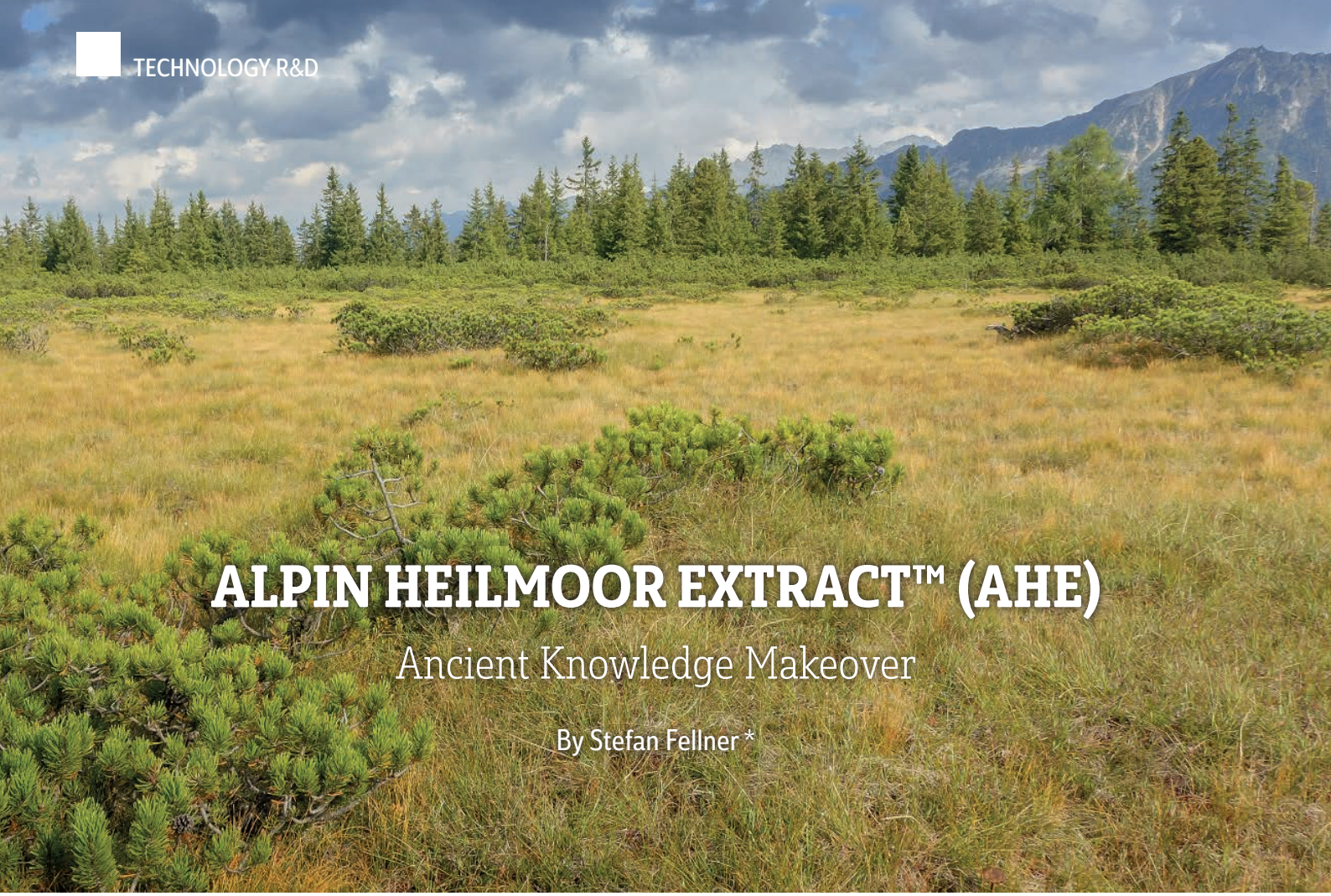


ALPIN HEILMOOR EXTRACT™ (AHE)

