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The Renaissance of Heilmoor – A New Efficient and Sustainable Extract

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abstract

Wound healing factors, skin barrier integrity and resistance to inflammation are determinants 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 Austrian healing Moor (Heilmoor) deposits at 500m above sea level, 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 improves wound healing, enhances membrane barrier integrity and suppresses 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 efficiency. Notably, AHE was generally very well tolerated and appreciated by study subjects.

Introduction

Historically, peloid (muds and healing earths) has been indicated for the treatment of chronic rheumatic processes, degenerative osteoarthritis, sequelae of osteo-articular 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 at the Dead Sea, Palestina, and the Urumieh Lake, Iran, as well as mud packs and baths that are practiced in rehabilitation centres in Austria, Germany and Hungary.

Cosmetic peloids, on the other hand, have a positive impact on 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 Austrian healing moor deposit (Heilmoor) at 500m above the sea level. We conducted a series of *in-vitro* studies to determine the effect of AHE on skin barrier enhancement, wound healing factors and anti-inflammatory efficacy as well as *in-vivo* clinical studies to ascertain pro-aging (elas-

ticity, 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.

Materials and Methods

A series of *in-vitro* studies with HaCaT cells and reconstructed human epidermis (RHE) to investigate 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 performed Western blot assays and MSD multiplexing analyses 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, as well as to determine AHE's applicability for future use.

Results and Discussion

Induction of Wound Healing Factors

In wound re-epithelialization, E-cadherin coordinates tractional forces promoting collective and directional migration of epithelial cells [2]. E-cadherin was found to be expressed

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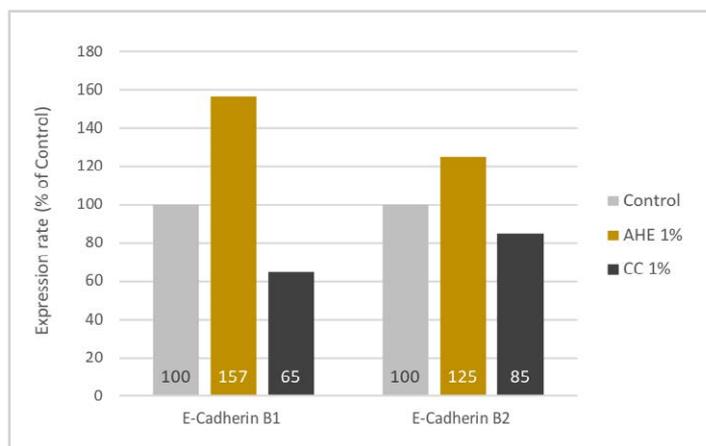


Fig.1 Expression rate of E-cadherin after 1% AHE treatment; 1,6-fold and 1,3-fold increase in expression of E-cadherin B1 and E-cadherin B2, respectively, compared to control, i.e. culture medium; (AHE – Alpin Heilmoor Extract, CC – activated charcoal).

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 (**Fig. 1**). Treatment with 5% AHE resulted

into 0,8-fold repression of E-cadherin 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*

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irritation study demonstrated the anti-inflammatory activity of AHE by suppressing the induction of inflammatory markers, such as tumor necrosis factor α (TNF- α), interleukin-1 β (IL-1 β), 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 (Fig. 2).

