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UNISOOTH EG-28

NATURAL COMPLEX WITH AN IMMEDIATE SOOTHING EFFECT TO REDUCE SIGNS OF SKIN IRRITATION AND DARK CIRCLES UNDER THE EYES

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1. Introduction

Skin is exposed to the external environment that brings daily aggressions such as UV light, chemicals, pollution, temperature, etc daily. These aggressions can create skin irritation especially in sensitive skin individuals leading to itching and discomfort. Moreover, in the long term the irritation would lead to skin damage and premature skin aging as a result of elastosis and matrix degradation.

It is therefore extremely important to stop the irritation rapidly in order to reduce skin discomfort and to avoid further skin damage.

Skin irritation is sustained by a cross-talk mechanism between the keratinocyte in the epidermis layer and the infiltrating immune cell (ex.T-lymphocyte). This cross-talk would create an amplification loop producing an over-reaction, alimenting the inflammatory process with consequent skin erythema and irritation. The skin is unbalanced and immediate re-balance is needed.

Our approach is to instantly interrupt the cross-talk between the keratinocyte and the immune cell by targeting specific mechanisms involved in the amplification loop with the goal to re-balance an unbalanced over-reacting skin.

The mechanism that is targeted with this product is the NF-kB signaling pathway. This mechanism is dramatically amplified in dermatological condition and plays a role in sustaining skin irritation (1).

In the NF-kB pathway a cytokine receptor expressed on the cell membrane of the keratinocyte will recognize cytokines ligands produced by skin infiltrating immune cells and trigger the release in the cytoplasm of the NF-kB unit, normally sequestered by a family of inhibitory proteins known as inhibitors of NF-kB (IkBs). The signaling pathway from the receptor will lead to the liberation and nuclear accumulation of NF-kB, which will then in turn activate transcription of pro-inflammatory molecules such as cytokines and chemokines (2,3). This cross talk mechanism would sustain the inflammation leading to skin irritation (Fig 1).

2. Concept of Unisooth EG-28

In order to target the NF-kB pathway and to reduce skin irritation immediately we have selected ingredients that have been described in the literature for their potential in soothing skin irritation and modulating the immune system and its mechanisms with in particular the NF-kB mechanism. We have formulated an innovative bioactive complex (Unisooth EG-28). This complex contains a balanced blend of Gallyl Glucoside, Epigallocatechin Gallatyl Glucoside and Propyl Gallate, all of natural origin.
Gallic Acid and its glucosylated form Gallyl Glucoside are extracted from Oak leafs and purified. Epigallocatechin Gallate was extracted from Green Tea and purified.
The glucosylation to Epigallocatechin Gallatyl Glucoside was obtained by the addition of a glucose group to the aglicone form by a bacterial enzyme.

The resulting glucosides proved to be more stable to degradation than the aglicone forms and extremely more water soluble (data not shown).

The choice of Gallic Acid as the aglicone for this development is derived by the extensive published evidences on its anti-inflammatory action. Gallic Acid has proven to be an anti-irritant in the rat (4), and an inhibitor of histamine release and cytokine production in mast cells (5) and in monocytes (6). Furthermore its activity in inhibiting NF-kB pathway has been suggested (7,8).

In the case of Epigallocatechin Gallate, well known are the immunomodulatory properties of green tea (9). Epigallocatechin Gallate is responsible for several actions against inflammation. It has shown to reduce immune cells infiltration in human skin (10), to decrease IL-8 release in human keratinocytes (11) and to inhibit NF-κB activation in human keratinocytes (12) and in the mouse (13,14).

Finally the addition of Propyl Gallate is based on historical evidences of this ingredient as a lipoxygenase inhibitor (15,16).

The hypothesis was then to test the combination of Gallyl Glucoside, Epigallocatechin Gallatyl Glucoside and Propyl Gallate (Unisooth EG-28) to inhibit the release of the pro-inflammatory mediators IL-8 and chemokine CXCL1 that would sustain a keratinocyte and immune cell cross-talk leading to increase inflammation and skin irritation.
(see Fig. 1)
During the experimental setting the keratinocytes were induced to a pro-inflammatory status by incubating them with a cytokine mixture known to induce the NF-kB pathway (17).
These experiments were then validated *in vivo* on human volunteers by testing Unisooth EG-28 as an immediate soothing agent after an induced irritation and by testing its effect in reducing dark circles under the eyes.

In Figure 1 the biological mechanism of Unisooth EG-28 action is depicted.

