Protection against stress by natural triterpene esters

Highly concentrated shea butter triterpene esters are shown to be promising new bioactives for protecting the skin against environmental stress conditions and reactive oxygen species. Shea butter triterpene esters, comprising cinnamic and acetic acid esters of lupeol and butyrospermol, demonstrate potent protease inhibiting and anti-inflammatory properties. These are properties of high value for caring cosmetics offering protection against premature skin ageing and treatment of mature skin.

Environmental stress and the human skin

The human skin, being the outermost shield protecting the body against various environmental conditions, responds to stress conditions by a series of inflammatory reactions aiming at tissue repair and regained homeostasis.
The inflammation can be induced by injurious agents, chemical irritants, toxins, pathogens, burns, UV exposure etc.
The condition is often caused by the formation of reactive oxygen species (ROS) followed by the biosynthesis of inflammatory mediators such as the leucotrienes and prostaglandins. Aged skin is characterised not only by a decreased thickness and increased sensitivity but also by a lower capability to manage the inflammatory reactions and show typically a low but constant inflammatory status.
The chronic inflammation further increases the breakdown of the skin collagen and elastin protein structures and results in a thinner, less elastic and wrinkled skin.

Table 1:
Approximate triterpene composition of investigated Lipex Shea Tris.

Triterpenes	Content (%)
Alpha-Amyrin	25
Butyrospermol	18
Lupeol	10
Parkeol	3
Beta-Amyrin	2

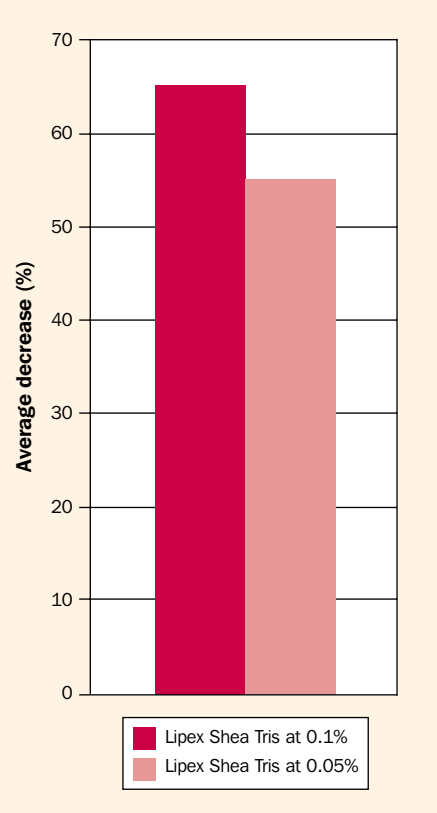

Fig. 1: *Inhibition of MMP-3 gene expression by shea butter triterpenes.*

Consequently, protection against premature ageing as well as treatment of sensitive and aged skin is of high priority for personal care formulations. Natural triterpenes are today well-researched for their bioactivity and show potential not only within the pharmaceutical industry but are also identified as potential new and valuable skin care ingredients.

Shea butter has high concentration of triterpene esters

Triterpenes originate from squalene and the biosynthesis of these pentacyclic hydrocarbon structures, consisting of six isoprene units, is considered to be one of the most complex reactions occurring in nature. Even though they are among the most common plant secondary metabolites, their function in plants has not yet been fully understood. They are usually concentrated in the plant's outermost layers such as the cuticle, fruit peel, and bark, mainly as alcohols. They are also found at a low concentration in plant oils.

Shea butter, known by the peoples of West Africa for centuries for its skin healing and protecting properties, is unique with its high content of esterified triterpenes, showing a typical content of 2% to 5% in the fruit oil. The dominating triterpenes are lupeol, α- and β-amyrin and butyrospermol belonging to the lupane, oleanane and ursane triterpene families respectively.

The bioactivity spectrum of triterpene esters

Triterpenes and their esters have been widely investigated in the search for new alternative pharmaceutical actives and are well-described in the scientific literature.

Lupeol, being the most well characterised and investigated triterpene, has been demonstrated to offer a broad spectrum of bioactivities, such as protease inhibiting, anti-inflammatory, anti-tumour and some minor antimicrobial properties. It is also proposed to be used as a chemopreventive in order to avoid several diseases and is regarded as safe for use thanks to its low cytotoxicity.
The various bioactivities of lupeol have been well-summarised in a review article by Gallo *et al*.[1]

Collagen protection by protease inhibition

Triterpene esters, such as acetates, palmitates and linoleates, are demonstrated to show potent protease inhibiting activity. Lupeol and other triterpene esters have shown potential for reducing arthritic joint destruction in rheumatoid arthritis and a direct pharmacological action mechanism of lupeol is proposed. A selective inhibiting activity of both metallo- and serine-proteases by lupeol esters is also described by Hodges *et al*.[2]

The protease inhibiting activity of triterpenes reported by several research teams indicates also potential for reducing

