Analysing a new, holistic anti-ageing skin bioactive

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Delaying skin ageing is a key global trend in the personal care industry. It is driven largely by growing ageing populations and increasing consumer concern regarding preserving general skin health as part of healthy ageing. Aged skin is characterised by dull, saggy skin with compromised skin barrier function. Therefore, products that effectively address ageing skin should target these different concerns.

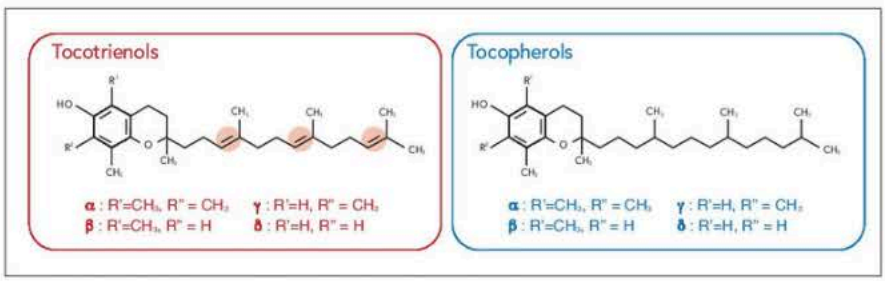

Figure 1: Chemical structure of Tocotrienols and tocopherols.

Oxidative stress and inflammation are root causes of skin ageing

Skin ageing is a gradual and inevitable biological process influenced by a combination of extrinsic (external environmental factors such as pollution, sun exposure, and poor diet) and intrinsic (genetics, cellular metabolism, and hormones) factors.[1] Intrinsic skin ageing is an inevitable natural physiological process governed by factors that are beyond our control. However, in extrinsic skin ageing, its factors are controllable to varying degrees. Successful control of these factors relies on addressing its root causes.

Constant exposure to extrinsic factors such as pollutants and UV lead to the formation of Reactive Oxygen Species (ROS) in skin. ROS are highly reactive and unstable chemical species. If the amounts of ROS exceed the capacity of exogenous and endogenous antioxidant systems to scavenge them, this leads to oxidative stress.

Oxidative stress is the cornerstone of skin ageing. Oxidative stress inhibits the activity of receptor protein tyrosine phosphatases, thereby elevating the level of phosphorylated receptor tyrosine kinases and triggering the activation of mitogen-activated protein kinase (MAPK), nuclear factor-κβ (NF-κβ) and transcription factor activator protein-1 (AP-1).[2] Activated NF-κB and AP-1 repress collagen production and increase Matrix metalloproteinases (MMP) gene transcription, resulting in the decrease of collagen content.[3] Gradual loss of collagen content may contribute to wrinkles by weakening the bond between the dermis and epidermis.

Oxidative stress also promotes low-grade chronic skin inflammation, in a phenomenon also termed as inflammaging. A proposed model of inflammaging states that oxidative stress results in damaged cells that have oxidised lipids.[1,5,6] Macrophages are activated to remove these damaged cells and oxidised lipids and, in the process, they degrade the extracellular matrix (ECM).[1] Chronic oxidative stress leads to overburdened macrophages which release proinflammatory cytokines and ROS, leading to long-term damage of ECM.[7,8] ECM components include elastin, collagens, and proteoglycans, which are required to provide elasticity, tensile strength and hydration to skin respectively. Thus, damage to ECM results in the phenotypic appearance of ageing.