Ancient Knowledge Makeover

By Stefan Fellner *

Abstract

Wound healing factors, skin barrier integrity and resistance to inflammation are essential factors for healthy skin. Additionally, a clean, pro-aged skin that is free from irritation and acne is cosmetically attractive. ALPIN HEILMOOR EXTRACT™ (AHE), a natural (organic) active ingredient (dark micronized powder) extracted from healing Moor (Heilmoor) deposits at 500m above sea level in Austria, was investigated for its efficacy in maintaining and enhancing these key-parameters of healthy and beautiful skin. A series of in-vitro *tests involving HaCaT cells and reconstructed human epidermis (RHE) revealed that AHE strongly improves wound healing, enhances membrane barrier integrity and supresses skin inflammation. An open, intra-individual* in-vivo *efficacy study confirmed the multiple beneficial effects of AHE: a pronounced pro-aging effect, remarkable cleansing/anti-pollution efficacy, both, an immediate and a preventive soothing effect, and a convincing anti-acne efficacy. Notably, AHE was generally very well tolerated and appreciated by subjects.*

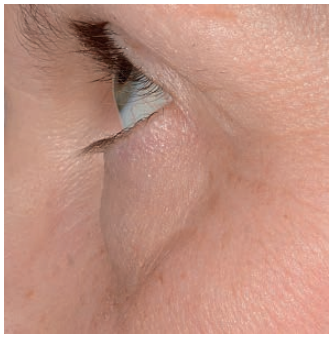
Introduction

Peloid has been indicated for the treatment of chronic rheumatic processes, degenerative osteoarthrosis, sequelae of osteoarticular injuries, fractures, dislocations, disorders following vasculopathies, dermatological diseases, etc [1]. These therapeutic benefits were already hypothesized centuries ago, for example by Abbot Thomas von Lambach in 1364, and the finding of a medieval bathtub is considered the oldest evidence of a moor bathing facility. Even currently, mud therapy (considered as natural pelotherapy) is in practice, such as the popular mud packs and baths practiced in the Bahrolmeyyet Lake, Palestina, the mud packs and baths practiced in the Urumieh Lake, Iran and mud packs and baths that are practiced in rehabilitation centres in Austria, Germany and Hungary.

Cosmetic peloids, on the other hand, have an essential function in the maintenance of healthy and pro-aged skin. Our objective was to investigate and demonstrate the dermocosmetic properties of ALPIN HEILMOOR EXTRACT™ (AHE). AHE is a micronized dark powder which concentrates the healing properties of medicinal peloid in a pure and potent form. It was sustainably extracted from a natural (organic) peloid of an accredited healing moor deposit (Heilmoor) at 500m above the sea level in Austria. We conducted a series of *in-vitro* studies to determine skin barrier enhancement, wound healing factors and anti-inflammatory efficacy as well as *in-vivo* clinical studies to determine pro-aging (elasticity, hydration, radiance under eye), soothing, and cleansing efficacy of cosmetic grades of ALPIN HEILMOOR EXTRACT™ (AHE). AHE was also investigated for its skin acceptability and future use.

* CEO Premium Organic GmbH, A-9313 St. Georgen am Längsee, Austria, www.premium-organic.com

Pro-aging eye-bag D0



Pro-aging eye-bag D28

Materials and Methods

A series of *in-vitro* studies with HaCaT cells and RHE to determine wound healing, skin barrier enhancement and anti-inflammatory efficacy of AHE were conducted. To determine the expression and/or activation of certain molecular factors upon AHE-treatment of cells and *in vitro* skin models, we used Western blot and MSD multiplexing involving the U-Plex platform. An open, intra-individual *in-vivo* efficacy study was conducted to demonstrate the anti-irritant, anti-acne, cleansing/anti-pollutant, pro-aging and skin tolerability properties of AHE to determine AHE's applicability for future use.

Results and Discussion

Induction of Wound Healing Factors

In wound re-epithelialization, E-cadherins coordinates tractional forces promoting collective and directional migration of epithelial cells [2]. E-cadherin was found to be expressed in two isoforms of different molecular weight: E-cadherin B1 (120kDa) and E-cadherin B2 (90–100kDa). By participating in multiple signalling cascades and the formation of focal adhesions (FA), paxillin plays a critical role during cellular migration and thus, wound healing. 1% AHE induced a 1.6- and 1.3-fold increase of E-cadherin B1 and B2 expression on the injured skin model (Figure 01). Treatment