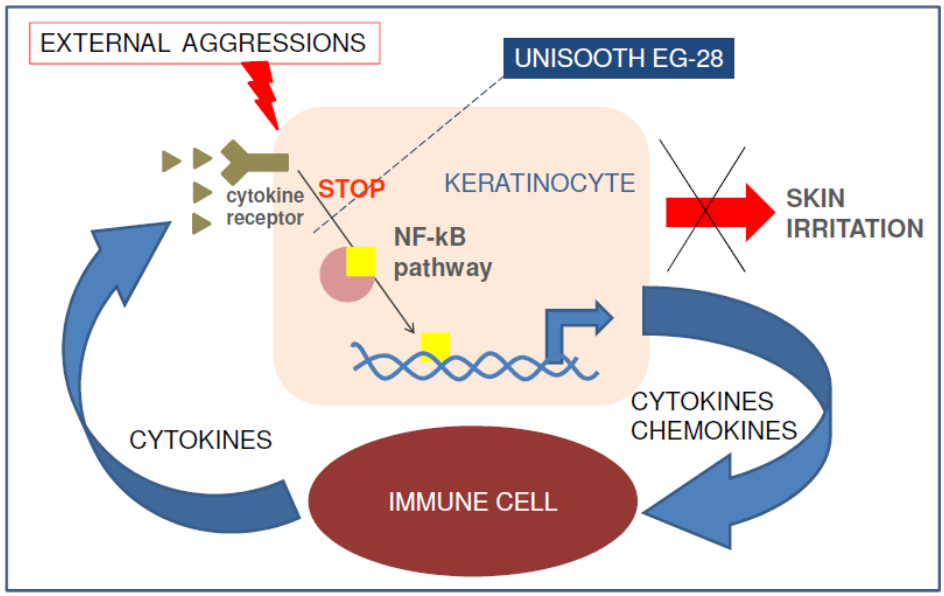

Figure 1: Unisooth EG-28 would inhibit NF-kB activated signaling pathway in keratinocytes. The cross-talking communication between the keratinocyte and the immune cell is interrupted with the result of inhibiting skin irritation to provide an immediate soothing effect.

3. Evidence of Effectiveness

3.1 In vitro studies – NF-kB activity, IL-8 and CXCL1 release

3.1.1 Culture of normal human epidermal keratinocytes (NHEK) and treatment

The keratinocytes were cultured in 96-well plates in culture medium for 24 hours. The medium was then removed and replaced by assay medium containing or not (control) Unisooth EG-28 at different concentrations (0.016%, 0.08%, 0.4%, based on cytotoxicity results) or the references (NF-kB inhibitor III at 5 μM and Dexamethasone at 100 μM). The cells were then pre-incubated for 24 hours. After the pre-incubation, the medium was removed and replaced by assay medium containing or not (control) Unisooth EG-28 at the same concentrations as above or the references, in association with TNF-α + IL-1α (both at 5 ng/ml). The cells were then further incubated for 48 hours. A non-stimulated control condition was also performed in parallel. All experimental conditions were performed at n=3. The association of TNF-α + IL-1α is later referred as cytokines mix (CTK mix).

The absence of cytotoxic effects was proven in preliminary studies.

3.1.2 Quantification of IL-8 and CXCL1 release in NHEK

At the end of NHEK treatment, the amount of the interleukin IL-8 and the chemokine CXCL1 in culture supernatants were measured using ELISA kits (for IL-8 PeproTech, for CXCL1 R&D systems). In the case of IL-8 measure, the lower limit of detection was 15.6 pg/ml and the highest limit 2000 pg/ml, however when diluted samples were tested, the limits of the detection range were adjusted by the dilution factor (1/2), i.e. from 31.2 to 4000 pg/ml. In the case of CXCL1 measure, the lower limit of detection was 31.3 pg/ml and the highest limit 2000 pg/ml, however when diluted samples were tested, the limits of the detection range were adjusted by the dilution factor (1/5), i.e. from 156.2 to 10000 pg/ml.

3.1.3 NF-kB Activity in human Ht29 cells

To test for NF-kB activation, transformed human cells (HT29) transfected with a NF-Kb reporter plasmid linked to alkaline phosphatase (pNiFty2SEAP, Invivogen) were incubated for 24 hours with the NF-kB cytokine activator TNF-α (20 ng/ml), in presence or in absence of Complex EG at different concentrations. A colorimetric reaction followed to determine the reporter gene activity and consequent NF-kB activity.

3.1.4 Results and Figures

Treatment of NHEK with TNF-α + IL-1α cytokine mix, dramatically increased the release of pro-inflammatory cytokine IL-8 and chemokine CXCL1. Increase vs. untreated control NHEK was +98.2% for IL-8 and + 66.6% for CXCL1 (Figure 2 and Figure 3). Treatment with references NF-kB inhibitor (5 μM) and Dexamethasone (100 μM) significantly inhibited cytokine mix (CTK mix) induced IL-8 and CXCL1 increase ($p<0.01$, Student's T test, Fig 2 and Fig 3).

Treatment with Unisooth EG-28 at increasing concentrations had strong significant inhibition activity when IL-8 release was measured (99% inhibition at 0.4% vs. CTK mix induced NHEK, $p<0.001$, Student's T test, Fig 2) and was even able to reduce the basal activity of CXCL1 release (135% inhibition at 0.4% vs. CTK mix induced NHEK, $p<0.001$, Student's T test, Fig 3). These data strongly support Unisooth EG-28 as an inhibitor of the NF-kB inflammation mediated pathway. Furthermore Unisooth EG-28 showed to be more effective then the NF-kB inhibitor III and Dexamethasone benchmarks.

Treatment of TNF-α induced HT29 with increased concentration of Unisooth EG-28 decreased NF-kB activation detected by the reporter plasmid activity. The decrease was dose dependent and statistically significant ($z<1.96$, Wilcoxon test) with a max inhibition at Unisooth EG-28 concentration of 1.1% (-85%, see Figure 4)

