Skin Pharmacology and Physiology

Research Article

Skin Pharmacol Physiol 2021;34:337–350
DOI: 10.1159/000518970

Received: May 20, 2021
Accepted: August 9, 2021
Published online: August 17, 2021

In vivo Skin Penetration, Radical Protection, and Structural Changes after Topical Application of a Herbal Oil Cream Compared to Topical Calcipotriol in Mild to Moderate Psoriasis

Juergen Lademann[a] Parvin Mansouri[b] Ali Nahavandi[c] Axel Ahlers[c] Fatemeh Zibakalam-Mofrad[c] Barbara Brower[c] Maryam Nahavandi[c] Frank Feddern[c] Maxim E. Darvin[a] Sabine Schanzer[a] Heike Richter[a] Martina C. Meinke[a] Seyed Ahmad Rezaii[d] Masoumeh Rohaninasab[b] Susan Farshi[b] Massimo Iacobelli[e] Sora Jung[a]

[a]Department of Dermatology, Venerology and Allergology, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, Berlin, Germany; [b]Tehran University of Medical Science, TUMS, Skin and Stem Cell Research Center, Tehran, Iran; [c]Alnapharm AG & Co. KG, Hamburg, Germany; [d]Tehran University of Medical Science, TUMS, Research Management Office, Tehran, Iran; [e]Techitra Srl, Milan, Italy

Keywords

Psoriasis · Topical treatment · Antioxidants · Reflectance confocal microscopy · Raman microscopy · Electron paramagnetic resonance spectroscopy · Skin penetration · Skin care · Herbal antioxidants · Calcipotriol

Abstract

Background: The chronicity of psoriasis often requires continuous topical treatment. ***Materials and Methods:*** Here, the radical protection of a cream containing various herbal oils was evaluated in vivo by electron paramagnetic resonance (EPR) spectroscopy and its skin penetration by Raman microscopy in intact and barrier-disturbed skin. Changes in skin barrier properties were evaluated after 4 weeks of daily topical application using in vivo laser scanning microscopy (LSM) and transepidermal water loss in 26 healthy volunteers. A randomized, controlled, double-blind, three-arm parallel clinical study evaluated the efficacy of the herbal oil cream compared to a 0.05% calcipotriol-containing cream and to a vehicle cream, in 135 patients with mild to moderate plaque psoriasis with the change in Psoriasis Area and Severity Index (PASI) from baseline to week 12 as the primary endpoint. ***Results:*** EPR spectroscopy disclosed a significantly higher radical formation in untreated than skin treated with the herbal oil cream ($p \leq 0.05$). LSM measurements indicated a protective skin barrier effect in treated compared to untreated skin. In the clinical trial, the topical application of herbal oils showed a significant reduction of the PASI score compared to topical calcipotriol at week 12 ($p = 0.016$). The mean reduction in PASI was 49% for the herbal oil cream, 38% for calcipotriol, and 55% for the vehicle cream. The percentage of patients, who reached PASI 50 and 75 at any time point, was 55.9% and 29.4% for the herbal oil cream, 47.4% and 15.8% for calcipotriol, and 23 (60.5%) and 13 (34.2%) for the vehicle, respectively ($p >$

karger@karger.com
www.karger.com/spp

Correspondence to:
Sora Jung, sora.jung@charite.de

Karger

0.05). The vehicle, originally designed as a placebo, contained a main ingredient of the herbal oil cream and therefore showed corresponding results. ***Conclusion:*** The herbal oil cream demonstrated effectiveness in the treatment of mild to moderate plaque psoriasis.

Introduction

Psoriasis is one of the most common chronic inflammatory skin diseases, which can affect the skin and the joints. The most common type of psoriasis, accounting for 80–90% of all cases, is psoriasis vulgaris with the typical clinical appearance of erythrosquamous plaques on the extensor sides of the extremities, but also the scalp, palmoplantar sites, the trunk, and nails can typically be affected [1–3]. There are various other types of psoriasis, such as guttate or inverse psoriasis. Being one of the most frequent inflammatory skin diseases, its prevalence ranges between 0.6% and 4.8%. The chronicity of symptoms, stigmatization, and affection of the joints can lead to a notable reduction of subjective life quality in affected patients regardless of the severity of the disease [4–7]. Although genetic-environmental interaction has been proposed as a model for the causation of psoriasis, the evidence for environmental factors is rather scarce [8, 9].

A wide range of effective topical and systemic therapies have already been in clinical use [4]. These therapies include topical agents, such as vitamin D analogs, such as calcipotriol or tacalcitol, topical steroids, coal tar, anthralin, or silicic acid, as well as phototherapy. Moreover, there are many available options for systemic treatment, such as fumaric acid, methotrexate, and especially biological response modifiers, including TNF-alpha, interleukin 17, and interleukin 23 inhibitors [10]. Depending on the psychological strain, limitation of life quality, and clinical symptoms, either topical or a combination of topical and systemic therapeutic options, can be indicated. For mild to moderate affection, topical treatments are the first choice, with phototherapy and systemic agents used in moderate to severe cases often requiring a long-term therapy [4, 11–15]. For patients presenting with extensive or moderate to severe disease, systemic therapies often combined with topical treatments are used [4, 13]. Topical treatments as a part of long-term management can ensure patients receiving appropriate proactive treatment and may help to optimize adherence and long-term outcomes [16].

Approximately 80% of affected patients show a mild to moderate form of the disease, where topical agents may be sufficient as first-line treatment [16–18] according to the current guidelines [16, 18–20]. Regarding the treatment of mild psoriasis, 3 topical options receive grade A recommendations for first-line use: corticosteroids, calcipotriol, and the calcipotriol-betamethasone dipropionate combination therapy for the acute treatment [21–23], since the long-term use of topical corticosteroids is generally not recommended [4], due to side effects including irreversible skin atrophy and the development of striae distensae [24, 25].

Topical herbal therapies have been used for the prevention and treatment of inflammatory skin diseases especially psoriasis in the past [26]. A variety of herbal products, for example, araroba tree, lace flower, barberry bark, indigo, turmeric, olibanum, and St. John's wort, have been studied in the treatment of psoriasis [26–28]. Almond oil has emollient properties for its rich concentration of oleic and linoleic essential fatty acid and has been used in ancient Chinese, ayurvedic, and Greco-Persian schools of Medicine to treat psoriasis [29].

It is known that the production of free radicals, which are at equilibrium with antioxidants, is enhanced in inflammatory skin lesions [30–33]. The excessive production of free radicals leads to oxidative stress, which can destroy cells and cell compartment and thus negatively influence the healing process [34]. The topical application of a cream containing high amounts of antioxidants can neutralize the production of free radicals [35]. Previous studies found increased levels of total oxidative stress and found evidence that the production of reactive oxygen species could be involved in the pathogenesis of psoriasis by, among others, modulation of cytokine pathways, such as MAPK/AP-1, NF-κB, and ERK [36–38], leading to the activation of Th1 and Th17 cells and the known pro-inflammatory cytokine and cell responses. Therefore, here it is hypothesized that a herbal-based topical cream (Alnovat®, Alnapharm AG & Co. KG, Hamburg, Germany) containing antioxidants can alleviate skin lesions in psoriatic patients without expecting strong adverse effects after long-term use.

The objective of this study was to determine the skin penetration depth and effects of a herbal oil containing cream (Alnovat®) on the skin barrier function and radical production in healthy volunteers and, in a second step, to investigate its efficacy and safety compared to topical calcipotriol 50 μ/g and a vehicle cream in patients suffering from mild psoriasis vulgaris. Here, a randomized, double-blind trial comparing the effect of the herbal oil formulation, of calcipotriol, and vehicle in patients with mild plaque-type psoriasis in a single center for 12 weeks was conducted.