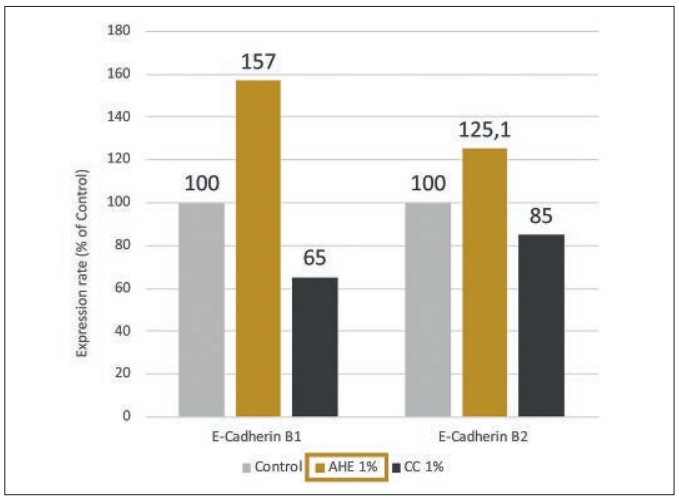


Figure 1: Expression rate of E-cadherin after 1% AHE treatment; 1.6-fold and 1.3-fold increase in expression of E-cadherin B1 and E-cadherin B2, respectively, compared to control, i.e. culture medium;
(AHE – ALPIN HEILMOOR EXTRACT™, CC – activated charcoal).

with 5% AHE resulted into 0.8-fold repression of B1 and complete repression of B2 on the injured skin model but 1.5-fold induction of B1 and 1.7-fold induction of B2 on the intact skin model. Paxillin, on the other hand, was 2.4-fold induced when cells were treated with 5% AHE. These results suggest that 1% and 5% AHE have a positive effect on wound healing by promoting E-cadherin and paxillin expression in human keratinocytes respectively.

Enhanced Skin Barrier Integrity

Cytokeratins 10 (CK10) and 16 (CK16) are essential for skin barrier integrity [3]. To understand the effect of AHE on membrane barrier integrity, we determined the expression pattern of CK10 and CK16 in HaCaT cells with and without AHE-treatment, respectively. Western blot analysis revealed an induction of CK10 in extracts obtained from HaCaT cells after treatment with AHE. Furthermore, this effect was reinforced when the cells were pre-stimulated with C. acnes.

Repression of Pro-Inflammatory Cytokines

A continuous expression of cytokines following noxious stress causes chronic inflammation and skin damage which in turn leads to skin disease and (premature) aging [4]. Our *in-vitro* irritation study demonstrated the anti-inflammatory activity of AHE by suppressing the induction of inflammatory markers, such tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and interleukin-8 (IL-8) in peripheral blood mononuclear cells (PBMCs) and reconstructed human epidermis (RHE) after stimulation with C. acnes. These results suggest a role for AHE in the prevention of inflammatory skin diseases and premature aging by suppressing pro-inflammatory cytokines (Figure 2).

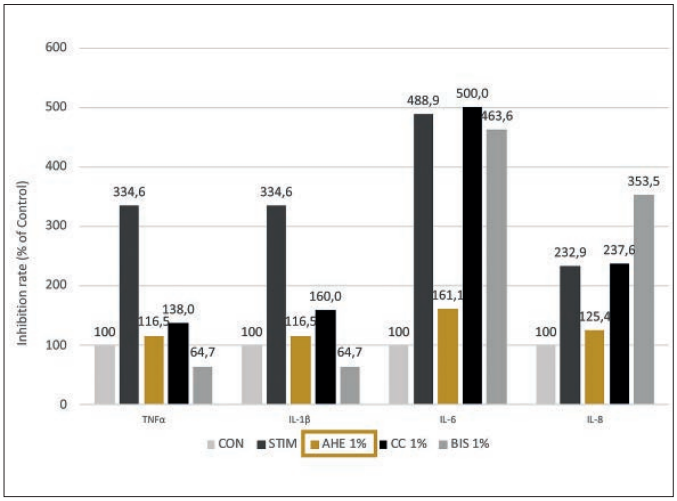


Figure 2: AHE-induced repression of inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-8 after stimulation of reconstructed human epidermis (RHE) with C. acnes. (control – non-stimulated, untreated RHE; STIM – stimulation with C. acnes, no treatment; AHE 1% - stimulation followed by treatment with 1% ALPIN HEILMOOR EXTRACT™, CC – stimulation followed by treatment with 1% charcoal; BIS – stimulation followed by treatment with 1% bisabolol).