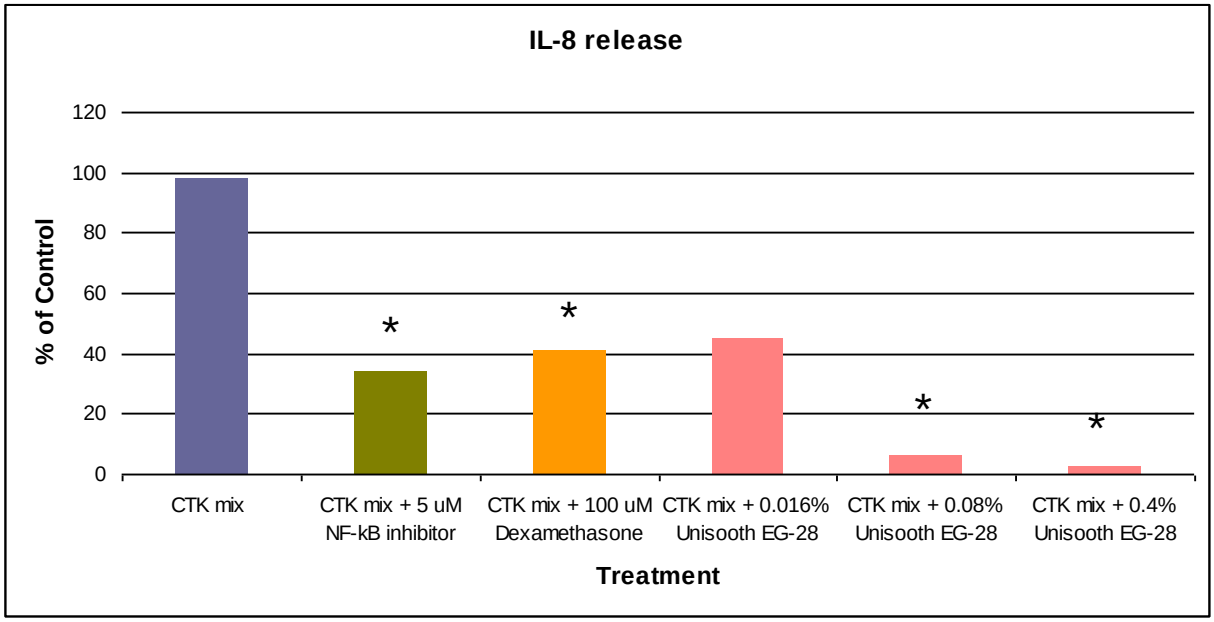

Figure 2: Cytokines mixture induced human keratinocytes treatment with increasing doses of Unisooth EG-28 strongly inhibits IL-8 release. (CTK mix = cytokine mixture). *Statistically significant vs. CTK mix control, Student's T test

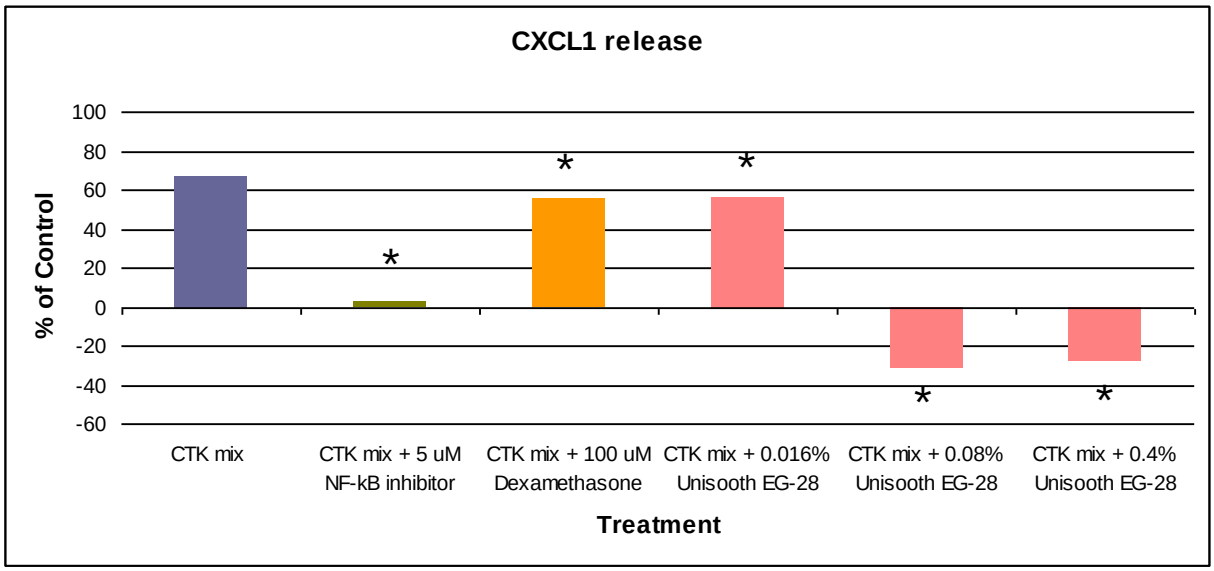

Figure 3: Cytokines mixture induced human keratinocytes treatment with increasing doses of Unisooth EG-28 strongly inhibits chemokine CXCL1 release. (CTK mix = cytokine mixture). *****Statistically significant vs. CTK mix control, Student's T test