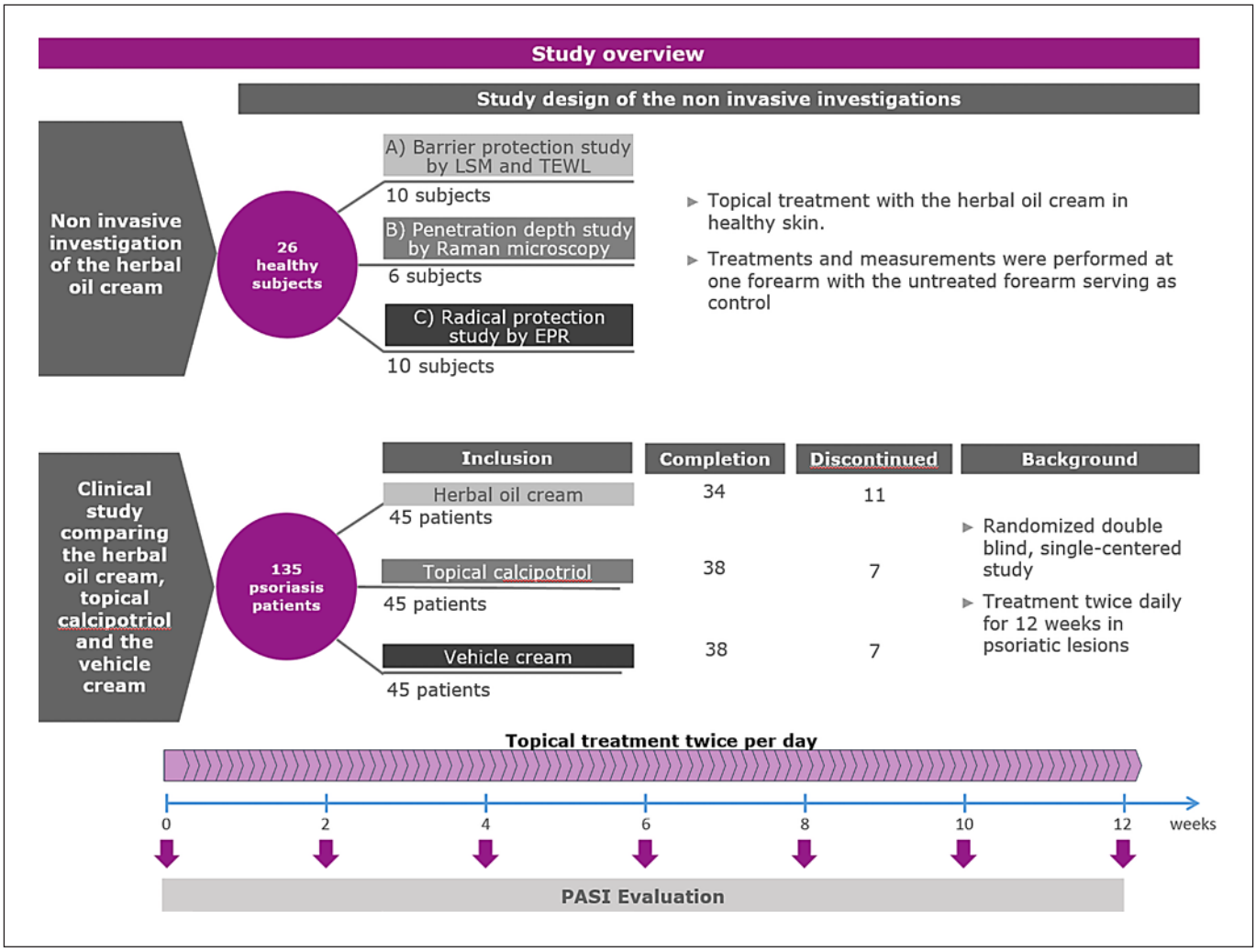

Fig. 1. Overview of the conducted investigations: first, noninvasive in vivo measurements in healthy volunteers comparing the herbal oil cream to untreated skin were performed, followed by a randomized, controlled, double-blind clinical study comparing the herbal oil cream to a calcipotriol cream and a vehicle in psoriasis patients. TEWL, transepidermal water loss; LSM, laser scanning microscopy; EPR, electron paramagnetic resonance.

Materials and Methods

Composition of the Topical Agents

The herbal oil cream to be investigated was provided by Alnapharm AG & Co. KG and was composed of various herbal, antioxidant-containing oils as follows:

Ingredients

Ingredients are coconut oil, stinging nettle oil, almond oil, bitter almond oil, hazelnut oil, sorbitanmonostearate, cetylstearyl alcohol, butylated hydroxytoluene, butylated hydroxyanisole, polysorbate 80, methyl-4-hydroxybenzoate, propyl-4-hydroxybenzoate, and water. The vehicle cream as placebo was composed similar to the herbal oil cream without the majority of its herbal oils. The main difference was the absence of most of the herbal oils (coconut oil, stinging nettle oil, almond oil, and hazelnut oil) except for bitter almond oil, which was necessary to provide a similar texture and smell in order to ensure the accuracy of the double-blind study protocol of the clinical study.

It contained bitter almond oil, middle-chain triglycerides, consisting of the saturated fatty acids caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, sorbitan monostearate, acetyl stearyl alcohol, butylated hydroxytoluene, butylated hydroxyanisole, polysorbate 80, riboflavin, methyl-4-hydroxybenzoate, propyl-4-hydroxybenzoate, and water. Both the verum herbal oil cream and the vehicle were provided by Alnapharm AG & Co. KG. The compared calcipotriol 50 μg/g containing cream is a product of LEO Pharma GmbH, Germany. Prior to the initiation of the study, ethical approval was obtained from the Ethics Committee of the Charité – Universitätsmedizin Berlin. Written informed consent of each participant was obtained prior to inclusion to the study. Figure 1 shows an overview of the conducted noninvasive in vivo investigations and the clinical trial study design.

Analysis of the Radical Formation in vivo

Using electron paramagnetic resonance (EPR) spectroscopy (L-band EPR spectrometer [LBM MT 03; Magnettech, Berlin, Germany]), free radicals were detected in the skin of 10 healthy female volunteers aged between 24 and 56 years [39, 40]. The measurements of radical formation in vivo describe the ability of substances to neutralize free radicals before they can develop their damaging effects [41, 42]. Since the experiments were carried out in healthy volunteers, radical formation in the skin was stimulated by exposing a skin area of 1.4 cm^2 in size on the forearm of the volunteers to visible and infrared radiation. The irradiation was carried out for 10 min at 120 mW/cm^2. In order to detect the short-lived, radiation-induced radicals, a stable radical was applied to the skin as a marker (PCA). Using EPR spectroscopy, its intensity was measured prior to and during the irradiation at intervals of 2 min, each. The short-lived radicals recombine with the marker, while the signal is becoming weaker during the irradiation. The measurements were taken in a dual mode. These investigations were carried out in 10 volunteers. The radical formation was measured prior to and 4 weeks after application of the cream twice daily. The untreated arm served as a control.

Investigation of the Skin Barrier and Skin Surface Structure by Laser Scanning Microscopy and Transepidermal Water Loss

Ten healthy subjects, 1 male and 9 females, aged between 25 and 62 years were instructed to apply the herbal oil cream to one of their forearms twice a day for a period of 4 weeks. The formation of the barrier film was investigated by transepidermal water loss (TEWL) and laser scanning microscopy (LSM) measurements the day before and 4 weeks after the application of the cream [43–45]. The TEWL measurements were carried out with a Tewameter TM 300 (Courage + Khazaka, Cologne, Germany). All volunteers were seated and asked to relax for at least 30 min prior to initiation of TEWL measurements in order to acclimatize to the standardized conditions in the laboratory of 20 ± 2°C and 50 ± 10% relative humidity.

An in vivo LSM (VivaScope 1500 Multilaser, MAVIG GmbH, Munich, Germany) was used to visualize the corneocytes representing the skin barrier. The VivaScope 1500 is a reflectance confocal LSM, which utilizes a wavelength of 488 nm for the skin structures. Changes regarding the LSM skin barrier properties at the second measurement time point were evaluated by 3 independent dermatologists using an assessment score for both arms as follows:

−1 = Deterioration
0 = Unchanged
1 = Slight improvement
2 = Clear improvement

The control investigations were performed on the untreated forearm of the volunteers.

Penetration Investigations

Six healthy female subjects aged between 28 and 47 years were enrolled in the investigations of the penetration depth of the herbal oil cream in healthy and barrier-disturbed skin. The penetration of the topically applied formulations into the skin was investigated [46–48] using a confocal Raman microscope (River Diagnostics, Model 3510, NL). The analysis was performed in the fingerprint region (400–2,000 cm^{-1}). The Raman spectra were evaluated using the Nonrestricted-Multiple-Least-Square-Fit method.

The analysis was carried out to determine whether the cream is localized exclusively on the skin surface and in the uppermost layers of the stratum corneum or whether it penetrates through the skin barrier. These investigations were carried out in 6 healthy volunteers on 2 skin sites: one was a healthy skin area, and on the other skin site tape stripping was performed 10 times prior to analysis. The tape-stripping procedure reduces the upper epidermal layers of the skin and therefore serves as a model for a disturbed skin barrier.