Advanced Pro-Aging

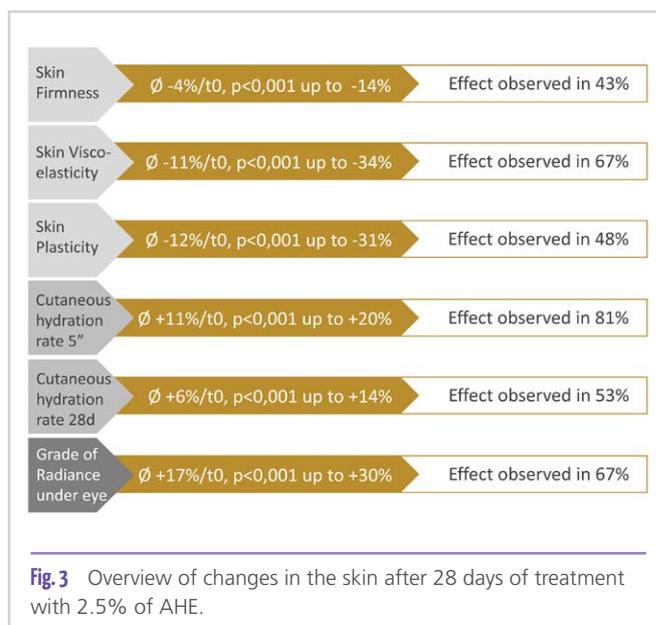
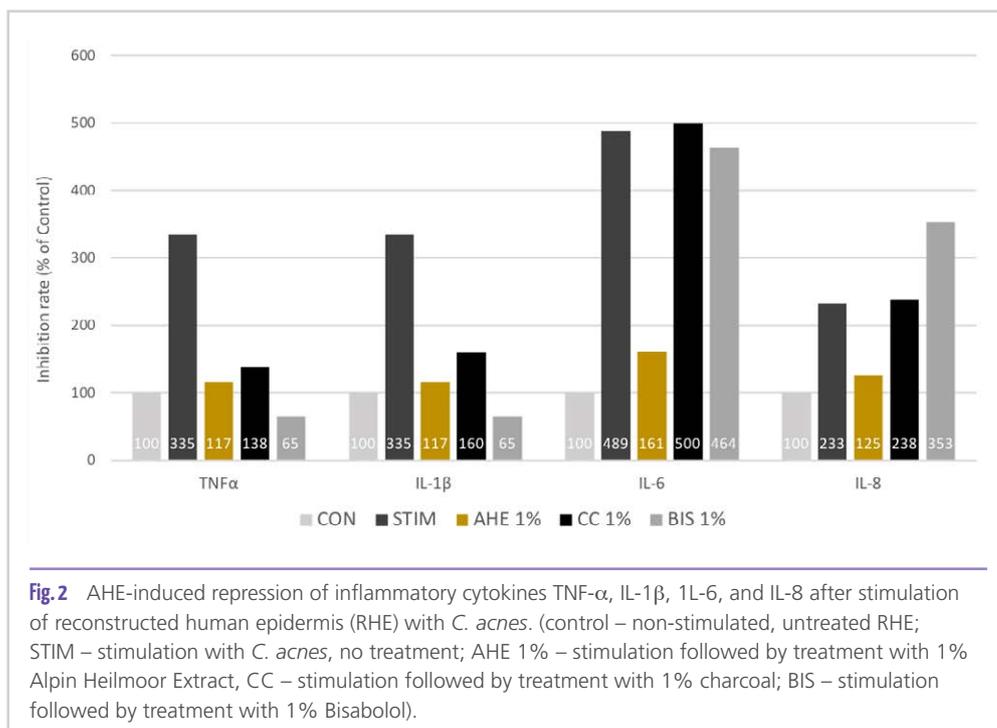
A reduction in the levels of functional dermal components such as collagens results in the emergence of clinical aging features, such as wrinkles and reduced elasticity [5]. After 28 days of treatment with a 2.5% AHE-containing day cream, there was a significant improvement in skin firmness (tensing effect of product) by 4%, viscoelasticity (anti-aging action of prod.) 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 (Fig. 3,4).

Enhanced Cleansing Efficacy

1.5% AHE-enriched facial cleanser exhibited a strong efficacy (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/anti-pollution 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 approximate-



ly 67%, in 97% of subjects after 28 days of use (Fig. 5, 6). 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 (Fig. 7). A significant decrease by 69% in the duration of stinging was also observed in 90% of subjects. Furthermore, there was a significant improvement by 48% on average in cutaneous reactivity observed in 68% of subjects after 28 days.

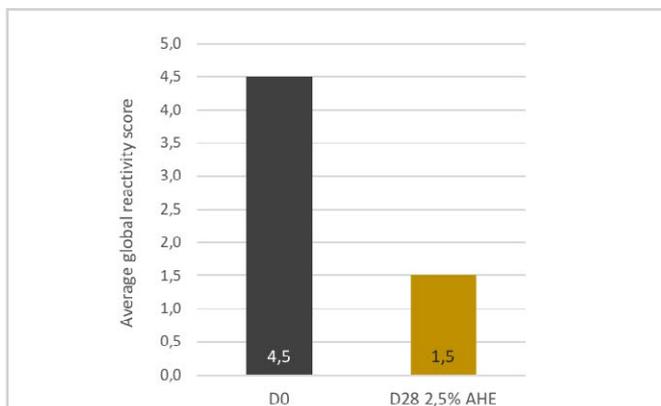


Fig. 5 Preventive soothing efficacy of 2.5% AHE application for 28 days; decrease in global reactivity score of about 67% in 97% of subjects ($p < 0,001$); (D0 – day before treatment onset, D28 2.5% AHE – day 28 of treatment with 2.5% Alpin Heilmoor Extract).