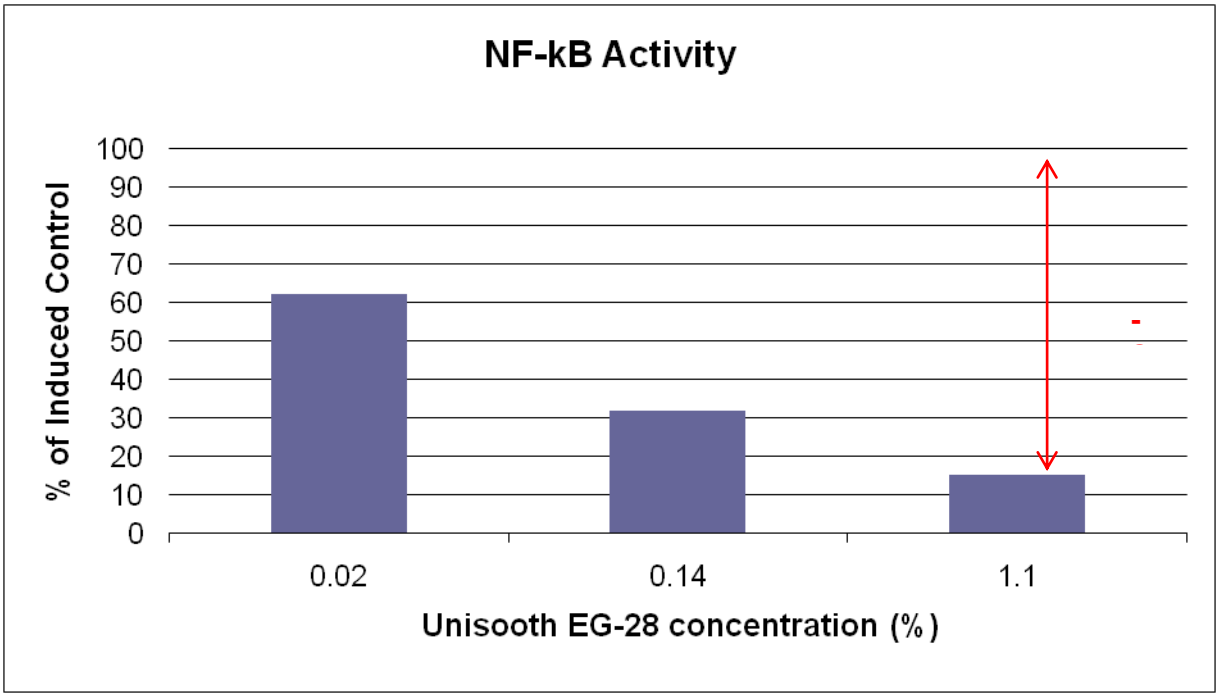

Figure 4: Treatment with increasing doses of Complex EG inhibits significantly NF-kB activity in human cells induced with TNF-α (20 ng/ml). *Data are significant vs. TNF-α-induced Control, Wilcoxon test

3.2 Studies in Human Volunteers – Double Blind Clinical Evaluation of Immediate Skin Soothing Repairing Effect and Dark Circles Reduction

3.2.1 Experimental group

For the immediate skin soothing test, twenty-five volunteers (males + females) were selected, while for the dark circles study additional twenty-five volunteers (only female) were chosen. All volunteers were informed about the importance and meaning of the study. Written informed consent was obtained from all the subjects prior to entry into the trial. The following criteria were used for subject inclusion in study: older than 18 years of age, clinically healthy, Caucasian, not on medications, negative for atopic skin; and for exclusion from study: skin diseases, allergies or sensitive skin, scars or lesions in the test areas. Moreover for the dark circles study, dark circles under the eyes was an additional selective criteria

3.2.2 Induction and Measurement of Irritation and Trans-Epidermal Water Loss

Skin irritation was induced through the application of an epicutaneous patch. Eight finn chambers of the patch were filled with 2% SLS aqueous solution and applied on the back of the volunteers. The eight patches were removed 24 hours after their application. As a measure of skin irritation and damage, Erythema index and Trans-Epidermal Water Loss (TEWL) were evaluated 15 min after the removal of patches (T0).

After skin irritation, gel samples (blind coded) were applied following their usual use. Samples were a gel containing/not containing Unisooth EG-28 at different concentrations.

On the irritated areas, 2 were treated with the gel containing Unisooth EG-28 at 3.0%, 2 with the gel containing Unisooth EG-28 at 1.0%, 2 with the placebo gel and 2 areas were left untreated. Samples were left for 15 min, 30 min, 60 min and 120 min time intervals. Erythema index and TEWL were evaluated after each time point. Comparisons were made between all samples and to the untreated areas. Erythema index was measured using a Mexameter MX 18 (Courage+Khazaka, electronic GmbH), while TEWL was measured using a Tewameter 300 (Courage+Khazaka, electronic GmbH). Data were analyzed and expressed as % variation vs. T0. Statistical significance was also calculated.

3.2.3 Measurement of Dark Circles – Quantitative and Qualitative Evaluation

Product efficacy was evaluated quantitatively 15 and 30 days after daily product use by means of instrumental analysis technique as described here below. The analysis was then completed with both clinical evaluation performed by the dermatologist and the self assessment of the subjects participating in the study. Two products (a water-based gel containing Unisooth EG-28 at 3% and a water-based placebo gel) were applied twice a day (morning and evening) on perfectly cleaned area around the eyes (following half-face method, according to the randomization scheme described in the information form given to the subject) and gently massaged on the skin. The measurement of the color of the eye circles was done by means of a spectrophotometer /colorimeter CM-700d (Konica Minolta). The instrument is able to evaluate the color in the CIELab chromatic space,

according to a standard method defined by the International Lighting Commission (CIE). CIELab is a standardized color space in which the color is defined - under standard illumination conditions (illuminant) and observer angle - by the values called a* and b* that defines hue and color saturation and by the value called L* that defines the color brightness.

The dermatologist evaluated the visibility of the eye dark circles in accordance with the clinical scores reported in the table (1) below.

Clinical Classification of dark circles T0	Score	Clinical Classification of dark circles at T15 and T30	Score
		No variation	1
The color of the eyelid skin is normal	1	Slight improvement	2
The under-eyes circles are slightly dark	2	Moderate improvement	3
The under-eyes circles are clearly dark	3	Remarkable improvement	4

3.2.4 Results and Figure

In human volunteers, SLS-induced skin irritation by a 24 hours patch occlusion, was treated with a gel containing Unisooth EG-28 at 3.0 or at 1.0% and produced an immediate soothing effect, measured by reduction of the erythema index, after only 15 min (Fig. 5). The data were significant when compared to a placebo gel, the highest dose of Unisooth EG-28 being significant all time, while the lowest dose of Unisooth EG-28 reaching significance after 120 min from application ($p<0.05$, $p<0.001$, Student's T test, Fig. 5). At 120 min from application, the gel containing Unisooth EG-28 at 3.0% reached a reduction of the erythema index of -15% (Fig.4).

