

A Multi-Pronged Topical Anti-Ageing Approach Using Plant-Derived Arctiin and Oligopeptides Improves Signs of Advanced Skin Ageing

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Introduction

Due to genetically determined intrinsic and environment induced extrinsic ageing-processes the structure of the skin changes over time leading to alterations of its complex function. Particularly after menopause a degradation of dermal and epidermal textures as a consequence of decreasing ovarian production of estrogen becomes evident. Declining hormone levels, exacerbating both intrinsic and extrinsic skin ageing effects, contribute to the reduction of collagen and elastic fibres as well as matrix components. The water-holding capacity abates. Furthermore a decreased skin thickness, a flattening of the dermo-epidermal junction with a diminished papillary density and a reduced cell turnover can be noted thus resulting in dry, pale, and thinned skin with loss of elasticity and density and the appearance of wrinkles. The quest for active ingredients to reduce or overcome these postmenopausal problems yielded to the extraction of arctiin, a highly effective component of the burdock fruit, and apiaceae peptides derived from ans fruits and seeds.

Arctiin has been shown to stimulate collagen neogenesis and reduce the production of proinflammatory cytokines in vitro. Since chronic inflammation is accused of promoting the ageing process this effect is of crucial importance. Apiaceae peptides on the other hand stimulate the metabolic activity of fibroblasts in cell cultures contributing to the formation of dermal connective tissue.

To link the in vitro proven benefits to a clinically measurable effect we conducted several studies investigating the influence of two newly developed face care creams containing these two active ingredients (Eucerin® DermoDensifyer Day and Eucerin® DermoDensifyer Night) on biophysical parameters of skin ageing.

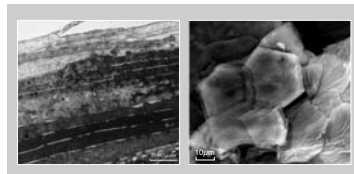
Material and Methods

Preparations: The O/W emulsions Eucerin® DermoDensifyer Day and Eucerin® DermoDensifyer Night containing arctiin and apiaceae peptides as active ingredients served as vera. Additionally, Eucerin® DermoDensifyer Day contains highly effective UVA and UVB filters with SPF 15 and Eucerin® DermoDensifyer Night dexpanthenol and vitamin E, respectively. The basis of the day cream without active ingredient (vehicle) was used as control.

Evaluation of facial wrinkle depth by PRIMOS method and expert grading: A total of 37 females between 51 and 67 years of age demonstrating facial wrinkles were enrolled in this blinded, single-center in-use study. After a five day preconditioning period, during which the use of any facial skin care product was prohibited, the test material Eucerin® DermoDensifyer Day was applied to the face twice daily over an eight-week period. Evaluation visits were conducted at baseline and after 4 and 8 weeks of treatment.

The intensity of facial wrinkles was assessed by PRIMOS [1] in the crow's feet region and by photo evaluation additionally on the forehead, in the nasolabial area, and around the mouth. For photo evaluation digital photographs of the face were taken on each visit. After blinding the classification of the pre- and post-treatment images was subsequently compared by 10 persons on the basis of a 9-point scale.

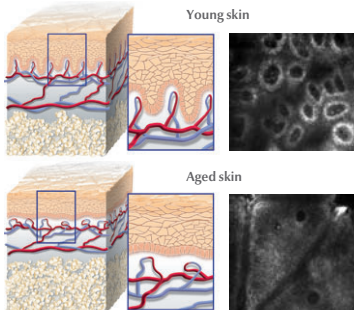
Evaluation of skin renewal rate by corneocyte image analysis: 35 female volunteers aged 57 to 70 were enrolled in this, in terms of test products blinded, randomized, single-center in-use-study. After a five day preconditioning period, during which the use of moisturising products was prohibited, the test products Eucerin® DermoDensifyer Day and Night were applied twice daily over an eight-week period to the inner forearm of each participant. An untreated area on the inner forearm served as control. Evaluation visits were conducted at baseline and after 4, 6 and 8 weeks of treatment.



Corneocyte size was assessed by means of image analysis of D-Squame® sheets [2]. A decrease in corneocyte size thereby indicates an improvement of the skin renewal rate.

Evaluation of epidermal thickness and papillary density: 32 volunteers ranging from 45 to 65 years of age with Fitzpatrick phototype II or III were enrolled in this blinded, vehicle controlled, single center in-use-study with semilateral randomization. The test product Eucerin® DermoDensifyer Day and the vehicle were applied twice daily for a total of 4 weeks, each to one of the inner forearms of every test person. The participants had to refrain from using any kind of skin care product on the test areas during a one week preconditioning period.

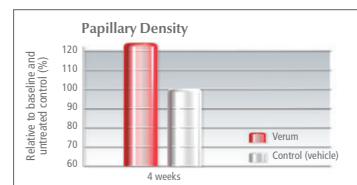
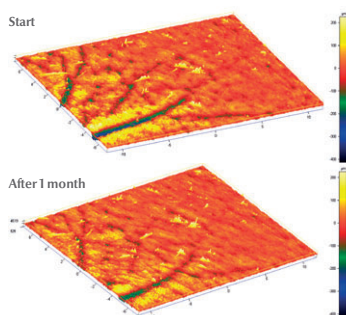
The thickness of the epidermis was measured with Optical Coherence Tomography (OCT) [3] at baseline and after expiration of the treatment period. To evaluate the flattening of the dermo-epidermal junction, a consistent feature of aged skin, the density of the dermal papillae was analyzed by means of confocal microscopic measurements [4].