To exclude an influence by a depth-dependent signal amplification, which may occur due to absorption and scattering of light in the skin, the Raman peaks were standardized to keratin that was postulated to be homogeneously distributed in the stratum corneum. The utilized Raman microscope was discussed in detail elsewhere [48].

Clinical Study

The clinical study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committees of the Tehran University of Medical Sciences (TUMS) and by the Iranian Ministry of Health, and listed in the Iranian Registry for clinical trials with the number IRCT201205209800N1 and the WHO-International Clinical Trials Registry Platform. A written informed consent was obtained for all patients in Persian. Statistical analysis was performed by Pharmalog, Institut für Klinische Forschung (Munich, Germany).

The herbal oil cream along with a 0.05% calcipotriol-containing cream and vehicle was applied, and its effect on the PASI was evaluated in patients suffering from psoriasis at the Dermatology Center of the Imam Hospital, Tehran University of Medical Sciences in Tehran, Iran. Participating subjects were included by the following criteria:

- Mild to moderate plaque psoriasis with an affected body surface area below 10%.
- Willingness to participate in this study.
- Ability to read, understand, and sign the informed consent form.

The exclusion criteria were as follows:

- Subjects treated with any systemic and/or topical medication and/or phototherapy in the last 4 weeks before inclusion into this study.
- Patients treated with medicines, which are known to potentially trigger psoriasis including β-blockers, lithium, and antimalarial drugs.
- Subjects with a hypersensitivity to creams.
- Women who were pregnant, breastfeeding, or have the intention of becoming pregnant within the next 12 weeks.
- Subjects younger than 18 years old.
- Patients suffering from other types of psoriasis such as pustular, palmoplantar, erythrodermic, and generalized cases in addition to the subjects with head and neck dermal signs and symptoms of disease.
- Patients with diagnosed chronic disease including but not restricted to any kind of cancer, HIV-AIDS, and any kind of viral and/or bacterial dermatological disease and eczema.
- Any kind of previous dermal disease subsequent to systemic disease such as SLE, dermatomyositis, etc.

Patients who met the inclusion criteria were randomized at a 1:1:1 ratio with a block size of 3 to receive the placebo, the herbal oil cream, or the calcipotriol cream according to a preplanned

computer-generated randomization table (groups A, B, and C). The subjects' identification was accomplished via a unique subject number.

The herbal oil cream and the vehicle were formulated and produced by C.P.M. ContractPharma GmbH & Co. KG, Aenova Group, Germany, with technical support (referring to the stability of both creams) of the Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran. The mixture of herbal oils described above, the calcipotriol 50 μg/g cream, and the vehicle cream were filled into 100 g tubes each. All 3 study medications were indistinguishable. The blinding of all 3 study medications was performed by C.P.M. ContractPharma GmbH & Co. KG, Aenova Group.

Study medications and placebo delivered to researchers were labeled with the randomization code. The codes were kept at the C.P.M. ContractPharma GmbH & Co. KG, Germany. The randomization list was prepared by a specialized CRO (Temmler Werke GmbH/Metronomia, Munich, Germany) and provided to C.P.M. A monitoring procedure assured that the randomization sequence was followed.

The randomization was performed using a random table provided to the study center, obtained using the Microsoft Excel 2010 RND function. All eligible subjects were randomly assigned to the following 3 groups A, B, or C:

Group A: The vehicle group, topical application of the vehicle cream composed as described above.

Group B: The herbal cream group, topical application of the herbal oil cream as described above.

Group C: The calcipotriol group, topical application of a cream containing 50 μg/g calcipotriol.

The patients were instructed to apply the medication to the affected skin twice daily for 12 consecutive weeks and were instructed not to use any emollient during the study. The instructions of cream application were given to the subjects by the investigators at the baseline visit.

The subjects were treated for 12 weeks and evaluated before the treatment and 2, 4, 8, and 12 weeks after initiation of the topical treatment. The primary efficacy endpoint was assessed using the PASI score (Psoriasis Area and Severity Index). The PASI score combines the extent of psoriasis with local skin signs (erythema, scale, and induration). The efficacy outcome was the change in PASI score from baseline to the measurement at week 12. Additional primary efficacy variable was the percentage of improvement in overall PASI scores over time by the treatment groups. The secondary efficacy endpoints were the number of patients who reached PASI 50 (>50% reduction of the PASI score from the start) and the number of patients who reached PASI 75 (>75% reduction of the PASI score from the start) at any time during the study.

Subjects complied with the study procedure in order to use creams twice daily to the affected areas of the body as per investigator instruction. Careful drug accountability has been performed, showing that all study products have been administered for a similar number of days and in a similar amount of cream. For concise evaluation of patients' adherence to treatment plan, the creams were weighed in each visit as a surrogate of compliance.

Safety was assessed for adverse drug events during 12 weeks and recorded in the specific form. Adverse events (AE) were divided into serious AE including a life-threatening experience and hospitalization and nonserious AE, which scored as mild, moderate, and severe. The definition of these categories was as follows:

Mild – no or transient symptoms, no interference with subject's daily activities.

Moderate – marked symptoms, moderate interference with subject's daily activities.

Severe – considerable interference with subject's daily activities.

Statistical Analysis

The sample size calculation was based on the comparison of mean PASI after 12 weeks of treatment. The following sample size calculation was based on the test on superiority of the herbal oil cream compared to vehicle in the mean PASI score after 12 weeks of treatment. The following parameter assumptions were used for the sample size calculation:

- Superiority margin (the difference in PASI which were assumed clinically significant): 1 score point,
- Type I error (α) = 0.05 (two-sided significant level of statistical test),
- Type II error (β) = 0.2, corresponding to a power of 80%, and
- Standard deviation: $S^1 = 1.2$ score points (vehicle) and $S^2 = 2.1$ score points (herbal oil cream).

With these parameters, the sample size has been calculated as 43 patients per treatment group for the evaluation of the primary endpoint with regard to the vehicle comparison of the herbal oil cream. Using the assumption that the herbal oil cream is at least noninferior to calcipotriol, with the possibility to show superiority, if noninferiority was shown, 45 subjects per treatment group were needed to be included in the study for the evaluation of the primary endpoint with regard to noninferiority comparison of the herbal oil cream to calcipotriol.

All statistical analyses were conducted using a two-sided test and a 5% significance level, unless otherwise stated. The analysis of efficacy outcome parameters was performed on the Efficacy Analysis Set. All patients who received at least 1 dose of any study treatment were included in the safety analysis.

Test on superiority of the herbal oil cream compared to vehicle using the analysis of covariance with the overall PASI score on week 12 as dependent variable, baseline as covariate, and treatment as effect. Testing on noninferiority was conducted with a noninferiority border 0.5 score points (=50% of the treatment difference that should be detected in the superiority test vs. vehicle according to sample size estimation) of the herbal oil cream compared to calcipotriol using the analysis of covariance with the overall PASI on week 12 as dependent variable, baseline as covariate, and treatment as effect.

Results

EPR Measurements of the Radical Formation in vivo

At the first measurement visits V1 – prior to the skin treatment – the investigations using EPR spectroscopy disclosed a significantly higher radical formation by visible and infrared irradiation in the untreated control arm than the verum treated arm. Therefore, the data were standardized to the initial results of V1 (V2–V1). Figure 2 illustrates the radical formation mean value of the 10 vol-

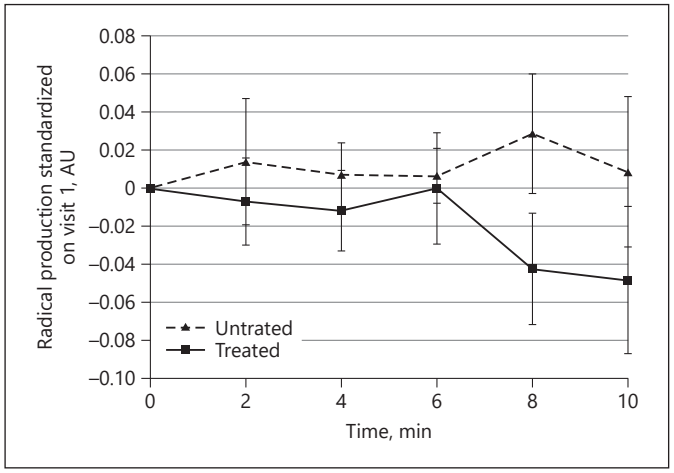

Fig. 2. Mean radical formation measured by EPR (n = 10) after 4 weeks of topical treatment twice daily with the herbal oil cream compared to untreated skin normalized to baseline values prior to treatment. Whiskers display the SD. EPR, electron paramagnetic resonance; SD, standard deviation.