In the same volunteers, SLS-induced skin trans-epidermal water loss (TEWL) was reduced by the gel containing Unisooth EG-28 at 3.0% or at 1.0% (Fig. 5).

Data were significant after 30, 60 and 120 min when compared to a placebo gel but only for the gel containing Unisooth EG-28 at 3.0% ($p<0.05$, $p<0.01$, Student's T test, Fig. 6). At 120 min from application, the gel containing Unisooth EG-28 at 3.0% reached a reduction of the TEWL of -23% (Fig.6).

The reduction of the erythema and of the TEWL shows not only an immediate soothing effect by Unisooth EG-28 but also a healing effect on the skin barrier.

Further on, treatment with a water-based gel containing 3% Unisooth EG-28 reduced dark circles around the eyes in human volunteers. The data were significant when compared to a Placebo treatment. The reduction was evaluated by the red and blue components, where they both decreased, with a resultant balanced color (less dark) as confirmed by dermatologist observation.

In particular, the a* value (red component) was significantly reduced vs. placebo at 15 days of treatment (-9.9%, $p<0.01$, Student's T test) and at 30 days of treatment (-11%, $p<0.01$, Student's T test) as shown if Fig.7.

The b* value was increased vs. placebo at 15 days (+13.2%, p<0.05, Student's T test) and 30 days of treatment (+13.6%, p<0.05, Student's T test) as shown in Fig.8. Note: an increase in b* value means a decrease in blue color component.

The quantitative observation was confirmed by a dermatological evaluation based on score, where a better score associated with volunteers treated with the gel containing Unisooth EG-28 (Table 1).

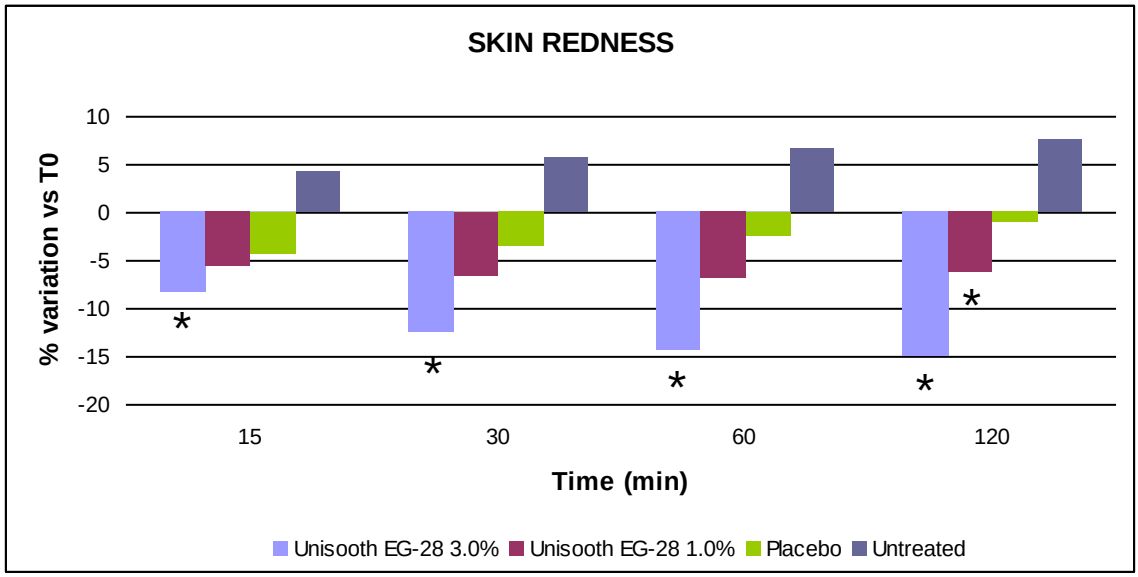

Figure 5: Treatment with a gel containing Unisooth EG-28 at 3.0% and 1.0% reduces SLS-induced erythema on skin of human volunteers. The reduction, expressed as % of variation vs. time of application (T0), was statistically significant when compared to a placebo gel.

*Significant data vs. placebo, Student's T test

REDNESS	TIME vs. T0 (%)			
Treatment	15	30	60	120
G-28 3.0%)	-8.3	-12.6	-14.4	-15.1
EG-28 1.0%)	-5.6	-6.7	-6.9	-6.2
Placebo	-4.3	-3.6	-2.6	-1.1
Untreated	4.2	5.6	6.6	7.5