Evaluation of skin elasticity by means of cutometry: 27 female volunteers aged 52 to 71 were enrolled in this randomized, in terms of test products double-blinded, single-center study. Test products Eucerin® DermoDensifyer Day and Night were applied twice daily to the inner forearm of each participant over a two-week period. An untreated area on the inner forearm served as control. Evaluation visits were conducted at baseline and after 1 and 2 weeks of treatment. The participants had to refrain from using skin care products during a one week preconditioning period on the test areas. Skin firmness was assessed by means of Cutometer® SM575 measurements [5].

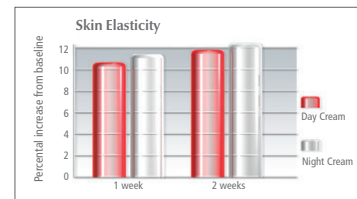
Results

Evaluation of facial wrinkle depth by PRIMOS method and expert grading: A significant decrease ($p \leq 0.05$) in topometric crow's feet wrinkle parameter as a result of diminished wrinkle volume compared to baseline could be revealed by PRIMOS methods after four and eight weeks of treatment. Likewise a significant reduction in wrinkle intensity was assessed by photo evaluation after four and eight weeks for crow's feet, forehead and around-the-mouth wrinkles; for nasolabial folds a significant improvement was found after eight weeks of treatment.



Likewise the application of the verum resulted in a significant ($p \leq 0.05$) increase of papillary density as a result of improved and rejuvenated dermo-epidermal junction. Treatment with vehicle on the other hand showed slightly declining levels of papillae quantity though this result was statistically not significant.

Evaluation of skin elasticity by means of cutometry: One- and two-week long application of Eucerin® DermoDensifyer Day as well as Eucerin® DermoDensifyer Night led to a significant ($p \leq 0.05$) increase in Cutometer SM575 Ur/Ue values compared to baseline and untreated control area thus demonstrating the test products potential to improve firmness and elasticity of the skin.



Discussion and Conclusion

The results from these clinical and dermatological studies including a total of 131 participants undoubtedly demonstrate the test formulations' strong capability of efficiently reducing wrinkle occurrence by contributing to the skin's thickness, elasticity and firmness and by improving the quality of the dermo-epidermal interdigitation. Furthermore, throughout the conducted trials a very good skin compatibility could be observed.

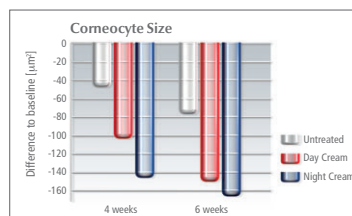
The thickening of the skin as well as the increase of the papillary density can clearly be attributed to the active ingredients since comparison to the ingredient-free basic emulsion showed an obvious superiority of the verum. Recollecting the verified in-vitro properties [6] the other benefits described above can probably be credited to these actives as well.

In summary, Eucerin® DermoDensifyer Day and Eucerin® DermoDensifyer Night can be regarded as effective and safe dermocosmetic treatment regimes against the symptoms of skin ageing.

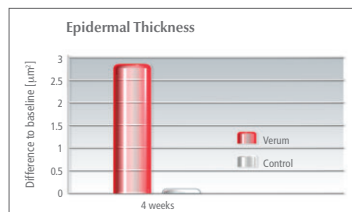
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Evaluation of skin renewal rate by corneocyte image analysis: At baseline the average size of a single corneocyte was about 1080 μm^2 . After 4, 6 and 8 weeks of treatment with Eucerin® DermoDensifyer Day and Eucerin® DermoDensifyer Night, respectively, a significant decrease ($p \leq 0.05$) in corneocyte size as a result of an improved renewal rate could be measured compared to baseline and untreated control.



Evaluation of epidermal thickness and papillary density: Compared to baseline a significant ($p \leq 0.05$) rise of epidermal thickness could be attained by application of Eucerin DermoDensifyer Day (+ 2.73 μm) over a four-week period whereas the vehicle showed no noteworthy effects at all (+ 0.12 μm).



Tolerance and Efficacy of a Moisturizing Body Cream for Children and Adults with Atopic Dermatitis

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RESULTS

Abstract

We evaluated the tolerance and efficacy of a soothing, oil-in-water moisturizing cream containing oatmeal. It was developed to help relieve dry, itchy skin, especially in patients with atopic dermatitis. Product safety was established by a battery of tests, including repeat insult patch, phototoxicity and photo-allergy tests, and controlled usage safety tests, conducted with atopic children and adults. Tolerance in children was demonstrated in 25 atopic and 25 normal children, aged 2 through 12, whose parents applied the product to the entire body and face. After two weeks of daily use, there were no clinically relevant irritation scores. In a similar study of 30 atopic adults, a lack of subjective and objective irritation was also demonstrated. Moisturization efficacy was demonstrated in a study of 30 adults with dry, itchy skin on the lower legs. Subjects applied the cream in the morning and evening, assessing itch intensity before application, and at 2, 10, 30, and 120 minutes after application. Itch was significantly relieved at each post-application time point, and improved significantly throughout the two weeks of product usage. After two weeks, significant moisturization was also observed, by skin hydration meter readings, clinical grading of dryness and cracking, and assessments of coarse and fine flakes, using image analysis of skin surface squame samples. When the product was discontinued for a week, regression of moisturization and itch relief benefits was observed. The subjects' responses to questions that probed product efficacy and aesthetics were highly favorable. Taken together, these data demonstrate exceptional tolerance and efficacy of the product formulation for dry, itchy skin, especially associated with atopic dermatitis.