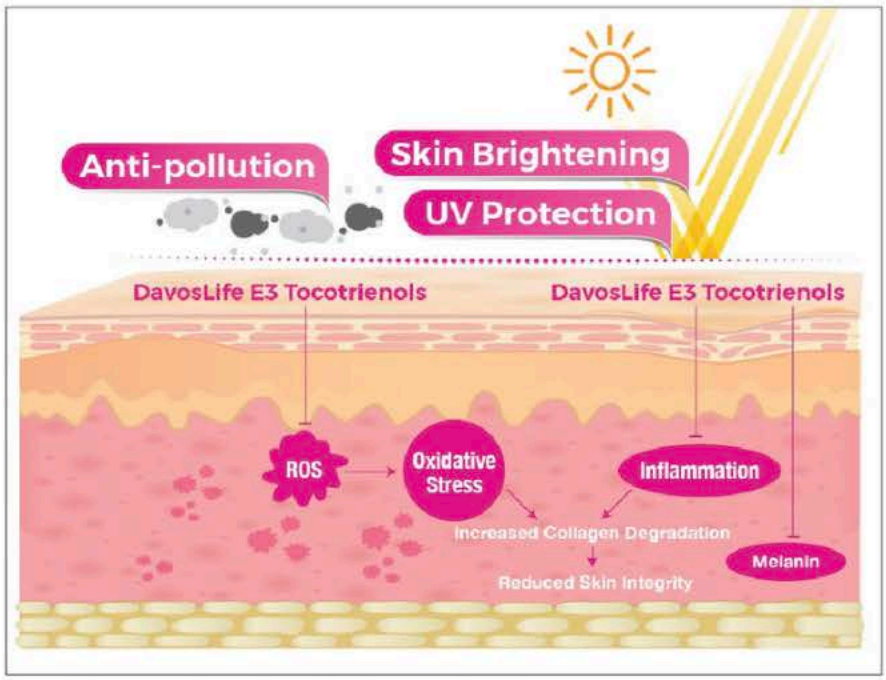

Figure 2: Protective effects of DavosLife E3 Tocotrienols in skin.

Tocotrienols alleviate oxidative stress and inflammation

Tocotrienols are members of the vitamin E family and are potent antioxidants. Tocotrienols and tocopherols both have a chromanol head and lipid-soluble isoprenoid side chain. Unlike tocopherols, Tocotrienols have unsaturated isoprenoid side chains, a key property that gives tocotrienols up to 60x more antioxidative potency (αTP)[9,10] and

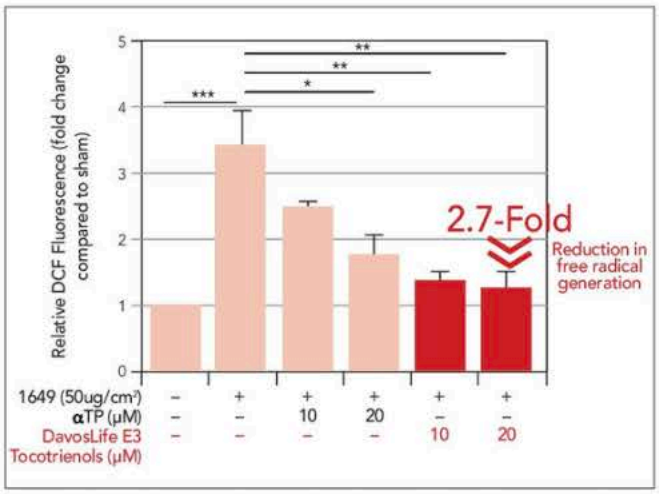

Figure 3: DCFH-DA assay showing that when NHDFs cells were treated with either 20 μM DavosLife E3 Tocotrienols or αTP for 24 hours, a significant reduction in PM2.5-induced intracellular oxidative stress was observed due to their antioxidative biological activities. However, when a lower concentration of treatment was used (10μM) the data showed that DavosLife E3 Tocotrienols were able to suppress the free radicals that generated by PM2.5 but not αTP.

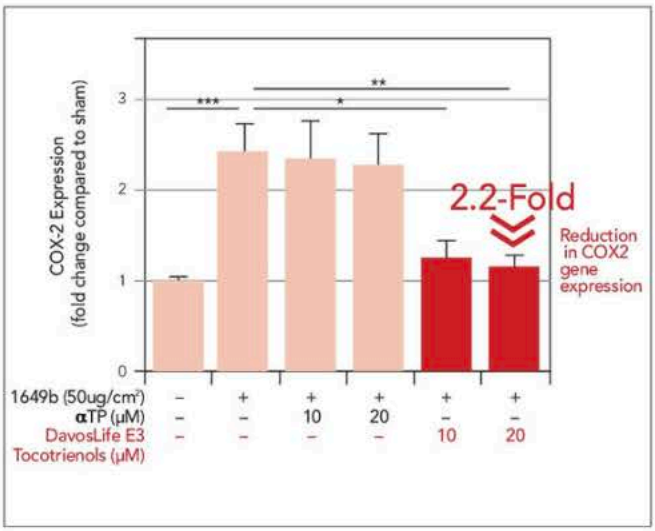

Figure 4: NHDFs cells that were treated with either 10 μM or 20 μM DavosLife E3 Tocotrienols for 24 hours. Only DavosLife E3 Tocotrienols treated cells showed a significant reduction in PM2.5- induced inflammation as evidenced by Cyclooxygenase-2 (COX2) activity. Dose-dependent treatment with αTP did not show amelioration of inflammation.

making them a more potent form of vitamin E (Fig 1).

