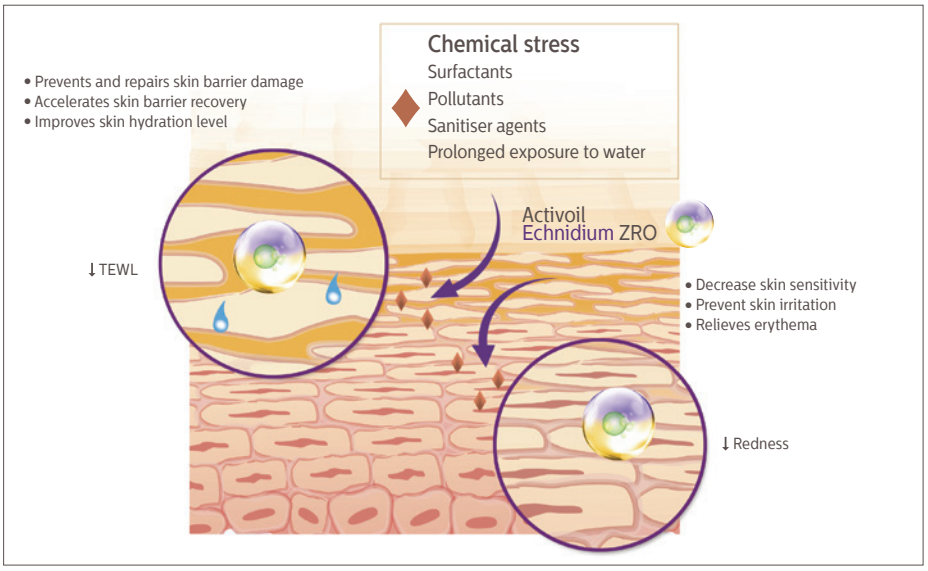

Figure 14: Clinical benefits of the lipophilic active upon exposure to chemical stress