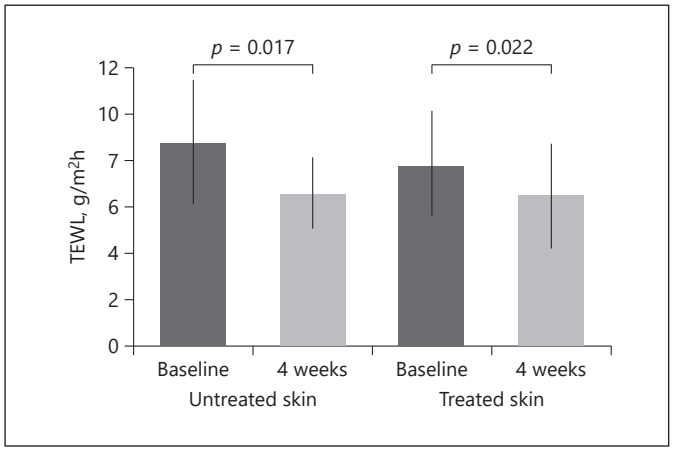

Fig. 3. Mean TEWL in 10 volunteers (absolute values) on the volar forearm in untreated skin and skin treated with the herbal oil cream prior to (baseline) and after 4 weeks of treatment twice daily (4 weeks). Whiskers represent the SD. TEWL, transepidermal water loss; SD, standard deviation.

unteers with standard deviation standardized to the baseline value prior to treatment, for the treated and untreated arms. The control arm exhibits an enhanced radical formation from V1 to V2, whereas the verum arm shows no changes, but rather a reduced radical formation.

Determination of the Transepidermal Water Loss

Regarding the evaluation of the skin barrier properties, the TEWL values were determined. The TEWL values of the treated and untreated arms declined significantly from visit 1 (V1) to visit 2 (V2) as it can be seen in Figure 3 (p = 0.017 and p = 0.022, respectively), indicating no significant differences between treated and untreated skin although an occlusion effect would be expected.

Table 1. Mean score of changes in the skin surface structure from visit 1 (baseline measurement before treatment) to visit 2 (after 4 weeks of treatment with the herbal oil cream twice daily) in both untreated and treated skin of the volar forearm, as rated by 3 independent dermatologists: –1 = deterioration, 0 = unchanged, 1 = slight improvement, 2 = clear improvement

Volunteer	Skin treated with the herbal oil cream	Untreated skin
	mean score of 3 measurement sites	mean score of 3 measurement sites
1	1.00	0.00
2	1.00	–0.67
3	0.67	–0.17
4	1.33	–0.33
5	2.00	0.67
6	1.67	0.67
7	0.00	0.17
8	0.67	1.00
9	1.83	0.00
10	3.00	1.17
Mean score of all volunteers	**1.32**	**0.25**
SD	0.80	0.57

Evaluation of the Skin Structure Analysis and the Barrier Function by in vivo LSM

The integrity of the skin barrier was determined by LSM. This method allows the analysis of the structure of the skin surface and possible barrier disruptions. The uppermost layer consists of corneocytes. An intact stratum corneum is characterized by a relatively uniform honeycomb-like structure. A disrupted skin barrier can show discontinuity of cell structures, bumpiness, clusters of cells, or crater-like structures within the stratum corneum.

Two representative LSM images of a volunteer before and after 4 weeks of daily treatment are shown in Figure 4. The images of all 10 volunteers were evaluated for their surface structure by 3 dermatologists, and a score was developed to assess the development from visit 1 to visit 2 for both arms (Table 1).

The score was averaged, connected to SPSS, and verified using the Wilcoxon test. For the treated arm, a mean score of 1.32 was determined, while the control arm ex-

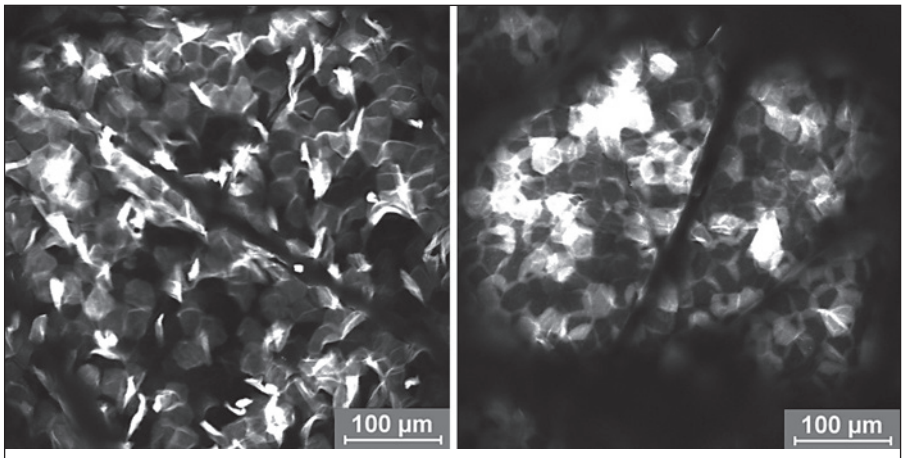

Fig. 4. Exemplary LSM image of one subject prior to and after 4 weeks of treatment with the herbal oil ointment. LSM, laser scanning microscopy.

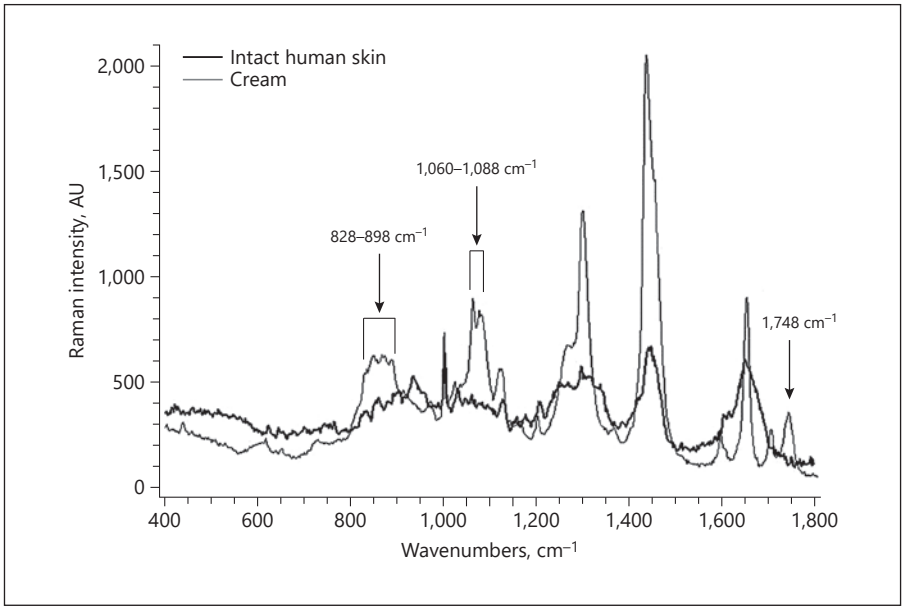

Fig. 5. Fingerprint Raman spectra of intact human skin (black line) and the herbal oil cream (gray line).

hibited a mean score of 0.25. The difference of 1.07 is significant ($p < 0.05$). Consequently, the treated arm had shown a significant improvement compared to the non-treated arm.

Penetration Investigations by in vivo Raman Spectroscopy

The penetration of the cream into the skin was measured in vivo. Figure 5 shows the spectra of the untreated human skin and the cream in the fingerprint region. The major differences in the spectra are marked by arrows. The cream application influences the intensity and shape of the Raman bands of the skin. These depth-dependent changes correlate with the cream that penetrated into the skin. Using the Nonrestricted-Multiple-Least-Square-Fit method, the measured Raman spectrum of the treated skin was compared to the known Raman spectra of the components of the stratum corneum, that is, of keratin, urea, ceramide, and cholesterol and for the applied cream in order to determine the penetration depth of the cream. As can be seen from Figure 5, the Raman spectra are superimposed with the Raman spectrum of the skin, except for the peak at 1,748 cm^{-1}. This band in the skin directly correlates with the cream penetrated into the skin.