Advanced Pro-Aging

A reduction in the levels of functional dermal components such as collagens results in the emergence of clinical aging features, such as wrinkles and reduced elasticity [5]. After 28 days of treatment with a 2.5% AHE-containing day cream, there was a signifi-

cant improvement in skin firmness (tensing effect of product) by 4%, viscoelasticity (anti-aging action of product) by 11%, and plasticity by 12% in 43%, 67% and 48% of subjects, respectively. There was also a significant increase in the cutaneous hydration rate by 6% and radiance under the eyes by 17%, consistent with the moisturizing and radiant skin effect of the product, respectively (Figure 3).

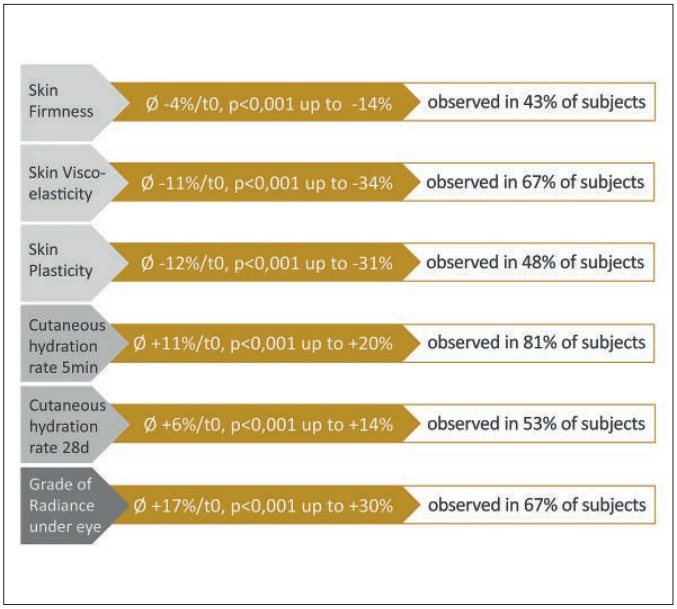


Figure 3

Enhanced Cleansing Efficacy

1.5% AHE-enriched facial cleanser exhibited a strong efficacy of about 98% in eliminating carbon microparticle deposit from skin. There was a significant improvement in the elimination index of the treated group compared to the control group ($p<0.006$). Thus, AHE exerts a highly efficient cleansing/antipollution effect. Notably, no clinical signs of intolerance or feeling of discomfort whatsoever were reported by the study subjects.

Immediate and Preventive Soothing Effect

Cutaneous irritation is a commonly known and widespread skin condition. AHE potently decreased the duration of lactic acid-induced stinging by 80% in 100% of subjects. What's more, AHE induced a significant decrease in the stinging intensity by 48%, 89% and 100% after 30 seconds, 5 and 15 minutes after AHE application, respectively, as has been reported by 86% to 100% of subjects. AHE also significantly reduced cutaneous reactivity score by approximately 67%, in 97% of subjects after 28 days of use (Figure 4). These results suggest that AHE is an anti-irritant with both immediate and preventive soothing effects.

Moreover, AHE induced a significant decrease of stinging intensity when applied to capsaicin-treated skin: an average 50% decrease was observed by 55% of subjects as soon as 30 seconds after administration, while 70% of subjects reported an average decrease of 70% of stinging intensity within 3 minutes after application of AHE. There was no difference in the decrease of stinging intensity reported after 6 and 9 minutes upon administration of 2,5% AHE, respectively (Figure 5). A significant decrease by 69% in the duration of stinging was also observed in 90% of

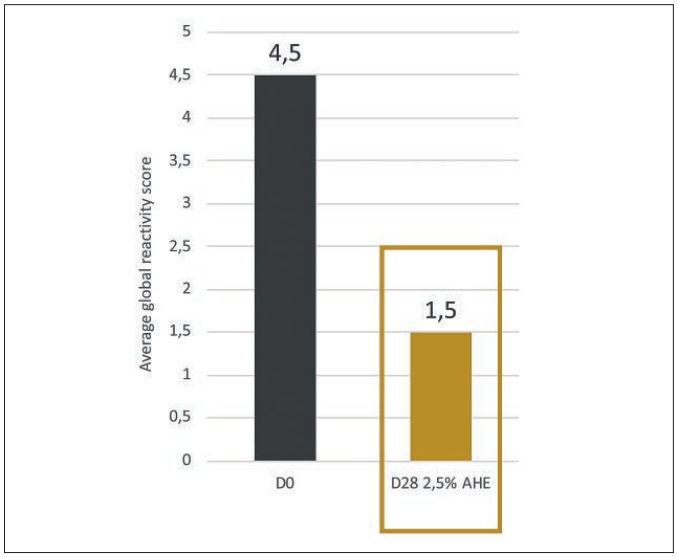


Figure 4: Preventive soothing efficacy of 2.5% AHE application for 28 days; decrease in global reactivity score of about 67% in 97% of subjects ($p<0.001$); (D0 – day before treatment onset, D28 2,5% AHE – day 28 of treatment with 2,5% Alpine Heilmoor Extract).