Introduction

Atopic dermatitis is a common immunologic skin disorder that results in a compromised barrier and increased sensitivity to external irritants. Itch is a prominent symptom that must be managed to limit the severity of recurrent flares, and scratching that can lead to excoriation and infection. While corticosteroids and immunomodulators are predominant therapies for pharmacologic management of the disease, emollient therapy is considered standard care¹. Emollient therapy enhances skin hydration that can support barrier repair and limit irritation that can exacerbate itch, and can be used on a daily basis for long-term management without concern of side effects. We examine a new oil-in water emulsion containing oatmeal for its efficacy to hydrate skin, decrease itch associated with dry skin, and tolerability of the cream for patients with atopic dermatitis.

Materials & Methods

Children's Safety In-Use Study. Twenty-six normal and twenty-nine atopic children, aged 2 to 12 years, participated in a two-week usage study. Atopic children were identified by physician diagnosis and/or positive assessment by the criteria of Hanifin and Rajka². Physician assessment of objective and subjective irritation of the face, hands, arms, legs, torso, and back was made at baseline and after two weeks of product usage. Clinical scores were assigned on a 4-point scale: 0=none, 1=mild, 2=moderate, 3=severe. At the final clinical visit, post-usage questionnaires were also completed.

Adult Atopic Safety In-Use Study. Twenty-six subjects, aged 18 to 65 years, completed a two-week safety in-use study to assess product tolerance on the face and body. Atopy was confirmed by subject history and evaluation according to the criteria of Hanifin and Rajka². Eleven of the subjects had active eczema, which was clinically evaluated on a 10 cm scale (0 to 1=none, 2 to 4=mild, 5 to 7=moderate, 8 to 10=severe). Clinical assessments of objective irritation (erythema, edema, and dryness/scaling) and subjective irritation (burning, stinging, itching, tightness, and tingling)

SAFETY IN-USE STUDIES — OBJECTIVE & SUBJECTIVE IRRITATION

Tolerance for the test cream was evaluated in a 2-week usage study of 26 normal and 29 atopic children, and a study including 26 atopic adults. In both studies, the subjects were clinically evaluated at a number of body sites for objective and subjective irritation. **Table 1** shows the panel average irritation scores for all body sites prior to treatment (baseline) and after two weeks of daily usage in the children's study. Average baseline scores were very low for most parameters in both groups. The atopic group, however, had an average baseline score of 1 (mild) that was significantly reduced at the 2-week visit. The average stinging score was significantly higher at week 2 than the baseline score of 0.00, although the mean value (0.27) was very low.

TABLE 1. Children's Study, Mean Clinical Irritation Scores

Symptom	Normal Children (n=26)		Atopic Children (n=29)	
	Baseline	Week 2	Baseline	Week 2
Erythema	0.03	0.01	0.08	0.07
Edema	0.00	0.00	0.00	0.00
Scaling	0.04	0.02	0.09	0.02 ↓
Burning	0.00	0.08	0.03	0.33
Stinging	0.00	0.04	0.00	0.27 ↑
Itching	0.08	0.12	1.03	0.13 ↓
Tightness	0.00	0.00	0.17	0.00
Tenderness	0.00	0.00	0.00	0.00
Tingling	0.00	0.00	0.00	0.00

↑ Indicates a statistically significant (p<0.05) increase compared to Baseline
↓ Indicates a statistically significant (p<0.05) decrease compared to Baseline

In **Table 2**, the adult atopics are seen to exhibit similarly low clinical irritation scores at baseline, and several statistically significant but small decreases were noted after 2 weeks of test cream usage (erythema, dryness/scaling, and itching).

TABLE 2. Adult Atopic Study, Mean Clinical Irritation Scores (n=26)