The unsaturated side chain makes Tocotrienols a smaller molecule compared to tocopherol. This smaller size ensures better flexibility with higher cellular activity. In addition to their antioxidant activity, Tocotrienols have anti-inflammatory properties. They regulate key players involved in pro-inflammatory pathways such as signalling pathways that involve NF-kβ cyclooxygenase 2 (COX2) and tumour necrosis factor (TNF).[11]

These key properties of Tocotrienols target the root causes of skin ageing, making them a potent bioactive ingredient with multifunctional targets for anti-ageing (Fig 2). DavosLife E3 Tocotrienols comprise the natural full spectrum of Tocotrienols isomers and αTP in a synergistic ratio for the promotion of overall skin health. Research studies using DavosLife E3 Tocotrienols have shown its efficacy in protecting skin from extrinsic factors (pollution and UV radiation), as well as promoting skin brightening.

Defending and repairing skin from environmental pollution

Environmental pollution (particulate matter and nitrogen dioxide) has been associated with premature skin ageing. Particulate Matter (PM) is involved in the initiation or exacerbation of skin inflammatory diseases.[12,13] The significant amount of polycyclic aromatic hydrocarbons (PAH) in PM produce excessive ROS, inflammatory cytokines and MMP which trigger oxidative stress and inflammation.[14,15] These signalling cascades will increase the production of Prostaglandin E2 and COX2, which subsequently decrease filaggrin expression thereby resulting in skin barrier function impairment.[16] Thus, naturally derived antioxidants and anti-inflammatory ingredients that potentially alleviate adverse skin reactions arising from PM are applicable in skin anti-ageing strategies.

In our study, we evaluated the protective effects of DavosLife E3 Tocotrienols (now referred to as 'Tocotrienols') on PM2.5-induced skin damage using Human Dermal Fibroblast cells (HDF). HDF cells were treated with 50μg/ml PM2.5 in the presence or absence of Tocotrienols. Oxidative stress level was determined by 2'-7'dichlorofluorescin diacetate (DCFH-DA) while COX2 was selected as the inflammation biomarker. The skin barrier function was assessed by investigating filaggrin protein expression.

Figure 3 shows that Tocotrienols inhibited PM2.5-induced ROS generation in HDF. When HDFs cells were treated with 20 μM Tocotrienols or αTP for 24 hours, a significant reduction in PM2.5-induced intracellular oxidative stress was observed due to their antioxidative biological activities. The data was evidenced by DCFH-DA assay. However, when a lower concentration of treatment was used (10μM), our data showed that only Tocotrienols were able to suppress the free radicals generated by PM2.5 but not for αTP.

As shown in Figure 4, Tocotrienols had a significant anti-inflammatory effect, as seen in the significant attenuation of PM2.5-induced up-regulation of COX2. However, treatment with αTP did not show amelioration of inflammation.

Our western blotting analysis (Fig 5) revealed 20 μM Tocotrienols could potentially restore skin barrier function following exposure to PM2.5, based on the increased levels of filaggrin protein expression. In contrast, this was not observed for αTP when NHF was treated at the same concentration.

Delaying UV irradiation-induced skin ageing

Excessive UV irradiation exposure is the primary cause of premature skin ageing (photoageing). Photoageing accounts for a wide range of biological effects, which includes physiological processes and alterations to the