The investigations were conducted on both healthy skin and skin damaged by tape stripping, the latter cor-

Table 2. Patient enrollment and follow-up of the prospective, controlled, randomized double-blind study

	Herbal oil cream		Calcipotriol		Vehicle		Total	
	N	%	*N*	%	*N*	%	*N*	%
Patients enrolled	45	100	45	100	45	100	135	100
Safety evaluable population*	45	100	45	100	45	100	135	100
Efficacy analysis population**	34	75.6	38	84.4	38	84.4	110	81.5
Patients discontinued	11	24.4	7	15.6	7	15.6	25	18.5
Primary reason for discontinuation								
Adverse drug reactions	4	8.9	3	6.7	2	4.4	9	6.7
Lost to follow-up	5	11.1	2	4.4	2	4.4	9	6.7
Other	2	4.4	2	4.4	3	6.7	7	5.2

* Safety analysis has been conducted in all patients who received at least 1 dose of study treatment. ** Received at least 8 weeks of treatment.

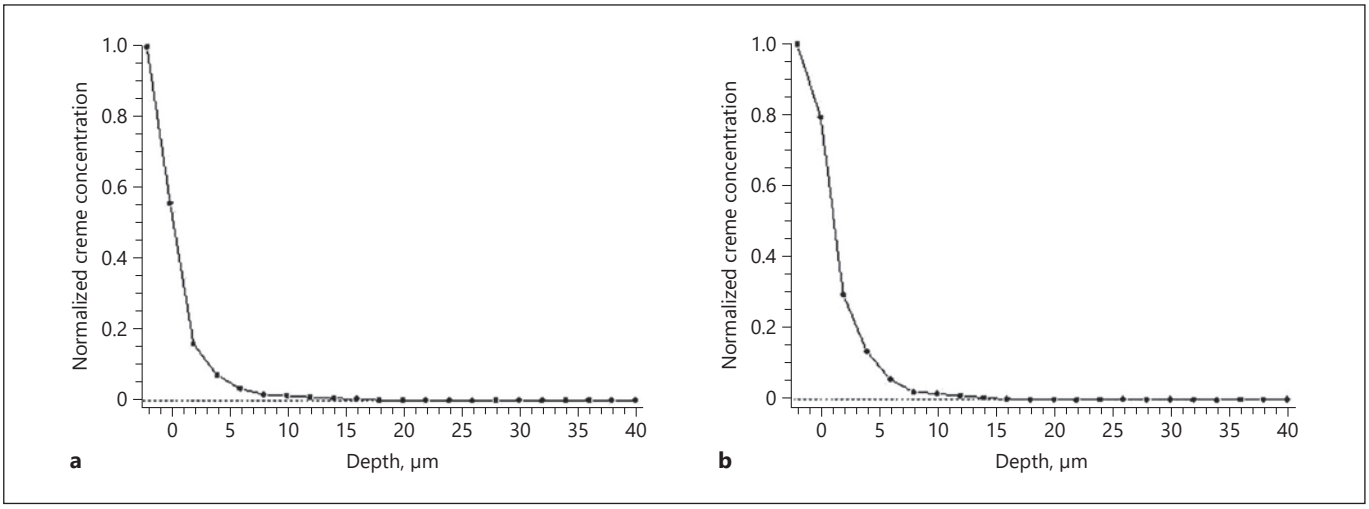

Fig. 6. Typical penetration profiles of the herbal oil cream in intact (**a**) and barrier-disturbed (after 10 tape strips) (**b**) skin. The MV of the penetration depths are 17.0 ± 1.7 µm in intact skin and 14.7 ± 1.6 µm in barrier-disturbed skin. MV, mean value.

responding more closely to the state of psoriatic skin. The penetration depths were measured in vivo using the Non-restricted-Multiple-Least-Square-Fit method for the pre-treated intact skin and for the skin that had been previously damaged by tape stripping. Here, it has to be considered that parts of the stratum corneum were removed by the 10 tape strips. An analysis of the water distribution in the skin in the wavelength range from 2,000 to 4,000 cm^{-1} permits to determine the thickness of the stratum corneum, which is, on average, 21.2 ± 2.8 µm for the intact skin and 17.0 ± 1.9 µm for the skin damaged by tape stripping, which is in accordance to published data.

Two typical penetration profiles are shown in Figure 6a (cream on intact skin) and Figure 6b (cream on barrier-disturbed skin). It is clearly visible that the cream is located in the uppermost layers of the stratum corneum generating a protective effect. The mean values of the penetration depth are 17.0 ± 1.7 µm for the intact skin and 14.7 ± 1.6 µm for the barrier-disturbed skin.

This distribution in the stratum corneum ensures that the cream does not reach the viable cells, neither in the case of the intact skin nor in the case of the barrier-disturbed skin. The cream forms a protective layer on the skin, and it is also ensured that it penetrates deeply enough into the stratum corneum to be not easily removed by sweating or fabrics. As a result, the protective efficacy of the cream in the skin might last longer.

Table 3. Number of patients who reached PASI 50 and PASI 75 at any time point during the study in the 3 treatment groups

	Herbal oil cream (*N* = 34), *N*	Calcipotriol (*N* = 38), %	Vehicle (*N* = 38), *N*	*p* value, %	*N*	%	Herbal oil cream vs. calcipotriol	Herbal oil cream vs. vehicle
PASI 50 reached								
Number of patients who reached PASI 50 at any time during the study								
Yes	19	55.9	18	47.4	23	60.5	0.47	0.69
No	15	44.1	20	52.6	15	39.5		
Total	34	100.0	38	100.0	38	100		
PASI 75 reached								
Number of patients who reached PASI 75 at any time during the study								
Yes	10	29.4	6	15.8	13	34.2	0.16	0.66
No	24	70.6	32	84.2	25	65.8		
Total	34	100.0	38	100.0	38	100.0		

PASI, Psoriasis Area and Severity Index.

Results of the Randomized, Controlled, Double-Blind Clinical Study

A total of 135 subjects were recruited in the study, with 45 subjects in each treatment group. Following randomization, 110 patients received at least 8 weeks of daily treatment with the study creams and constituted the efficacy analysis population (34 subjects in the herbal oil cream group, 38 subjects in the calcipotriol group, and 38 in the vehicle group). Twenty-five subjects discontinued the study treatment at different times before week 12. Eleven patients in the herbal cream group were discontinued due to AE (4 subjects), incompliance (5 subjects), and other reasons (2 subjects). Seven patients in the calcipotriol group discontinued the study due to AE (3 subjects), incompliance (2 subjects), and other reasons (2 subjects), as well as 7 patients in the vehicle group due to AE (2 subjects), incompliance (2 subjects), and other reasons (3 subjects) (Table 2).

The primary efficacy analysis showed a statistically significant higher efficacy of the herbal oil cream than calcipotriol on the reduction of the PASI score at week 12 ($p = 0.016$, 95% CI −1.11 to −0.12) (Fig. 7). In the herbal oil cream group, the mean baseline PASI score of approximately 3 reduced to 1.3. Topical calcipotriol leads to a reduction of the mean PASI to 2.2 after 12 weeks of treatment. Nevertheless, the superiority of the herbal oil cream over the vehicle could not be shown.

The number of subjects who reached PASI 50 (>50% reduction of the PASI) and PASI 75 (>75% reduction of the PASI) at any time during the study showed that the herbal oil cream is not significantly inferior to calcipotriol, but again not superior of the vehicle containing bitter almond oil, as shown in Table 3. Here, no significant differences were detected between the 3 treatment arms.

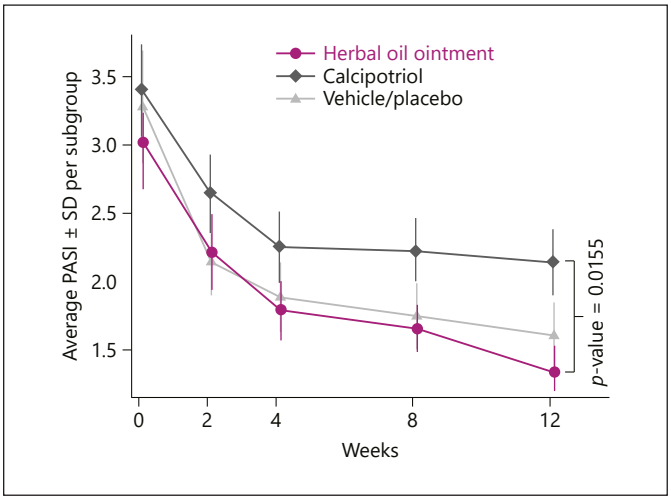

Fig. 7. Reduction of the mean PASI score with SD (whiskers) in the 3 treatment arms over 12 weeks. PASI, Psoriasis Area and Severity Index; SD, standard deviation.