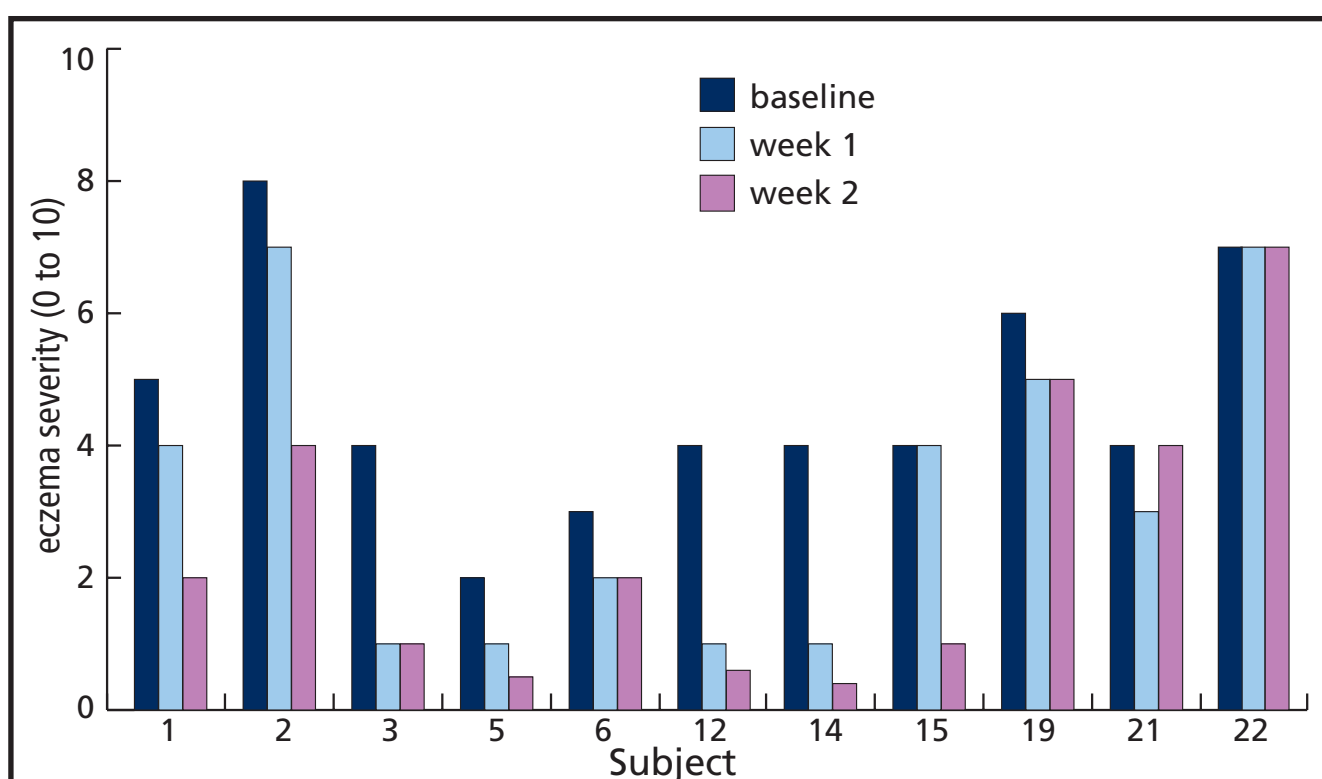
Symptom	Baseline	Week 1	Week 2
Erythema	0.29	0.23	0.21 ↓
Edema	0.00	0.00	0.00
Dryness/Scaling	0.38	0.07 ↓	0.02 ↓
Burning	0.03	0.02	0.00
Stinging	0.01	0.00	0.00
Itching	0.35	0.07 ↓	0.04 ↓
Tingling	0.00	0.00	0.00
Tightness	0.00	0.00	0.01

were made on a 4-point scale (0=none, 1=mild, 2=moderate, 3=severe) at baseline and after two weeks of twice-daily product usage on the face and body. Subjects completed a post-usage questionnaire at the study's end.

24-hour Kinetic Moisturization Test. Thirty subjects with mild-to-moderate dryness on the lower legs participated in the study. At the first clinical visit, baseline corneometer readings were taken in triplicate after subjects equilibrated to

room air conditions for 30 minutes. Twenty-five cm² test sites were marked on the lower leg, and 100 microliters of the test cream was applied and gently rubbed into the skin; other sites remained untreated. The subjects remained in the clinic, and Corneometer readings were repeated at 4, 8, 12, and 14 hours post-application. Subjects then left the clinic, but were instructed not to wet the test sites until the study conclusion. Subjects returned approximately 24 hours after product application, and final Corneometer measurements were made after a 30-minute equilibration period.

FIGURE 1. Eczema Severity in Adult Subjects, Before & After Twice-Daily Product Use



At the final clinical visit, subjects completed post usage questionnaires, evaluating a series of statements about the test products. The response choices to each statement were: agree completely, agree somewhat, neither agree nor disagree, disagree somewhat, and disagree completely. **Figures 2 & 3** show the percentage of positive responses of the atopic subjects. For all questions, the positive responses were significantly greater than the negative responses.