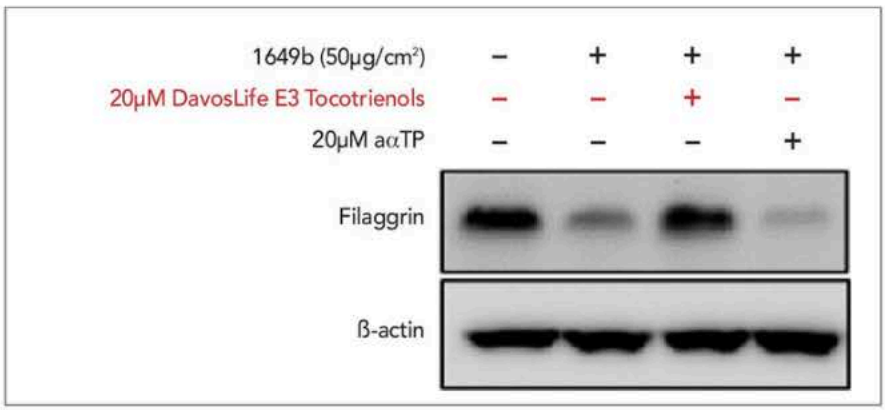

Figure 5: Western blotting analysis showing that DavosLife E3 Tocotrienols was able to increase the protein expression of Filaggrin upon the PM2.5 stimulation but not for αTP which was treated at the same concentration.

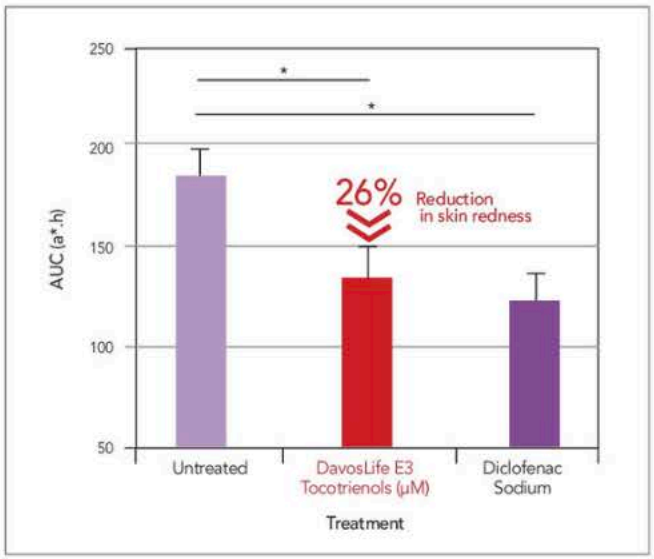

Figure 6: Comparison of the AUC for the change in skin redness over time is shown. AUC was calculated using the trapezium rule. The smaller the AUC value, the faster the skin returned to normalcy. Results were analysed using repeated measures ANOVA. Data are shown as means ± SEM.

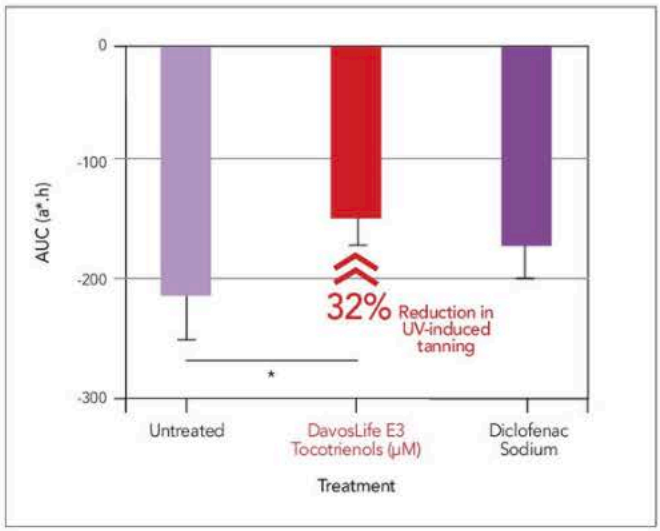

Figure 7: Comparison of the AUC for the change in skin tan over time. Smaller AUC values correspond to darker skin tones. Significant differences between DavosLife E3 Tocotrienols-treated and untreated skin over the whole test period of 120 h is indicated as $P < .05$. Results were analysed using repeated measures ANOVA. Data are shown as means ± SEM.

skin. UV irradiation, even at insufficient levels to cause a sunburn, induces small amounts of damage to the skin. These damages initiate a sequence of events that can lead to inflammation and free radical generation (i.e. ROS and reactive nitrogen species [RNS]), which stimulate skin's barrier function restoration. Photodamaged skin also shows signs of mild but chronic inflammation (inflammaging), which phenotypically manifest as coarse and deep wrinkles, mottled pigmentation, sallowness and telangiectasia.[22] The antioxidative and anti-inflammatory properties of tocotrienols can play a vital role to ameliorate UV-induced inflammaging. We carried out a human study to investigate the protective effects of 1% Tocotrienols in formulation against UV-induced erythema and pigmentation. This formulation and a commercial sunblock containing Diclofenac Sodium (Feniderma sun repair gel, Novartis, Switzerland) were coded and compared.