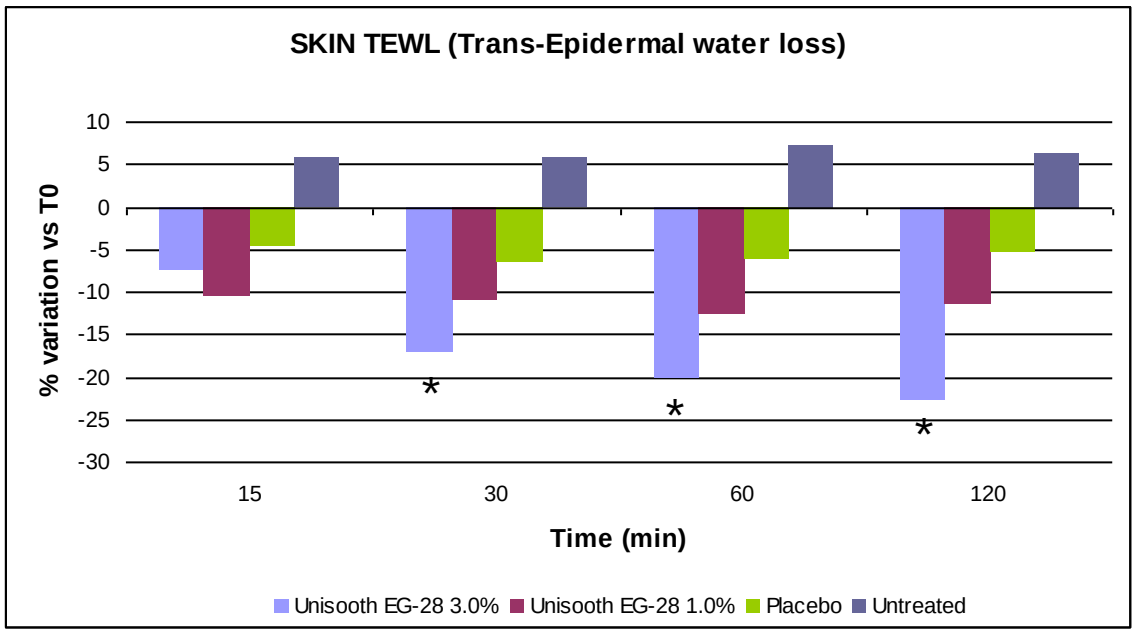

Figure 6: Treatment with a gel containing Unisooth EG-28 at 3.0% and 1.0% reduces SLS-induced TEWL on skin of human volunteers. The reduction, expressed as % of variation vs. time of application (T0), was statistically significant when compared to a placebo gel.

*Significant data vs. placebo, Student's T test.

TEWL	TIME vs. T0 (%)			
Treatment	15	30	60	120
EG-28 (3.0%)	-7.4	-17.1	-20.2	-22.7
EG-28 (1.0%)	-10.4	-10.9	-12.6	-11.5
Placebo	-4.5	-6.5	-6.3	-5.2
Untreated	5.8	5.8	7.1	6.3

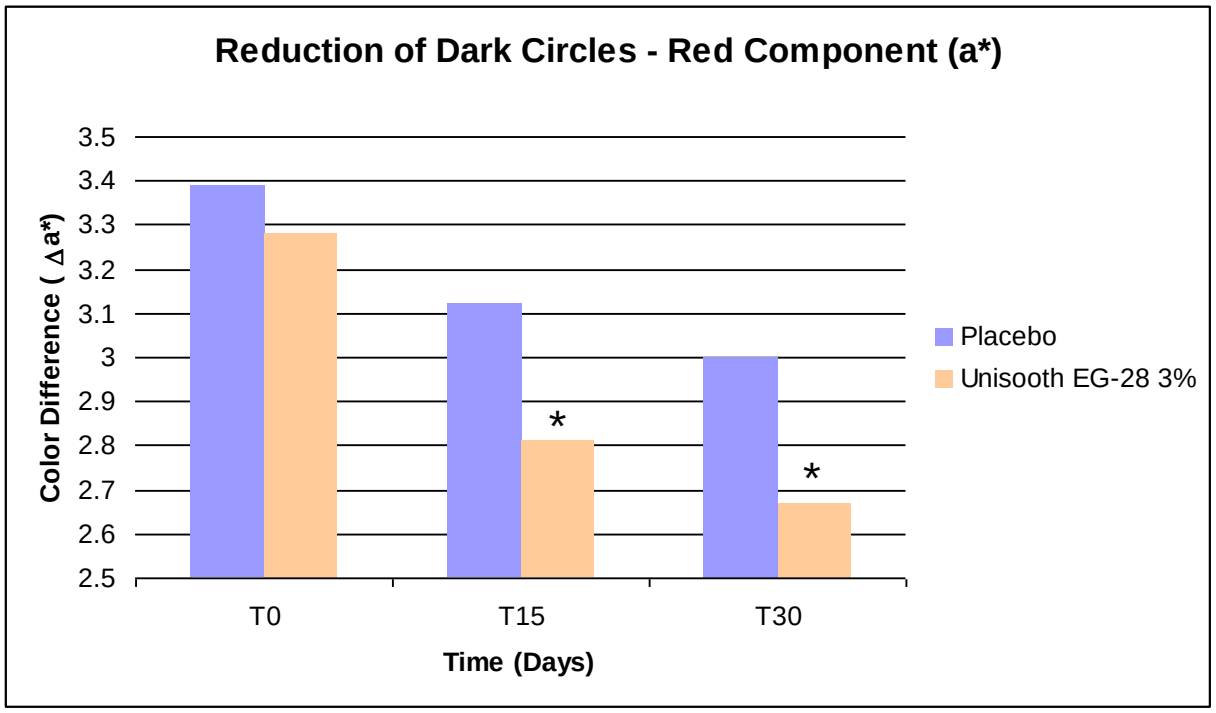

Figure 7: Treatment with a gel containing Unisooth EG-28 at 3.0% reduces red color of dark circles on under eye skin of human volunteers. The reduction was statistically significant when compared to a placebo gel.

*Significant data vs. placebo, Student's T test.