FIGURE 2. Atopic Adults, Post-Usage Questionnaire

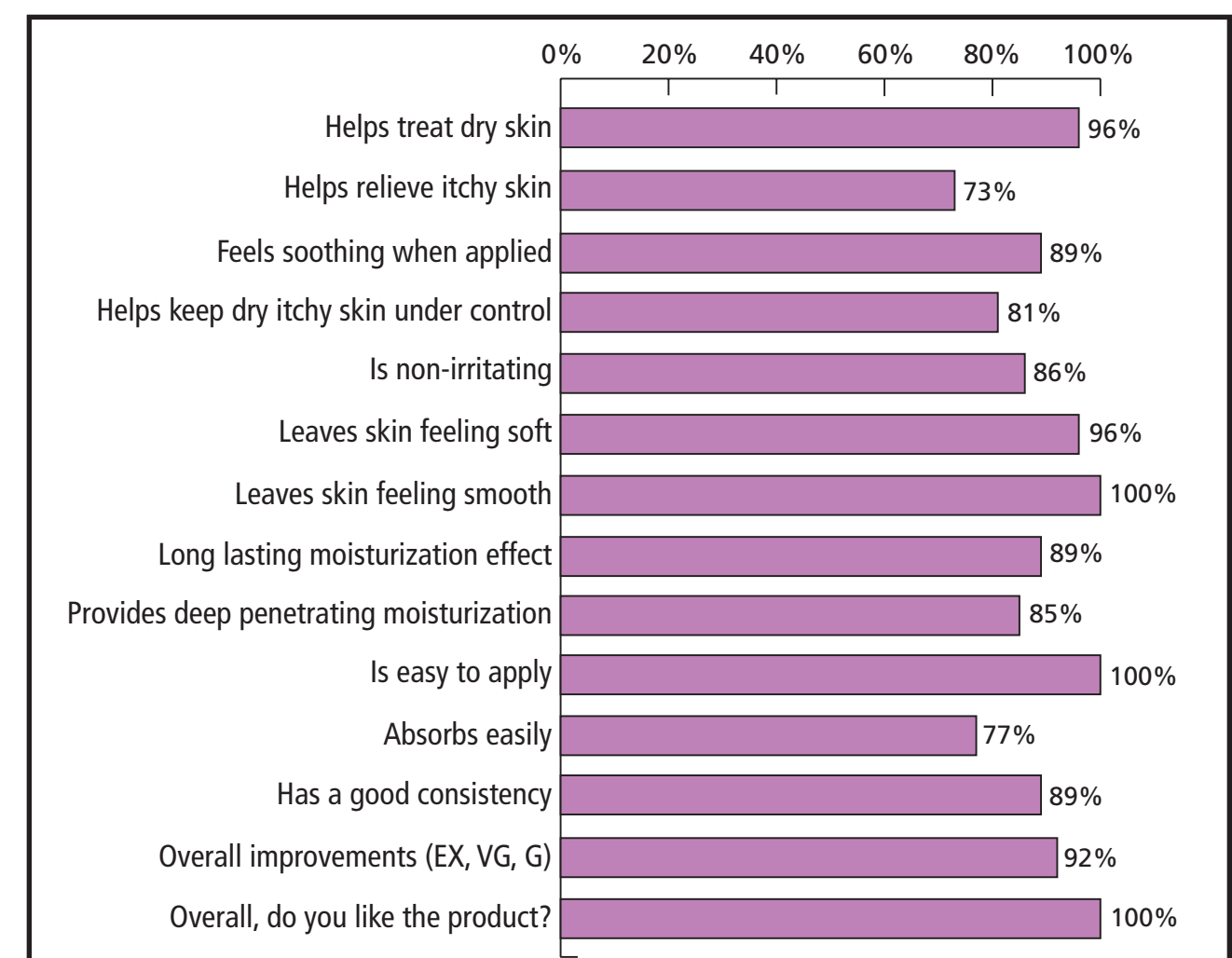
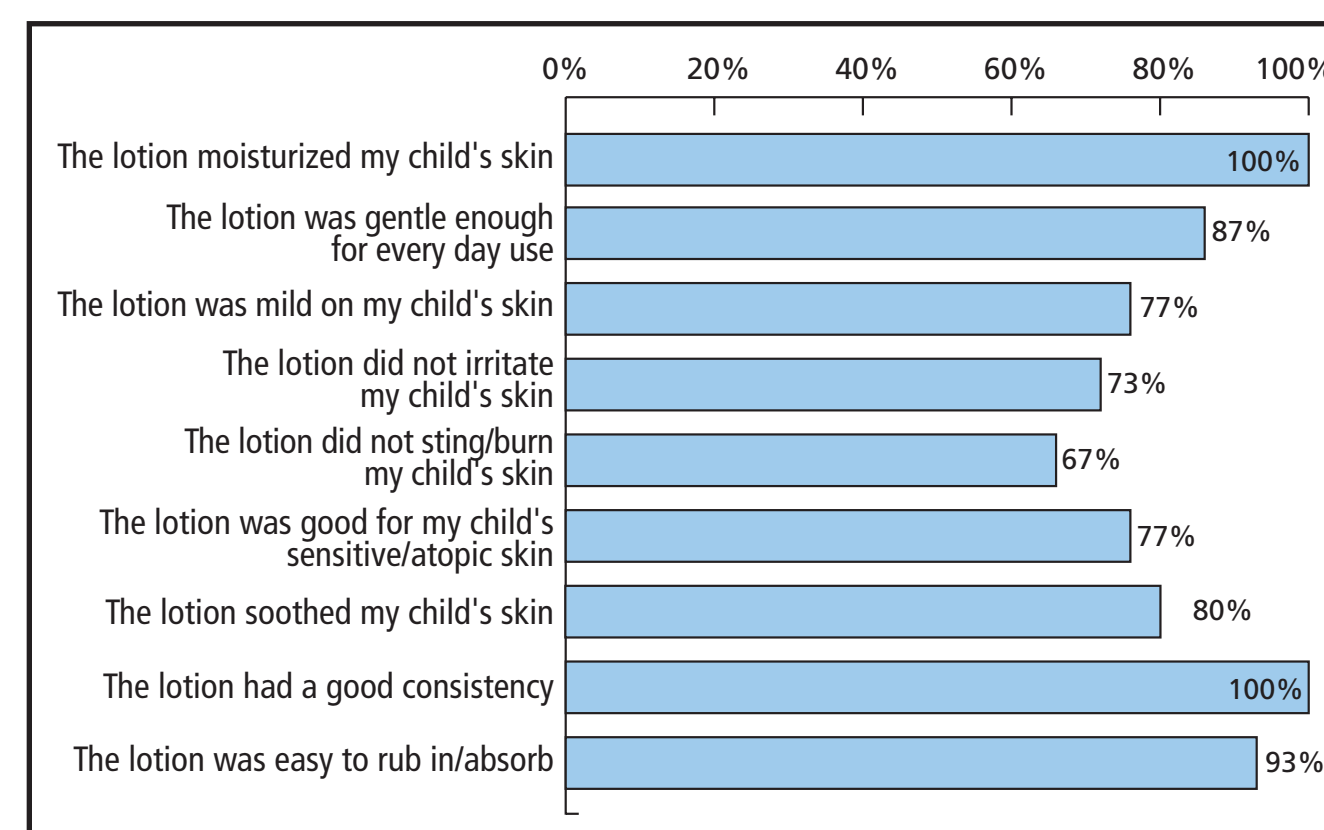