In this study, 20 subjects were instructed not to wash the test area for 8 hours before the commencement of the trial. The test products were randomly applied to the test areas. To avoid distortions and inhomogeneity of product application at the edges, colour measurements were restricted to a 3x 3 cm square in the centre of each test area. Baseline measurements were obtained before the test areas were irradiated with 1.5 Minimal Erythema Dose (MED). Products were applied 10 minutes after the completion of irradiation. Upon completion of application, the subject remained in the prone position for 20 minutes to allow absorption of the test materials. Skin colour was measured and the product re-applied 6 hours after irradiation. Subsequent skin colour measurements were made every morning at 24, 48, 72, 96, and 120 hours after UV irradiation. Subjects were requested not to wash the test area for 2 hours after each application.

An integrated analytical approach – Area Under the Curve (AUC) was adopted to compare the changes in redness and skin colour over the whole test period of 120 hours for different test products. In this analysis, the smaller the AUC, the faster the skin returned to normalcy. As shown in Figure 6, 1% Tocotrienols was as effective as Diclofenac Sodium in reducing skin redness after UV irradiation. As for skin colour (Fig 7), results showed that 1%Tocotrienols in formulation appeared to decrease UV-induced tanning significantly over the whole test period of 120 hours. However, no significant differences were observed for 0.1% diclofenac sodium-treated skin compared with untreated skin.

Our work shows that the biological activities of Tocotrienols in photo-protection are different than other sunscreens. Sunscreens physically block or absorb UV rays, but they do not ameliorate UV-induced inflammation. Tocotrienols not only scavenge free radicals formed by UV irradiation, they have anti-inflammatory properties that are comparable to over-the-counter drugs and are very well tolerated by the skin. Thus, complementing conventional sunscreens or after-sun products with Tocotrienols may serve as a good strategy for protection from photoageing.

Promoting skin brightening

Pigmentary change is the most important cutaneous manifestation in premature photoageing. Ingredients that can even out skin tone are commercially used to help brighten the appearance of skin that has been exposed to excessive ultraviolet light. They

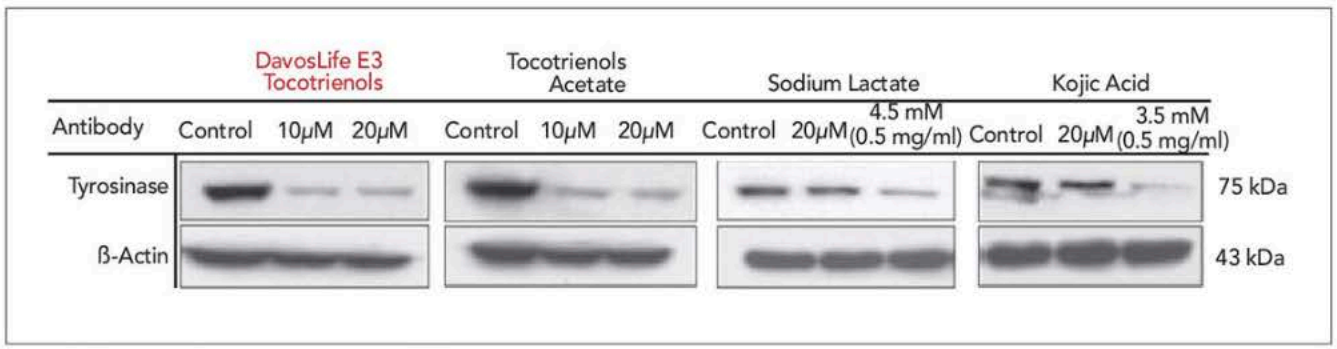

Figure 8: Treatment of B16 cells with DavosLife E3 Tocotrienols, Tocotrienols acetate, sodium lactate and kojic acid for 24 h inhibited tyrosinase protein expression.