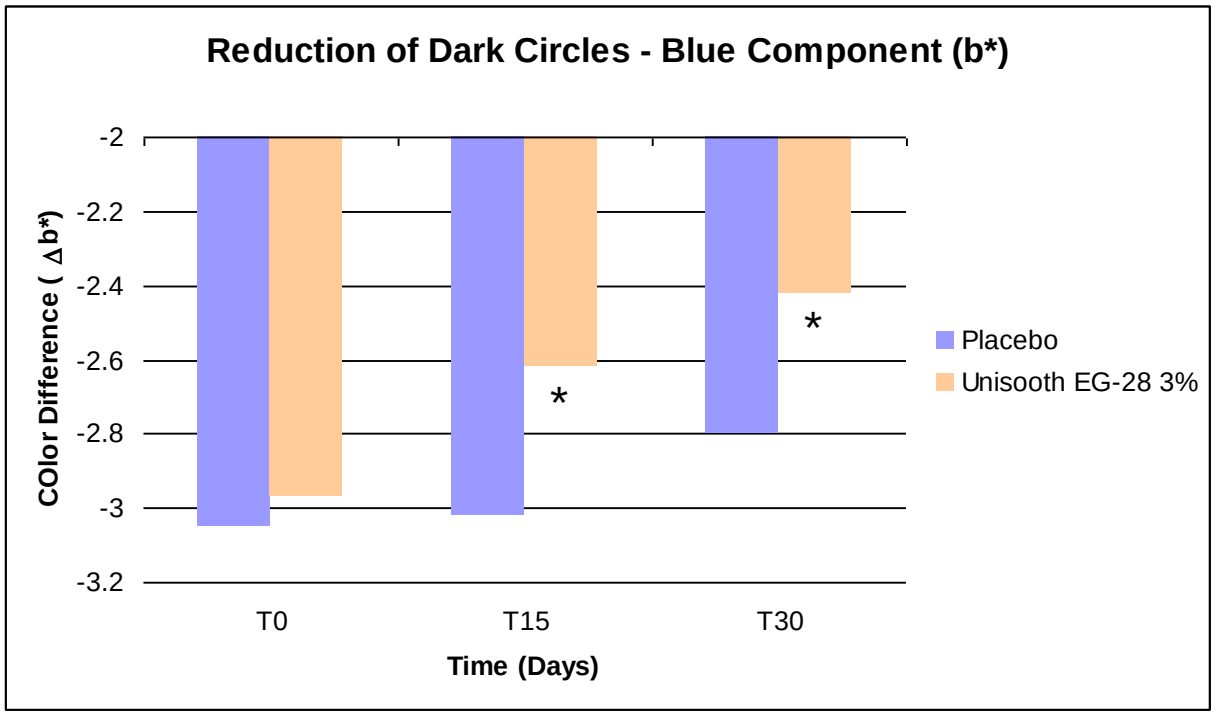

Figure 8: Treatment with a gel containing Unisooth EG-28 at 3.0% decreases blue color component of dark circles on under eye skin of human volunteers. The decrease was statistically significant when compared to a placebo gel.

*Significant data vs. placebo, Student's T test.

Placebo	No improvement	Slight improvement	Moderate improvement
T15	75%	25%	0
T30	58.3%	25%	16.7%

Unisooth EG-28 3%	No improvement	Slight improvement	Moderate improvement
T15	50%	50%	0
T30	33.3%	37.5%	29.2%

Table 1: Dermatological Analysis (based on score) of treated volunteers. Improvement relates to Dark Circles.

4. Statistical Analysis

The inter-group comparisons were performed by Student's T test.

5. Conclusions

Unisooth EG-28 strongly inhibits NF-kB activity in HT29 human cells as detected by a reporter plasmid, confirming the specificity of Unisooth EG-28 to target the NF-kB pathway. This inhibition was dose dependent and strongly significant.
Unisooth EG-28 further inhibits the release of pro-inflammatory molecules such as interleukine IL-8 and chemokine CXCL1 in normal epidermal keratinocytes (NHEK).
To induce the release of IL-8 and CXCL1, NHEK were induced with a cytokine mixture (IL-1α and TNF-α) mimicking the cross-talk between skin infiltrating T lymphocytes and keratinocytes (2,3). This cytokine mixture was chosen because of its association with the NF-kB pro- inflammatory signaling pathway (17), being the components of Unisooth EG-28 involved in the modulation of the NF-kB pathway as shown in Figure 4 and as suggested by the literature (7,8,12-14).
The cytokine mixture produced a strong increase in IL-8 and CXCL1 release from NHEK as measured by ELISA. Unisooth EG-28 tested at 0.016%, 0.08% and 0.4% was able to reduce in a statistical significant way the IL-8 and CXCL1 increase.

The effect was dose dependent and reached 99% inhibition at 0.4% of Unisooth EG-28 regarding IL-8 release, and was even below untreated control (that had a basal CXCL1 release) at 0.08% and 0.4% Unisooth EG-28.
The results obtained with the highest doses of Unisooth EG-28 were superior in inhibition than the benchmarks NF-kB inhibitor III and Dexamethasone.

When Unisooth EG-28 was incorporated in a gel at concentration of 1.0% and 3.0% and applied on irritated skin of human volunteers, it was able to rapidly sooth the irritation as fast as 15 min from application. The soothing effect increased with time from application and seemed to go to plateau after 120 min from application. The increase was more relevant for the highest dose than the lowest dose of Unisooth EG-28 (dose dependency) and statistically significant when compared to a placebo gel (not containing Unisooth EG-28), while for the lowest dose (1.0%) significance was observed after 120 min from application.

Interestingly, Unisooth EG-28 was also able to decrease the trans-epidermal water loss (TEWL) induced by the irritation treatment. This effect was statistically significant after 30 min from application of the cream containing Unisooth EG-28 compared to the placebo cream, it was dose dependent, and did not seem to reach plateau after 120 min (so possibly lasting and further increasing at later time points). The lowest dose was effective but did not reach statistically significance vs. the placebo gel.

Finally, treatment of a separate group of volunteers with a gel containing Unisooth EG-28 at 3% around the eye area was able to reduce the red and blue color components of dark circles after 15 and 30 days. This effect was superior and statistically significant when compared to a placebo gel treatment. These quantitative data were further confirmed by a qualitative evaluation by a dermatologist.

In conclusion, we have demonstrated that Unisooth EG-28 is indeed a strong anti-irritation ingredient by *in vitro* and *in vivo* studies. It rapidly soothes skin redness and its effect in reducing the TEWL suggests a role as a healing agent in restoring the damaged skin barrier. Moreover, it reduces dark circles around the eyes.