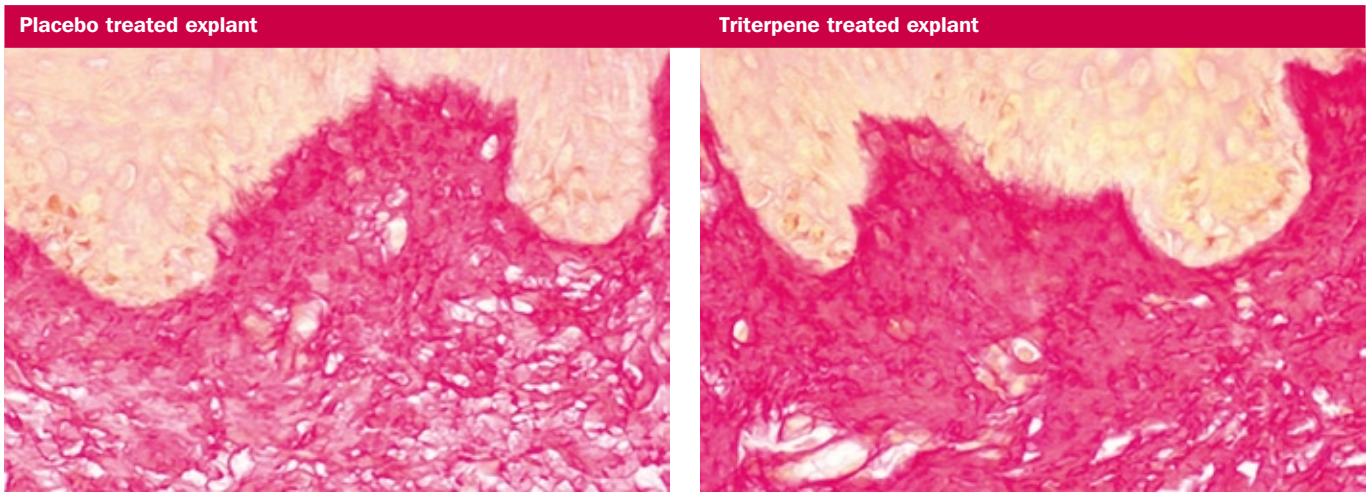

Fig. 2: *Increase of collagen network by shea butter triterpenes. Living human skin explants treated during six days with placebo cream versus cream with shea triterpenes at 3000 ppm. The collagen structures (coloured in red) are clearly over-expressed in all the papillary dermis after treatment with the triterpenes.*

skin stress induced by environmental factors. This effect shows a possible use of triterpene esters in skin care formulations for protecting against stress-related breakdown of skin collagen and elastin structures and suggests a new, well-characterised, natural tool for protection against premature ageing and treatment of mature skin.

Shea butter triterpene esters preserve collagen status in skin

The collagen-protecting activity of Lipex Shea Tris, an extract enriched in shea butter triterpene acetates and cinnamates (Table 1), has recently been investigated by both *in vitro* and *ex vivo* methods. The results illustrate protease inhibiting and possible collagen stimulating properties of importance for maintaining a healthy and elastic skin.

Since a required condition for collagen protection would be a deactivation of matrix metalloproteinases (MMPs), a gene expression analysis of normal human epidermal keratinocytes was initiated. The results demonstrated a significant reduced expression of the *MMP-3* gene after exposure to the shea butter triterpenes. MMP-3 is known for being up-regulated in aged skin, after UV-exposure, and being responsible for break-down of the skin collagen type III. An inhibiting effect of >50% was shown already at 300 ppm shea triterpenes (Fig. 1). The RT-qPCR method used in this test is a highly sensitive method illustrating the gene expression profile at the actual time and under defined conditions, in this evaluation without any added external stress factor.

Further *ex vivo* study of the same shea butter triterpene extract confirms the collagen protecting activity of shea butter triterpenes after exposure to collagenases. Human skin explants, kept in survival for eight days, were exposed to 3000 ppm triterpenes and twice exposed to collagenase (50 U/ml). A 30% reduction of the total collagenase activity versus placebo was confirmed in the treated explants.

A significant increase of dermal collagen network in the papillary dermis of human skin explants was also seen after six days of treatment with the triterpene ester formulation. The explants were grown under normal growth conditions and without any added external stress conditions. Figure 2 compares the explants treated with the active cream versus placebo cream and illustrates a well-functioning dermis after the treatment with the active cream. This observation further confirms the reported collagen protecting activity of shea butter triterpenes and might also indicate a collagen stimulating effect.

Anti-inflammatory action leads to skin stress reduction

Numerous studies of lupeol and amyrins indicate potent anti-inflammatory properties of the triterpenes both as alcohols and as esters. Recently investigated lupeol acetates are reported to offer anti-inflammatory action in several models of inflammation.[3]

Lupeol is also shown by several authors to inhibit the release of pro-inflammatory mediators such as PGE2, TNF-α and IL-1β but with less or no effect on the lipoxygenase (LOX) metabolites. The mechanism of activity of lupeol is proposed by Huguet *et al*[4] to be explained by the inhibition of protein kinase C (PKC) with potential reduced formation of intracellular reactive oxygen species (ROS) and an early inhibition of the inflammatory cascade.