are also utilised for hyperpigmentation and melasma treatment. Many of these brightening ingredients act at various levels of melanin production in the skin and are known as competitive inhibitors of tyrosinase (TYR) - a key enzyme in melanogenesis.[19] Another common mechanism is to inhibit the maturation of TYR or the transport of pigment granules (melanosomes) from melanocytes to surrounding keratinocytes.[20]

To study the dose response of tyrosinase suppression by Tocotrienols, B16 melanoma cells were treated with an increasing dosage of treatments. Our data shows that treatment of B16 melanoma cells with Tocotrienols and tocotrienols acetate resulted in consistent suppression of tyrosinase protein expression in a dose dependent manner (Fig 8). This finding suggested tocotrienols acetate had been absorbed and hydrolysed by B16 melanoma cells to its native form of Tocotrienols,[21] thus exerting an inhibitory effect on the tyrosinase gene. Alternatively, the anti-melanogenesis effect may be associated with tocotrienols' unsaturated isoprenoid side chain. In contrast, 20 µM treatments with kojic acid and sodium lactate did not result in observable down-regulation of tyrosinase protein levels. Using a higher dose of kojic acid and sodium lactate (3.5 mM and 4.5mM respectively), however, led to significant inhibition of tyrosinase protein expression.

Total melanin content per cell was also quantified in both control medium and treated medium. After melanin content was normalised for differences in cell growth by dividing by total cell numbers, it was found that B16 melanoma cells treated with the Tocotrienols on day 9 had 55% lower melanin content compared to controls (Fig 9). It is noteworthy that the melanin content of B16 melanoma cells following Tocotrienols treatment was marginally lower than that of treatment samples using 4.5 mM sodium lactate and 3.5 mM kojic acid respectively. This finding shows that naturally derived Tocotrienols is more effective in brightening compared to commercially-known brightening agents.

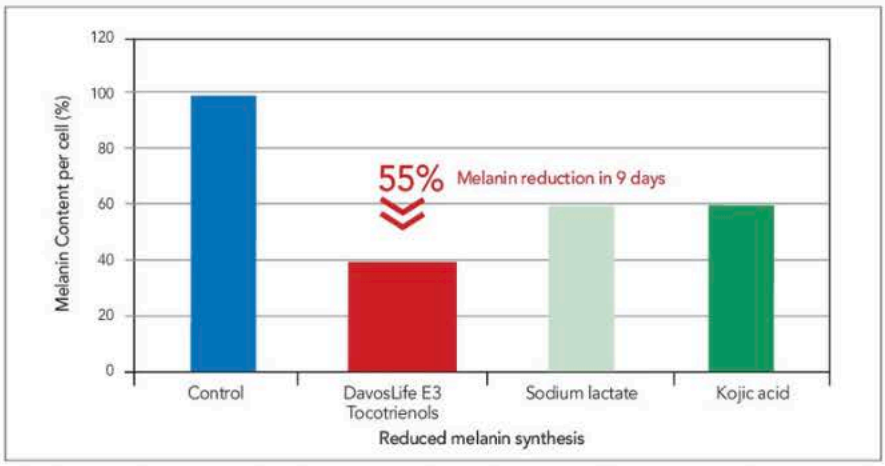

Figure 9: Melanin content on Day 9 after treatment with DavosLife E3 Tocotrienols, sodium lactate and kojic acid.

Conclusion

DavosLife E3 Tocotrienols from Davos Life Science targets the underlying root causes of skin ageing. *In vitro*, this bioactive ingredient worked to reduce PM2.5-induced intracellular oxidative stress and inflammation. There was a 2.7-fold reduction in free radical generation and a 2.2-fold reduction in COX2 gene expression. It also promotes skin barrier function restoration by increasing filaggrin protein expression. It is clinically-proven to protect skin from UV-induced erythema by up to 26% and pigmentation by up to 32%. It naturally balances skin pigmentation to promote brighter skin by inhibiting tyrosinase protein expression, resulting in up to 55% reduction in melanin content. DavosLife E3 Tocotrienols is a comprehensive bioactive ingredient targeting multiple extrinsic factors for anti-ageing products to promote younger-looking skin. PC

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