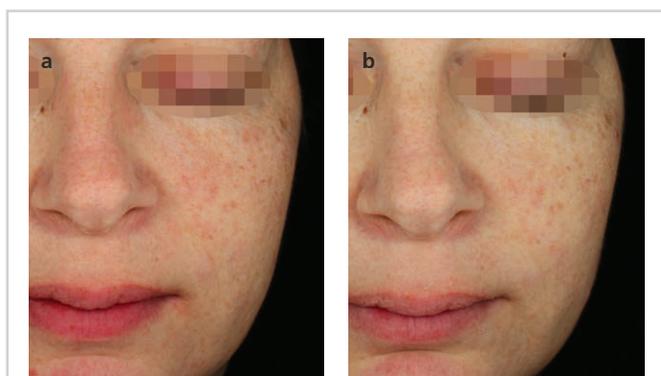


Fig. 6 a: Preventive Soothing Effect of 2.5% AHE; D0; b: Preventive Soothing Effect of 2.5% AHE; D28.

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 microcyts, papules and pustules. Moreover, a significant decrease in the total number of lesions (global non-inflammatory and inflam-

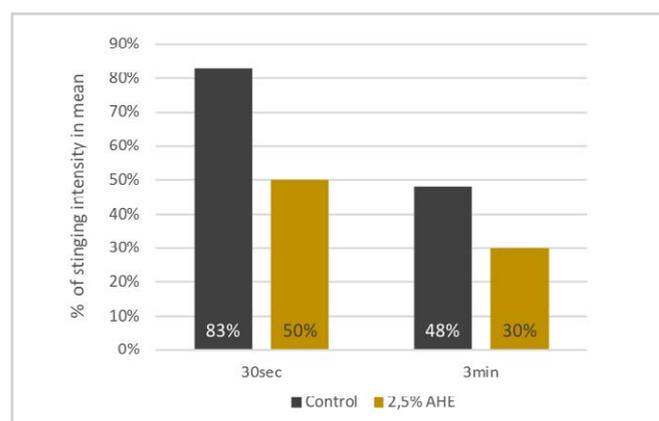


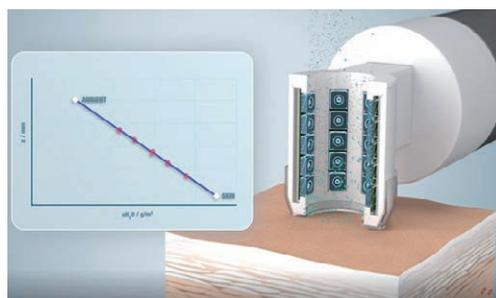
Fig. 7 Immediate soothing effect induced by 2.5% AHE; statistically significant decrease in the mean of intensity of stinging score after 30 sec ($p < 0,001$) and 3 min ($p < 0,001$) in 55% and 70% of subjects, respectively.

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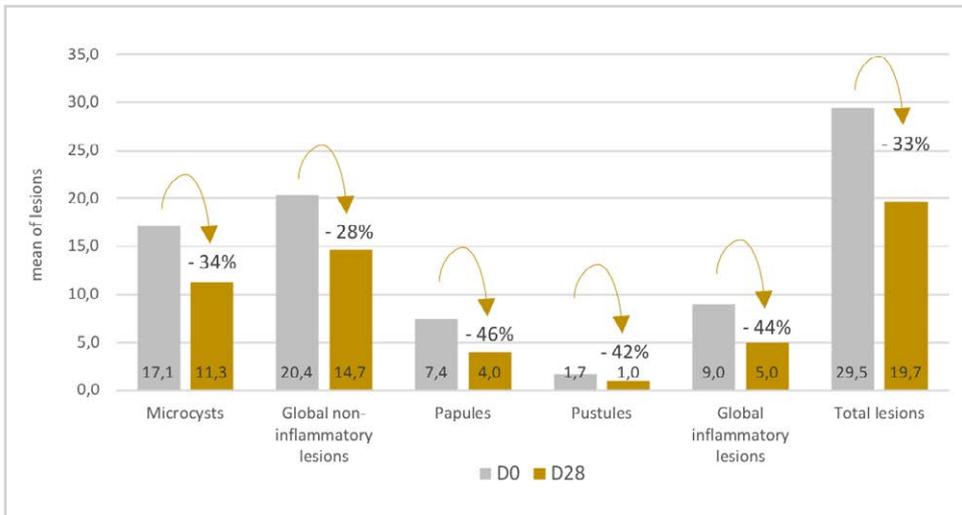


Fig. 8 Anti-acne efficacy of 1.5% AHE application for 28 days; statistically significant decrease in the number of microcysts, global inflammatory lesions, global non-inflammatory lesions, papules and total lesions ($p < 0.0001$); (D0 – day before treatment onset, D28 – day 28 of treatment with 2.5%).



Fig. 9 a: Anti-Acne efficacy of 1.5% AHE; D0; b: Anti-Acne efficacy of 1.5% AHE; D28.

matory) was observed (Fig. 8, 9). 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.

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. Furthermore, AHE was shown to enhance skin barrier integrity by inducing the expression of two different cytokeratins. 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).

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