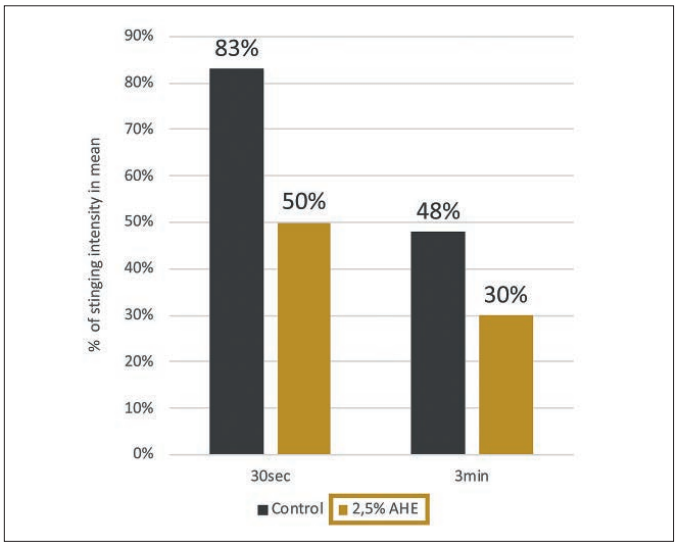
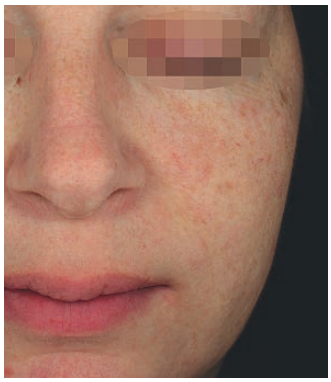
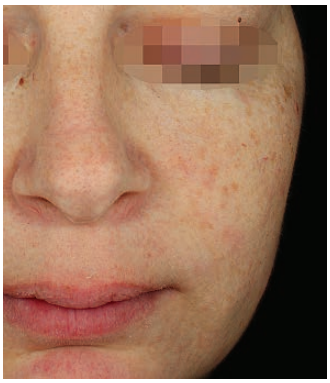


Figure 5: Immediate soothing effect induced by 2.5% AHE; statistically significant decrease in the mean of intensity of stinging score after 30sec ($p<0.001$) and 3min ($p<0.001$) in 55% and 70% of subjects, respectively.

subjects. Furthermore, there was a significant improvement by 48% on average in cutaneous reactivity observed in 68% of subjects after 28 days.