Additional analyses with exploratory purposes have been performed. The analysis of the single components of the PASI score showed a significant improvement of erythema and desquamation on the legs ($p = 0.015$ and $p = 0.004$, respectively) after application of the herbal oil cream compared to calcipotriol.

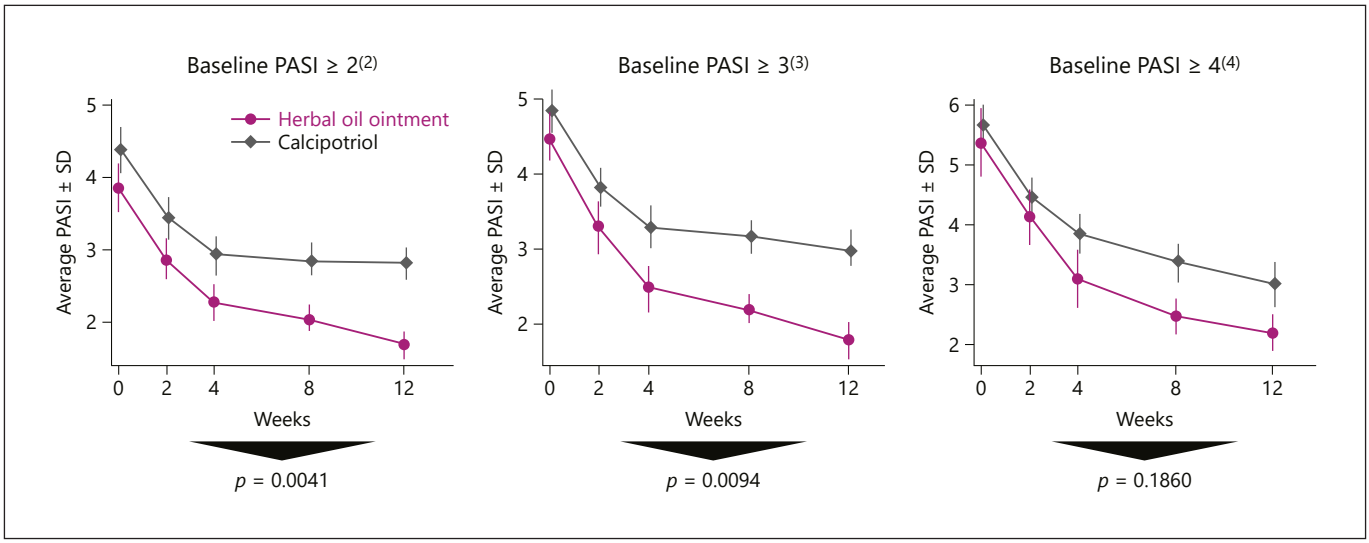

Fig. 8. Reduction of the mean PASI score from baseline to week 12 in patients treated with calcipotriol and the herbal oil cream with PASI ≥2, PASI ≥3, and PASI ≥4 at baseline. PASI, Psoriasis Area and Severity Index; SD, standard deviation.

Table 4. Observed ADE within the 3 treatment groups

AE	Herbal oil cream (*N* = 45)		Calcipotriol (*N* = 45)		Vehicle (*N* = 45)		Total (*N* = 135)	
	ADE	patients, *n* (%)	ADE	patients, *n* (%)	ADE	patients, *n* (%)	ADE	patients, *n* (%)
Erythema	3	3 (6.7)	4	4 (8.9)			7	7 (5.2)
Pruritus	4	4 (8.9)	3	3 (6.7)	3	3 (6.7)	10	10 (7.4)
Skin burning sensation	1	1 (2.2)	3	3 (6.7)	2	2 (4.4)	6	6 (4.4)
Skin exfoliation	2	2 (4.4)	1	1 (2.2)			3	3 (2.2)
Skin edema	1	1 (2.2)	3	3 (6.7)			4	4 (3.0)

ADE, adverse drug events; AE, adverse event.

Time to achieve response to PASI 50 and PASI 75 was calculated as the time from enrolment to the first documentation of PASI 50 and PASI 75. Here, the 3 groups showed a similar efficacy (log-rank test, $p = 0.47$ and $p = 0.09$).

Subgroup analyses on reduction of the PASI score at week 12, in patients with a PASI ≥2, PASI ≥3, and PASI >4 at baseline are presented in Figure 8. In patients with PASI ≥2 and PASI ≥3, there is a significant improvement after treatment with the herbal oil cream compared to calcipotriol ($p = 0.004$ and 0.009, respectively). In patients with PASI ≥4, the improvement of PASI score with the herbal oil cream compared to calcipotriol showed a reduced superiority of the herbal oil cream ($p = 0.186$), probably due to the small number of patients in this subgroup.

Although the sample size is low in the subgroup with PASI ≥4, there is an improvement after application of the herbal oil cream compared to placebo ($p = 0.083$) regarding the reduction of overall PASI score from baseline to week 12. The analysis in PASI >1 and PASI >5 subgroups has not been considered due to the small number of subjects in these groups. The subgroup analysis comparing PASI <10 to PASI >10 patients was not conducted, as no patient with a PASI >10 at baseline was included in the study.

The observed AE are presented in Table 4. Eleven patients experienced AE: 4 (8.9%) of the 45 subjects in the herbal oil group, 4 (8.9%) of the 45 patients in the calci-

potriol group, and 3 (6.7%) of the 45 subjects in the vehicle group. Thirty AE have been reported and all recovered.

All AE were considered possibly related by the investigator, and most of the patients with AE (9/11) discontinued treatment. The number and type of AE were comparable between the 3 groups.

Discussion

Using natural oils containing a high amount of antioxidants, a positive effect after topical treatment could be shown in psoriatic patients. Due to the radical formation during the psoriatic inflammatory processes and resulting oxidative stress [49, 50], it can be assumed that these free radicals are at least partially neutralized by the daily application of the herbal oil cream as topical antioxidants, which may enhance the healing process of the lesional skin more effectively.

Furthermore, a positive effect on the skin barrier could be found by LSM, but not by TEWL measurements, although at least an occlusion effect would have been expected. It has to be stated that TEWL measurements are very sensitive to external factors, such as humidity and even subject-related factors, such as excitement, sweating, and speaking so that occlusion effects or other influence factors can be pronounced rapidly [51]. The LSM measurements showed an improved skin barrier structure in untreated skin, which is most probably to enhanced air humidity, showing the importance of external factors, which are not only applicable in healthy, but especially in psoriatic skin, which is known to improve during the summer months compared to colder weather conditions.

All measurements of the RPF, skin barrier, and penetration depths were conducted regarding the herbal oil cream. Further studies should include a comparison with topical calcipotriol or the vehicle. All measurements were conducted in healthy skin, which could be extended and compared to psoriatic or inflammatory skin in future studies.

The enrolled subjects showed very low PASI scores at baseline, since they were only eligible without systemic treatment. Therefore, the improvement of the PASI score is very limited to a few points. In addition to that, the PASI score itself always shows a subjective variation. Given the low clinical PASI scores at baseline and during follow-up in this study, changes in the PASI score can be very investigator-dependent.

Emollients, moisturizers, and keratolytic agents are essential in the topical treatment of psoriasis. They are adjuvants for classic treatments and help to reduce the scaling and dryness of individual patients. The major role for emollients and moisturizers is the supportive role in normalizing hyperproliferation, differentiation, and apoptosis; furthermore, they exert anti-inflammatory effects, for example, through physiological lipids. Subsequently, an improved barrier function and stratum corneum hydration make the epidermis more resistant to external stressors and reduce the induction of Koebner phenomena [52].

In healthy as well as psoriatic skin, a topical treatment may induce AE. It turned out that such events occur to a small extent when applying both calcipotriol and the herbal oil cream. In this context, it is interesting that the different creams also promote different skin irritations. However, adding up these AEs, their percentage is the same for both creams. Safety was comparable between treatment groups, all reported AEs were local, and patients completely recovered. No death or serious AEs were observed during the study period. The number of AEs during the treatment with the herbal oil cream might be, among others, due to allergic reactions or cross-reactions with the herbal components. Nevertheless, no irreversible reactions were observed, while topical corticosteroids can lead to irreversible skin atrophy after daily application for several weeks. Therefore, corticosteroids are not intended to be used for a long period of time, while topical application of herbal oils cream can provide a daily long-term treatment option.