FIGURE 3. Atopic Children, Post-Usage Questionnaire

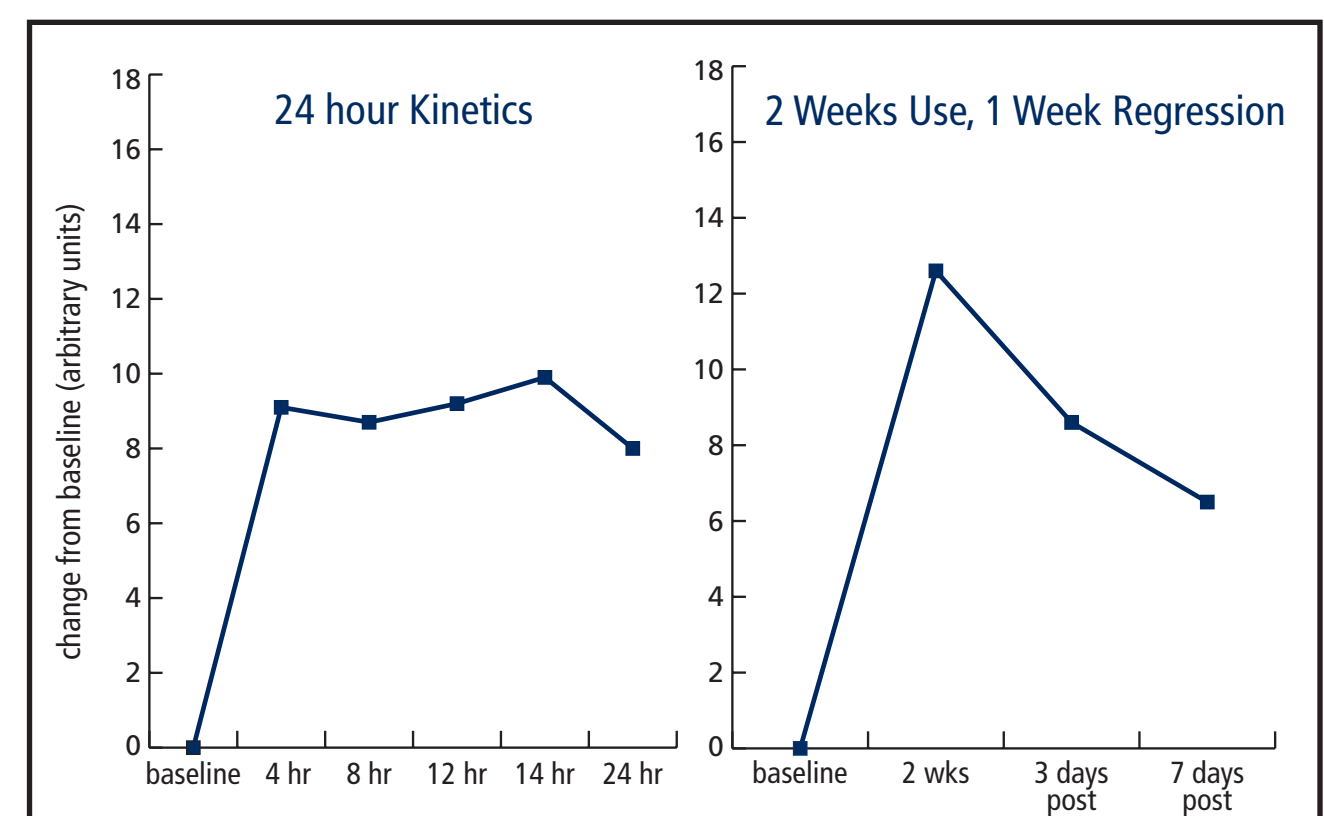


MOISTURIZATION STUDIES

Skin moisturization was evaluated by measuring skin capacitance, an indirect measure of stratum corneum hydration, during a 24-hour period following a single application, and by evaluating capacitance and other clinical measures after two weeks of daily usage and a week-long regression period.