Soothing Preventive Lactic d0



Soothing Preventive Lactic d28

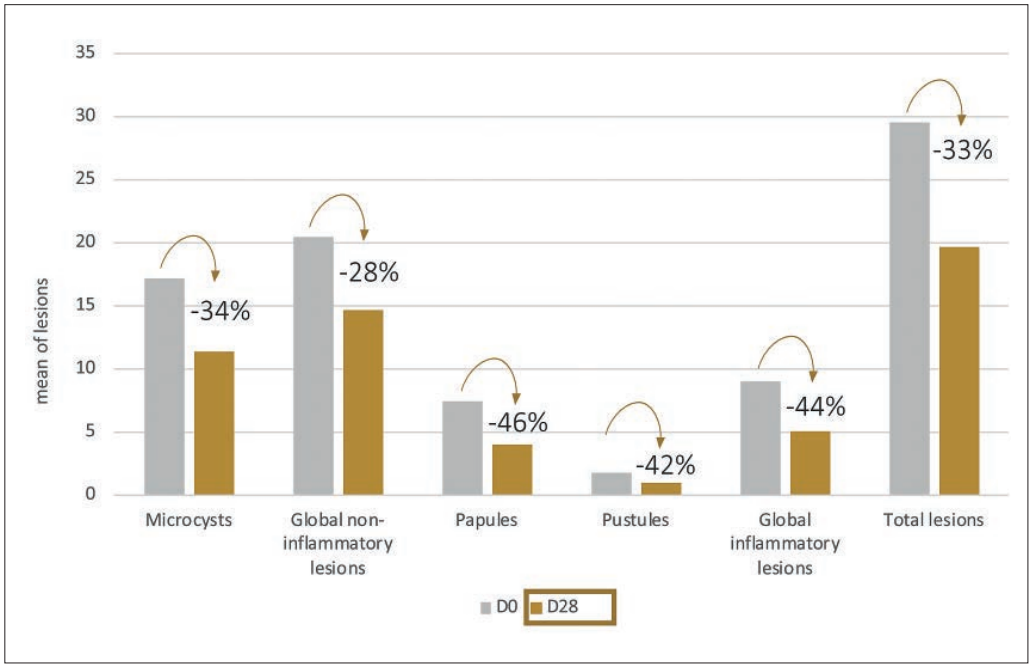
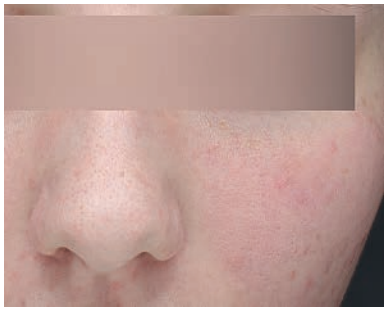


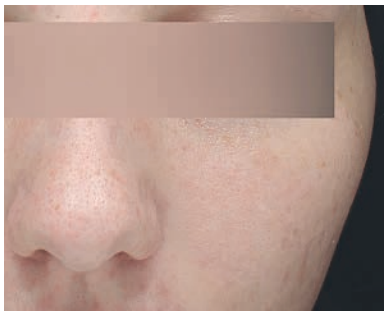
Figure 6: Anti-acne efficacy of 1.5% AHE application for 28 days; statistically significant decrease in the number of microcysts, global inflammatory lesions, global non-inflammatory lesions, papules and total lesions ($p<0.0001$); (D0 – day before treatment onset, d28 – day 28 of treatment with 1,5%).

Anti-Acne Efficacy and Cutaneous Tolerability

Activation of innate immunity via the expression of cytokines by keratinocytes, resulting in the hyperkeratinization of the pilosebaceous unit, plays an essential role in acne formation[6]. After 28 days of twice daily application, 1.5% AHE induced a statistically significant decrease in the number of microcysts, papules and pustules. Moreover, a significant decrease in the total number of lesions (global non-inflammatory and inflammatory) was observed (Figure 6). AHE also induced a decrease in porphyrin expression, which characterizes a reduction of susceptibility to acne lesions, though results were not statistically significant. AHE was very well tolerated and about 73% and 82% of subjects stated that the product prevents inflammatory acne if used regularly and reduces inflammatory acne, respectively.



Acne D0



Acne D28

Conclusion

We found AHE to be implicated in the promotion of wound healing factors E-cadherin and paxillin, and the suppression of cytokines involved in inflammation *in vitro*. Furthermore, AHE was shown to enhance skin barrier integrity by inducing the expression of two different cytokeratines. In addition, AHE displays anti-irritant, anti-acne, anti-pollutant, and a pro-aging property when tested *in vivo*. AHE was also very well tolerated stressing its beneficial characteristics for future use in different application forms (rinse-off and leave-on).

References

1 *www.echa.europa.eu/documents/10162/1b8ab766-b3ff-840f-a0bd-f1ad1) 1. Gomes C, et al. Appl Clay Sci. 2013 May 1;75–76:28–38.*

2 *Li L, et al. Cell Mol Life Sci [Internet]. 2012;69(16):2779–89. Available from: https://doi.org/10.1007/s00018-012-0951-3*

3 *Kumar V, et al. J Cell Biol [Internet]. 2015 Dec 7;211(5):1057–75. Available from: https://pubmed.ncbi.nlm.nih.gov/26644517*

4 *Borg M, et al.Climacteric. 2013 Oct;16(5):514–21.*

5 *Shin J-W, et al. Int J Mol Sci. 2019 Apr;20(9).*

6. *Dréno B. J Eur Acad Dermatol Venereol. 2017 Sep;31 Suppl 5:8–12.*

Stefan Fellner