In a study conducted by Ramsay et al. [53], lesional and perilesional irritation was reported in 25% of patients who received calcipotriol for 12 months. Asymptomatic hypercalcemia and hypercalciuria were developed in some patients who treated with the higher doses of calcipotriol [54]. In comparison with our study, calcipotriol was more tolerable than in these studies. Vitamin D analogs are recommended to be used on a maximum of 30% of the body surface, while the use of topical herbal oils cream is not restricted.

Overall, the efficacy analysis demonstrated that the investigated cream containing a combination of various herbal oils is not inferior to calcipotriol and showed a statistically improved outcome compared to calcipotriol in reducing the PASI score at week 12, which was the primary end point for this study. Although different from the expected results, the superiority of the herbal oil cream over the vehicle containing bitter almond oil could not be shown. In this study, the vehicle has shown

an effective activity similar to the other 2 treatment agents, which is certainly due to the composition of the vehicle. In order to ensure blinding, the vehicle cream contained the vehicle base of the herbal oil cream excluding the majority of herbal oils, but contained additional bitter almond oil to recreate the same cream scent as the verum cream. Having antioxidant effects, bitter almond oil could lead to anti-inflammatory changes and has skin-moisturizing effects. In addition to that, the vehicle cream contained, among others, middle-chain triglycerides, C = capric acid, lauric acid, and myristic acid, which are known as transdermal penetration enhancers, and most of the esters of myristic acid are used as skin-conditioning agents in many types of cosmetics in a range of concentrations [55]. Among other lipids, free fatty acids are required for permeability barrier homeostasis in the stratum corneum [56–58]. Based on this knowledge, a therapeutic effect of the middle-chain triglycerides contained in the vehicle cannot be excluded. In fact, most of these components have emollient effects, which should improve clinical manifestations of the mild psoriasis. For further and more detailed analysis of the clinical improvement, in addition to the PASI, histological evaluations could be useful in future studies, including the assessment of inflammatory psoriatic biomarkers of improvement, which were not applied in the present study.

Here, the investigated naturally derived emollient composed of various herbal oils was assessed for the first time in a clinical study setting and was designed in a small scale. Therefore, the small sample size could also be considered as another limitation, favoring the conduction of a bigger trial to confirm the obtained results. The results show the benefit of further studies involving the effects of alternative topical agents and antioxidative components exhibiting a high radical protection factor in inflammatory skin diseases.

Conclusion

The topical application of a cream containing various herbal oils with antioxidant properties showed a significantly higher efficacy than calcipotriol regarding the reduction of the PASI score at week 12. Therefore, it can be used for the long-term treatment in psoriatic patients as an effective alternative to current standard therapies including calcipotriol.

Acknowledgment

In memoriam of Nader Nahavandi, the authors thank him for his contributions and extensive general support during the study.

Statement of Ethics

The noninvasive in vivo measurements in healthy volunteers was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Committee of the Charité – Universitätsmedizin Berlin (Decision reference number: EA1/230/18 and EA1/58/14). A written informed consent was obtained from all participating subjects before inclusion to the study. The clinical study in psoriasis patients was conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committees of the Tehran University of Medical Sciences (TUMS) and by the Iranian Ministry of Health, and listed in the Iranian Registry for clinical trials with the number IRCT201205209800N1 and the WHO-International Clinical Trials Registry Platform. A written informed consent was obtained for all patients in Persian.

Funding Sources

The study was financially supported by Alnapharm AG & Co. KG, Hamburg, Germany.

Conflict of Interest

The coauthors Ali Nahavandi, Axel Ahlers, Fatemeh Zibakalam-Mofrad, Barbara Brower, Maryam Nahavandi, and Frank Feddern are employed by Alnapharm AG & Co. KG, Hamburg, Germany.

Author Contributions

J.L., A.N., A.A., F.Z., B.B., M.N., and F.F. designed the study; P.M., S.S.R., M.R., M.D., S.S., H.R., M.M., and S.F. conducted the research; J.L., M.D., P.M., A.A., A.N., B.B., F.F., S.J., and M.C. analyzed the data; J.L., A.N., A.A., B.B., F.F., M.D., S.F., M.I., and S.J. wrote the paper; and J.L., A.N., A.A., B.B., F.F., M.D., and S.J. are responsible for the final content. All listed authors of the study read and approved the content of the final manuscript.

Data Availability Statement

All datasets of the conducted investigations of this study are available upon request.