Unisooth EG-28 can be incorporated in products for delicate skin, especially for the eye area, products for immediate soothing such as after sun, after-shave, etc. or products for sensitive skin individuals that easily develop skin reactions to external inducers thus needing immediate soothing effect.

Unisooth EG-28 has been tested for skin tolerance, mutagenicity and biodegradability, and has provided an excellent safety profile. Recommended usage levels are between 1.0% and 3.0%.

6. Characteristics

Composition	Unisooth EG-28 is a balanced blend of water soluble active substances consisting of Propyl Gallate, Gallyl Glucoside and Epigallocatechin Gallatyl Glucoside
Appearance	Clear faint yellowish liquid
Analytical data	see specifications
Solubility	readily soluble in water
Safety	see safety data sheet
Dosage	1-3%
Storage	see safety data sheet
Shelf life	see specification

Identification

INCI Monograph ID	INCI name	CAS No.
2641	Propyl Gallate	121-79-9
23717	Gallyl Glucoside	131579-69-6
23716	Epigallocatechin Gallatyl Glucoside	1236072-19-7

7. References

1. Bell S, Degitz K, Quirling M, Jilg N, Page S, Brand K. Involvement of NF-kappaB signalling in skin physiology and disease. Cell Signal 15(1):1-7, 2003

2. Wan F, Lenardo MJ. The nuclear signaling of NF-kappaB: current knowledge, new insights, and future perspectives. Cell Res 20(1):24-33, 2010

3. Pasparakis M. Regulation of tissue homeostasis by NF-kappaB signalling: implications for inflammatory diseases. Nat Rev Immunol 9(11):778-88, 2009

4. Franzone JS, Natale T, Cirillo R, Torrielli MV. Influence of propyl-gallate and 2-mercaptopropionylglycine on the development of acute inflammatory reactions and on biosynthesis of PGE2. Boll Soc Ital Biol Sper. 56(24):2539-2545, 1980

5. Kim SH, Jun CD, Suk K, Choi BJ, Lim H, Park S, Lee SH, Shin HY, Kim DK, Shin TY. Gallic acid inhibits histamine release and pro-inflammatory cytokine production in mast cells. Toxicol Sci 91(1):123-131, 2006

6. Kuppan G, Balasubramanyam J, Monickaraj F, Srinivasan G, Mohan V, Balasubramanyam M. Transcriptional regulation of cytokines and oxidative stress by gallic acid in human THP-1 monocytes. Cytokine 49(2):229-234, 2010

7. Choi KC, Lee YH, Jung MG, Kwon SH, Kim MJ, Jun WJ, Lee J, Lee JM, Yoon HG. Gallic acid suppresses lipopolysaccharide-induced nuclear factor-kappaB signaling by preventing RelA acetylation in A549 lung cancer cells. Mol Cancer Res 7(12):2011-2021, 2009

8. Lee KW, Kundu JK, Kim SO, Chun KS, Lee HJ, Surh YJ. Cocoa polyphenols inhibit phorbol ester-induced superoxide anion formation in cultured HL-60 cells and expression of cyclooxygenase-2 and activation of NF-kappaB and MAPKs in mouse skin in vivo. J Nutr 136(5):1150-1155, 2006

9. Katiyar SK. Skin photoprotection by green tea: antioxidant and immunomodulatory effects. Curr Drug Targets Immune Endocr Metabol Disord 3(3):234-242, 2003

10. Katiyar SK, Matsui MS, Elmets CA, Mukhtar H. Polyphenolic antioxidant (-)-epigallocatechin-3-gallate from green tea reduces UVB-induced inflammatory responses and infiltration of leukocytes in human skin. Photochem Photobiol 69(2):148-153, 1999

11. Trompezinski S, Denis A, Schmitt D, Viac J. Comparative effects of polyphenols from green tea (EGCG) and soybean (genistein) on VEGF and IL-8 release from normal human keratinocytes stimulated with the proinflammatory cytokine TNFalpha. Arch Dermatol Res 295(3):112-116, 2003

12. Afaq F, Adhami VM, Ahmad N, Mukhtar H. Inhibition of ultraviolet B-mediated activation of nuclear factor kappaB in normal human epidermal keratinocytes by green tea constituent (-)-epigallocatechin-3-gallate. Oncogene 22(7):1035-1044, 2003

13. Kundu JK, Surh YJ. Epigallocatechin gallate inhibits phorbol ester-induced activation of NF-kappa B and CREB in mouse skin: role of p38 MAPK. Ann N Y Acad Sci. 1095:504-512, 2007

14. Afaq F, Ahmad N, Mukhtar H. Suppression of UVB-induced phosphorylation of mitogen-activated protein kinases and nuclear factor kappa B by green tea polyphenol in SKH-1 hairless mice. Oncogene 22(58):9254-9264, 2003

15. Kuhn C, Bekemeier H, Hirschelmann R, Giessler AJ. Action of cyclooxygenase (COX) and lipoxygenase (LOX) inhibitors as well as of oxygen free radical scavengers (OFRS) in the inflammation-induced vasodepression. Biomed Biochim Acta 47(10-11):S320-S323, 1988

16. Bosman B. Testing of lipoxygenase inhibitors, cyclooxygenase inhibitors, drugs with immunomodulating properties and some reference antipsoriatic drugs in the modified mouse tail test, an animal model of psoriasis. Skin Pharmacol 7(6):324-334, 1994

17. Vallabhapurapu S, Karin M. Regulation and function of NF-kappaB transcription factors in the immune system. Annu Rev Immunol 27:693-733, 2009