Figure 3 illustrates schematically the inflammatory pathways in a keratinocyte cell with proposed inhibitory mechanisms of shea butter triterpenes.

Reducing skin stress using shea butter triterpene esters

The anti-inflammatory activity of shea butter triterpenes has recently been thoroughly investigated by Akihisa *et al*.[5] In this study, lupeol cinnamate showed the highest activity against TPA- and carrageenan-induced *in vivo* inflammation. It was shown to be even more effective than the reference compound indomethacin.

An *in vitro* evaluation of a commercially available shea butter triterpene extract, Lipex Shea-U, on human epidermal keratinocytes exposed to croton oil, illustrates a significant anti-inflammatory effect of shea butter triterpenes. A reduced release of intracellular IL-1α cytokine at 25% versus control cells was confirmed within the investigated range of 100 ppm to 500 ppm triterpenes.

Proven activity

The reported protease inhibiting and anti-inflammatory activity of shea butter triterpenes correlates well with published studies on activities of similar triterpene and triterpene esters. These findings further highlight the new enriched shea extract as a potential bioactive ingredient of high value for protecting the skin against environmental stress conditions and of potential for treatment of aged skin.

Conclusion

The high concentration of effective and safe triterpene esters makes Lipex Shea Tris a suitable bioactive ingredient for caring and anti-ageing applications. It is a natural choice for formulations aiming at reducing premature breakdown of skin structures

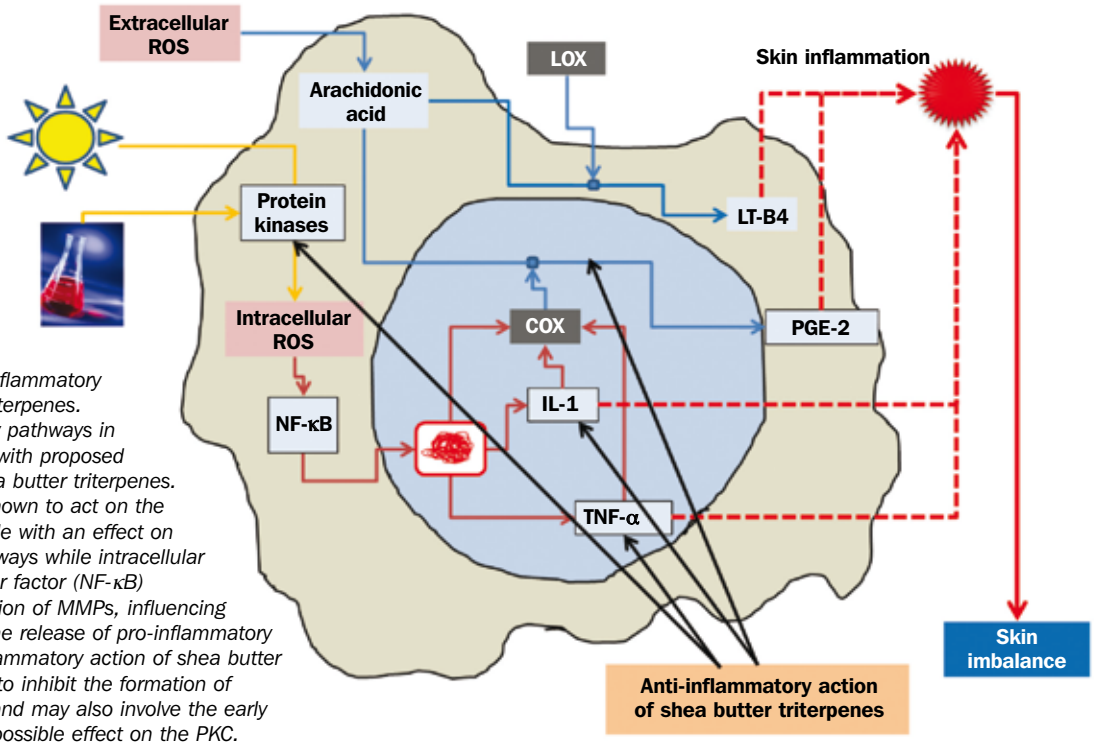

Fig. 3: *Proposed anti-inflammatory action of shea butter triterpenes. Schematic inflammatory pathways in a keratinocyte skin cell with proposed inhibiting activity of shea butter triterpenes. Extracellular ROS are shown to act on the arachidonic acid cascade with an effect on both LOX and COX pathways while intracellular ROS acts via the nuclear factor (NF-κB) regulating the transcription of MMPs, influencing the COX pathway and the release of pro-inflammatory mediators. The anti-inflammatory action of shea butter triterpenes is proposed to inhibit the formation of PGE2, IL-1 and TNF-α and may also involve the early formation of ROS by a possible effect on the PKC.*

and support a healthy and well-balanced skin. The new enriched shea extract is well soluble in vegetable oil and ester based emollients at the recommended use level of 0.1% to 0.5%. At this low level of incorporation, it is easy to formulate light and elegant formulations for facial care, eye care and body lotions. It is also well compatible with ingredients used in colour cosmetics and can therefore be added as a bioactive ingredient in lipsticks and lip-care formulations. PC

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