References

1 Griffiths CE, Barker JN. Pathogenesis and clinical features of psoriasis. Lancet. 2007; 370(9583):263–71.
2 Nestle FO, Kaplan DH, Barker J. Psoriasis. N Engl J Med. 2009;361(5):496–509.
3 Mrowietz U, Reich K. Psoriasis: new insights into pathogenesis and treatment. Dtsch Arztebl Int. 2009;106(1–2):11–9.
4 Murphy G, Reich K. In touch with psoriasis: topical treatments and current guidelines. J Eur Acad Dermatol Venereol. 2011;25(Suppl 4):3–8.
5 Belinchon I, Rivera R, Blanch C, Comellas M, Lizan L. Adherence, satisfaction and preferences for treatment in patients with psoriasis in the European Union: a systematic review of the literature. Patient Prefer Adherence. 2016; 10:2357–67.
6 Farzanfar D, Dowlati Y, French LE, Lowes MA, Alavi A. Inflammation: a contributor to depressive comorbidity in inflammatory skin disease. Skin Pharmacol Physiol. 2018;31(5): 246–51.
7 Tohid H, Aleem D, Jackson C. Major depression and psoriasis: a psychodermatological phenomenon. Skin Pharmacol Physiol. 2016; 29(4):220–30.
8 Naldi L. Epidemiology of psoriasis. Curr Drug Targets Inflamm Allergy. 2004;3(2): 121–8.
9 Gelfand JM, Weinstein R, Porter SB, Neimann AL, Berlin JA, Margolis DJ. Prevalence and treatment of psoriasis in the United Kingdom: a population-based study. Arch Dermatol. 2005;141(12):1537–41.
10 Griffiths CEM, Armstrong AW, Gudjonsson JE, Barker JNWN. Psoriasis. Lancet. 2021; 397(10281):1301–15.
11 Rendon A, Schäkel K. Psoriasis pathogenesis and treatment. Int J Mol Sci. 2019;20(6).
12 Martins AM, Ascenso A, Ribeiro HM, Marto J. Current and future therapies for psoriasis with a focus on serotonergic drugs. Mol Neurobiol. 2020;57(5):2391–19.
13 Nast A, Boehncke WH, Mrowietz U, Ockenfels HM, Philipp S, Reich K, et al. S3 – Guidelines on the treatment of psoriasis vulgaris (English version). Update. J Dtsch Dermatol Ges. 2012;10(Suppl 2):S1–95.
14 Smith J, Cline A, Feldman SR. Advances in psoriasis. South Med J. 2017;110(1):65–75.
15 Rapalli VK, Singhvi G, Dubey SK, Gupta G, Chellappan DK, Dua K. Emerging landscape in psoriasis management: from topical application to targeting biomolecules. Biomed Pharmacother. 2018;106:707–13.
16 Segaert S, Calzavara-Pinton P, de la Cueva P, Jalili A, Lons Danic D, Pink AE, et al. Long-term topical management of psoriasis: the road ahead. J Dermatolog Treat. 2020:1–10.
17 Stein Gold LF. Topical therapies for psoriasis: improving management strategies and patient adherence. Semin Cutan Med Surg. 2016;35(2 Suppl 2):S36–44; quiz S5.
18 Menter A, Korman NJ, Elmets CA, Feldman SR, Gelfand JM, Gordon KB, et al. Guidelines of care for the management of psoriasis and psoriatic arthritis. Section 3. Guidelines of care for the management and treatment of psoriasis with topical therapies. J Am Acad Dermatol. 2009;60(4):643–59.
19 Burden AD, Hilton Boon M, Leman J, Wilson H, Richmond R, Ormerod AD, et al. Diagnosis and management of psoriasis and psoriatic arthritis in adults: summary of SIGN guidance. BMJ. 2010;341:c5623.
20 National Institute for Health and Care Excellence: Clinical Guidelines. Psoriasis: assessment and management. London: Royal College of Physicians; 2012.
21 Mason J, Mason AR, Cork MJ. Topical preparations for the treatment of psoriasis: a systematic review. Br J Dermatol. 2002;146(3): 351–64.
22 Ashcroft DM, Po AL, Williams HC, Griffiths CE. Systematic review of comparative efficacy and tolerability of calcipotriol in treating chronic plaque psoriasis. BMJ. 2000; 320(7240):963–7.
23 Papp KA, Guenther L, Boyden B, Larsen FG, Harvima RJ, Guilhou JJ, et al. Early onset of action and efficacy of a combination of calcipotriene and betamethasone dipropionate in the treatment of psoriasis. J Am Acad Dermatol. 2003;48(1):48–54.
24 Mason AR, Mason J, Cork M, Dooley G, Edwards G. Topical treatments for chronic plaque psoriasis. Cochrane Database Syst Rev. 2009(2):CD005028.
25 Lebwohl M, Ting PT, Koo JY. Psoriasis treatment: traditional therapy. Ann Rheum Dis. 2005;64(Suppl 2):ii83–6.
26 Hoffmann J, Gendrisch F, Schempp CM, Wölfle U. New herbal biomedicines for the topical treatment of dermatological disorders. Biomedicines. 2020;8(2).
27 Bahraini P, Rajabi M, Mansouri P, Sarafian G, Chalangari R, Azizian Z. Turmeric tonic as a treatment in scalp psoriasis: a randomized placebo-control clinical trial. J Cosmet Dermatol. 2018;17(3):461–6.
28 Najafizadeh P, Hashemian F, Mansouri P, Farshi S, Surmaghi MS, Chalangari R. The evaluation of the clinical effect of topical St Johns wort (Hypericum perforatum L.) in plaque type psoriasis vulgaris: a pilot study. Australas J Dermatol. 2012;53(2):131–5.
29 Ahmad Z. The uses and properties of almond oil. Complement Ther Clin Pract. 2010;16(1): 10–2.
30 Man MQ, Ye L, Hu L, Jeong S, Elias PM, Lv C. Improvements in epidermal function prevent relapse of psoriasis: a self-controlled study. Clin Exp Dermatol. 2019;44(6):654–7.
31 Muller K. Antipsoriatic and proinflammatory action of anthralin. Implications for the role of oxygen radicals. Biochem Pharmacol. 1997; 53(9):1215–21.
32 Rocha-Pereira P, Santos-Silva A, Rebelo I, Figueiredo A, Quintanilha A, Teixeira F. The inflammatory response in mild and in severe psoriasis. Br J Dermatol. 2004;150(5):917–28.
33 Ishibashi T, Ichikawa M, Sato B, Shibata S, Hara Y, Naritomi Y, et al. Improvement of psoriasis-associated arthritis and skin lesions by treatment with molecular hydrogen: a report of three cases. Mol Med Rep. 2015;12(2): 2757–64.
34 Rashmi R, Rao KS, Basavaraj KH. A comprehensive review of biomarkers in psoriasis. Clin Exp Dermatol. 2009;34(6):658–63.
35 Lademann J, Vergou T, Darvin ME, Patzelt A, Meinke MC, Voit C, et al. Influence of topical, systemic and combined application of antioxidants on the barrier properties of the human skin. Skin Pharmacol Physiol. 2016; 29(1):41–6.
36 Plenkowska J, Gabig-Ciminska M, Mozolewski P. Oxidative stress as an important contributor to the pathogenesis of psoriasis. Int J Mol Sci. 2020;21(17).
37 Zhou Q, Mrowietz U, Rostami-Yazdi M. Oxidative stress in the pathogenesis of psoriasis. Free Radic Biol Med. 2009;47(7):891–905.
38 Cannavo SP, Riso G, Casciaro M, Di Salvo E, Gangemi S. Oxidative stress involvement in psoriasis: a systematic review. Free Radic Res. 2019;53(8):829–40.
39 Haag SF, Tscherch K, Arndt S, Kleemann A, Gersonde I, Lademann J, et al. Enhancement of skin radical scavenging activity and stratum corneum lipids after the application of a hyperforin-rich cream. Eur J Pharm Biopharm. 2014;86(2):227–33.
40 Meinke MC, Friedrich A, Tscherch K, Haag SF, Darvin ME, Vollert H, et al. Influence of dietary carotenoids on radical scavenging capacity of the skin and skin lipids. Eur J Pharm Biopharm. 2013;84(2):365–73.
41 Belikov AV, Schraven B, Simeoni L. T cells and reactive oxygen species. J Biomed Sci. 2015;22:85.
42 Syring F, Weigmann HJ, Schanzer S, Meinke MC, Knorr F, Lademann J. Investigation of model sunscreen formulations comparing the sun protection factor, the universal sun protection factor and the radical formation ratio. Skin Pharmacol Physiol. 2016;29(1):18–23.
43 Desmet CM, Danhier P, Acciardo S, Levêque P, Gallez B. Towards in vivo melanin radicals detection in melanomas by electron paramagnetic resonance (EPR) spectroscopy: a proof-of-concept study. Free Radic Res. 2019;53(4): 405–10.
44 Lademann J, Otberg N, Richter H, Meyer L, Audring H, Teichmann A, et al. Application of optical non-invasive methods in skin physiology: a comparison of laser scanning microscopy and optical coherent tomography with histological analysis. Skin research and technology. Off J Int Soc Bioeng Skin. 2007; 13(2):119–32.

45 Archid R, Duerr HP, Patzelt A, Philipp S, Röwert-Huber HJ, Ulrich M, et al. Relationship between histological and clinical course of psoriasis: a pilot investigation by reflectance confocal microscopy during Goeckerman treatment. Skin Pharmacol Physiol. 2016;29(1):47–54.
46 Vergou T, Schanzer S, Richter H, Pels R, Thiede G, Patzelt A, et al. Comparison between TEWL and laser scanning microscopy measurements for the in vivo characterization of the human epidermal barrier. J Biophotonics. 2012;5(2):152–8.
47 Choe C, Schleusener J, Lademann J, Darvin ME. Human skin in vivo has a higher skin barrier function than porcine skin ex vivo – comprehensive Raman microscopic study of the stratum corneum. J Biophotonics. 2018;11(6):e201700355.
48 Choe C, Schleusener J, Lademann J, Darvin ME. Age related depth profiles of human Stratum Corneum barrier-related molecular parameters by confocal Raman microscopy in vivo. Mech Ageing Dev. 2018;172:6–12.
49 Choe C, Lademann J, Darvin ME. Analysis of human and porcine skin in vivo/ex vivo for penetration of selected oils by confocal Raman microscopy. Skin Pharmacol Physiol. 2015;28(6):318–30.
50 Langley RG, Feldman SR, Nyirady J, van de Kerkhof P, Papavassilis C. The 5-point Investigator's Global Assessment (IGA) Scale: a modified tool for evaluating plaque psoriasis severity in clinical trials. J Dermatolog Treat. 2015;26(1):23–31.
51 Barel AO, Clarys P. Study of the stratum corneum barrier function by transepidermal water loss measurements: comparison between two commercial instruments: evaporimeter and tewameter. Skin Pharmacol. 1995;8(4):186–95.
52 Fluhr JW, Cavallotti C, Berardesca E. Emollients, moisturizers, and keratolytic agents in psoriasis. Clin Dermatol. 2008;26(4):380–6.
53 Ramsay CA, Berth-Jones J, Brundin G, Cunliffe WJ, Dubertret L, van de Kerkhof PC, et al. Long-term use of topical calcipotriol in chronic plaque psoriasis. Dermatology. 1994;189(3):260–4.
54 Bleiker TO, Bourke JF, Mumford R, Hutchinson PE. Long-term outcome of severe chronic plaque psoriasis following treatment with high-dose topical calcipotriol. Br J Dermatol. 1998;139(2):285–6.
55 Becker LC, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Marks JG Jr, et al. Final report of the amended safety assessment of myristic acid and its salts and esters as used in cosmetics. Int J Toxicol. 2010;29(4 Suppl):162S–86.
56 Man MM, Feingold KR, Thornfeldt CR, Elias PM. Optimization of physiological lipid mixtures for barrier repair. J Invest Dermatol. 1996;106(5):1096–101.
57 Feingold KR. The outer frontier: the importance of lipid metabolism in the skin. J Lipid Res. 2009;50(Suppl):S417–22.
58 Feingold KR. Thematic review series: skin lipids. The role of epidermal lipids in cutaneous permeability barrier homeostasis. J Lipid Res. 2007;48(12):2531–46.