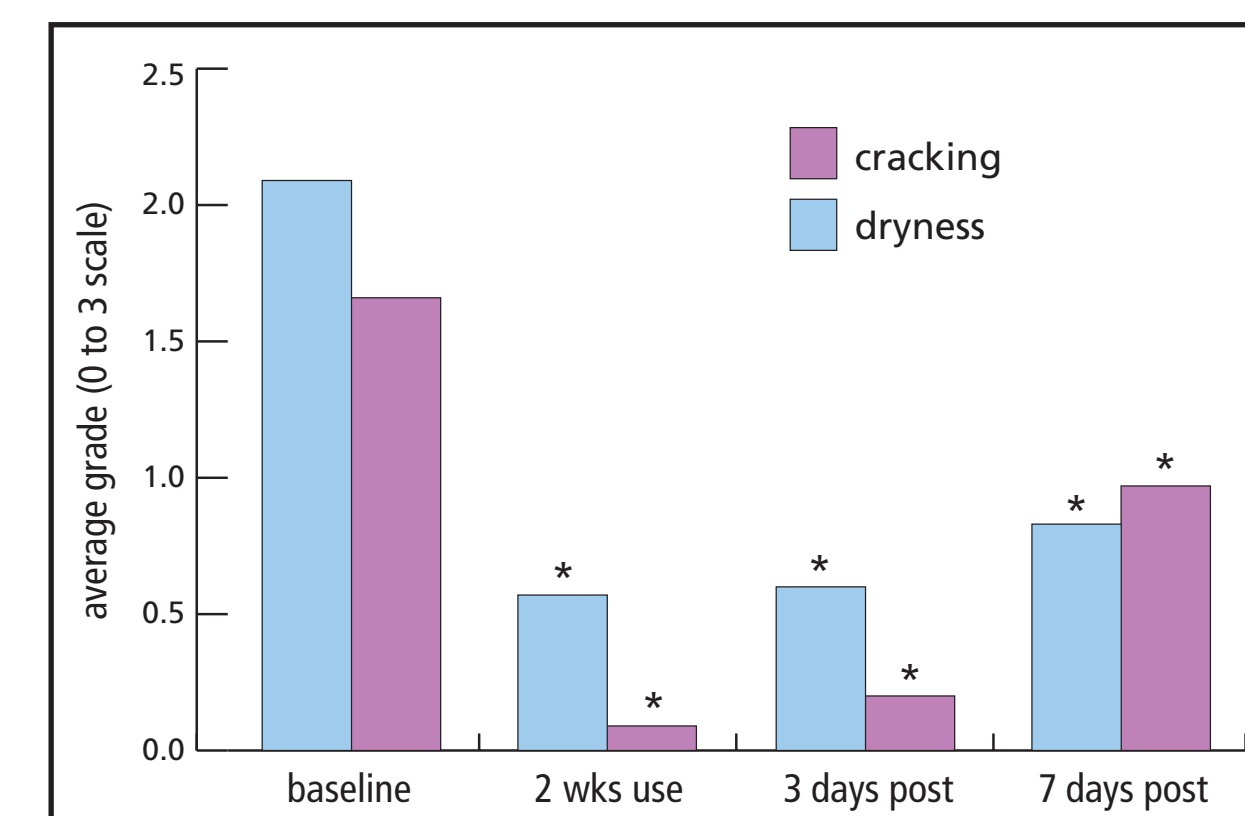
A single application of the test cream showed a significant and sustained improvement over untreated skin sites for the entire 24-hour test period (**Figure 4, left**). A significant increase in skin hydration relative to pretreatment Corneometer values was also observed after two weeks of daily usage (**Figure 4, right**). Skin hydration declined, but was still significantly improved over baseline, at 3 and 7 days after discontinuing treatment.

FIGURE 4. Skin Hydration – Single Application (left) and 2 Weeks of Daily Use (right)



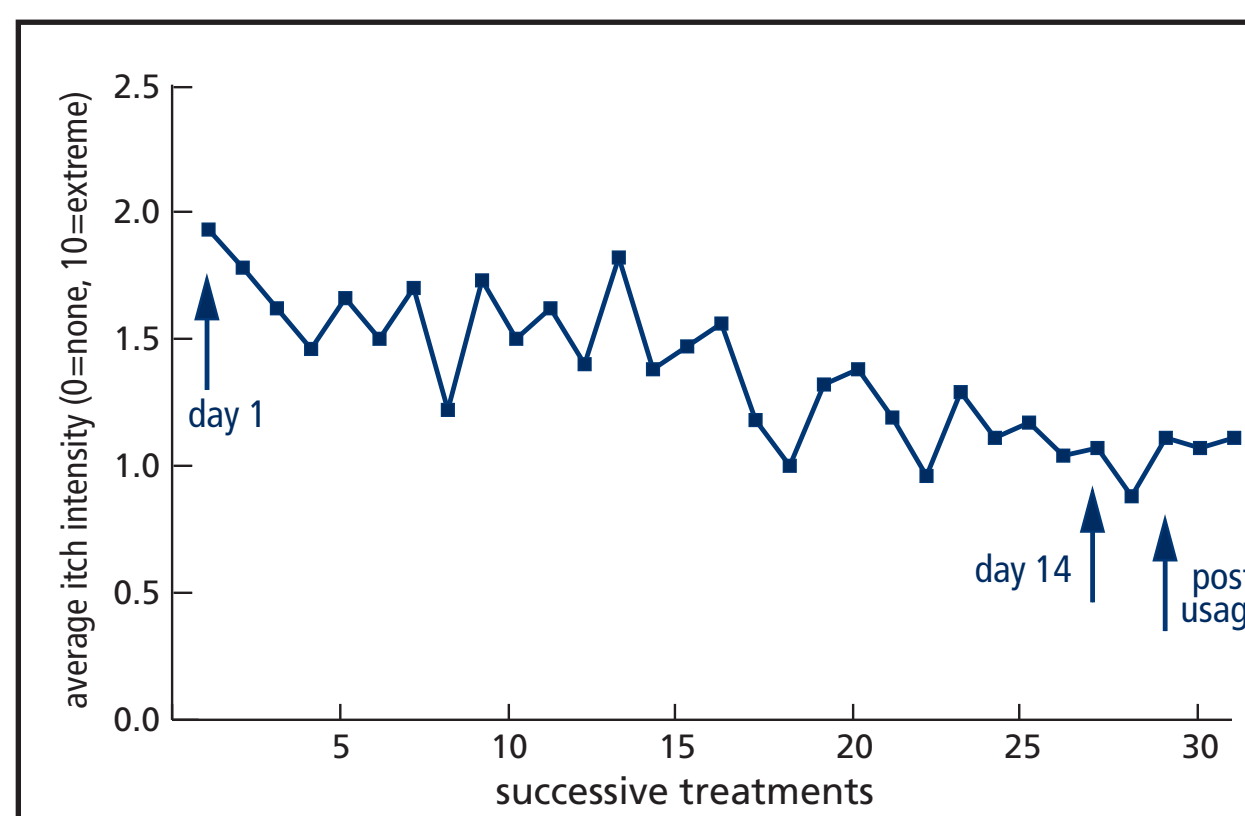
Significant skin moisturization was also observed by clinical assessment and D-Squame® analysis. Average clinical scores for skin dryness and cracking were significantly lower than baseline after two weeks of treatment, and also showed a loss of improvement during the regression phase (**Figure 5**). Significant decreases in desquamation were observed from the D-Squame® after 2 weeks of moisturization—an 88% decrease in the quantity of coarse flakes, a 40% decrease in fine flakes, and a 67% decrease in the desquamation index, relative to pretreatment values (data not shown).

FIGURE 5. Clinical Grading – Dryness & Cracking



Finally, a gradual and progressive decrease in itch intensity was observed by analyzing itch perception recorded in subject daily diaries. Subjects rated itch intensity before product application, and at several time points after morning and evening applications. **Figure 6** illustrates the group average perceptions for the two-hour time period. Each of the post-application time periods showed a similar profile, suggesting a decrease in overall itch intensity during the moisturization period.

FIGURE 6. Itch Perception, 2 hours After Product Application



Dryness Assessment	Clinical Score	Cracking Assessment
None	0	None
Fine, powdery appearance	1	Fine, superficial cracking
Obvious powdery appearance	2	Narrow, shallow cracking
Small scales (<1mm) firmly attached; edges curling and lifting	3	Widened, deepened cracking; no breaks in skin
Obvious large scales (≥1mm), curled, uplifted, and loosely attached	4	Obvious cracking with small breaks in the skin

Conclusions

We tested the moisturization efficacy of an oil-in-water cream for subjects with dry, itchy skin, and tolerability for patients with atopic dermatitis. Corneometer readings showed significant skin hydration for 24-hours after a single application. After two weeks of daily usage, significant moisturization over baseline levels was confirmed by Corneometer readings, clinical grading of dryness and cracking, and D-Squame® analysis. As expected, the moisturizing effects of the cream declined during the week-long regression period, but still demonstrated significant improvements compared to pre-treatment levels. Improvement in moisturization was also accompanied by a decrease in subjects' itch intensity, as noted in daily diaries. In post-usage questionnaires, 85% of subjects indicated that use of the cream relieved their itchy skin.

Tolerance to the cream among children and adults with atopic dermatitis was very good. Most post-usage clinical irritation scores were negligible; average stinging scores for the atopic children were statistically higher than baseline scores, although the absolute value (0.27) was not clinically relevant. Questionnaire responses also demonstrated that the subjects tolerated the test cream well, and perceived it to be moisturizing, soothing, and having favorable aesthetics. Some stinging irritation was reported from the adult subjects with severe eczema and several atopic children. The parents reported the burning was confined to sites with broken skin, and that this occurs with all moisturizing products. In addition, adult subjects with active eczema experienced improvement in the severity of their condition.

These studies demonstrate the safety and moisturization efficacy of the oatmeal containing water-in-oil test cream. Its moisturizing efficacy, ability to reduce itch intensity, and good tolerability among atopic subjects, recommend it as an appropriate choice for basic emollient therapy for patients with atopic dermatitis and very dry skin.

References

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Materials & Methods (continued)

The following assessments were made at baseline (pretreatment), after 2 weeks of product usage, and at 3 and 7 days of regression:

- Measurement of skin hydration using the Corneometer CM825 (Courage + Khazaka, Köln, Germany), an electrical capacitance instrument.
- D-Squame® disc (Cuderm, Dallas, TX) samples of the lower legs to quantify fine and coarse flakes, and desquamation index (baseline and week 2 only).

Subjects also completed an Itch Perception and Usage Diary each day. Itch perception was recorded on a 10 cm analog scale where 0=no itch and 10=extreme itch.

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