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Moisturizing effect of cosmetic formulations containing *Aloe vera* extract in different concentrations assessed by skin bioengineering techniques

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Background/purpose: The polysaccharide-rich composition of *Aloe vera* extracts (*Aloe barbadensis* Miller), often used in cosmetic formulations, may impart moisturizing properties to the product. The aim of this study was to evaluate the effect of cosmetic formulations containing different concentrations of freeze-dried *Aloe vera* extract on skin hydration, after a single and a 1- and 2-week period of application, by using skin bioengineering techniques.
Methods: Stable formulations containing 5% (w/w) of a trilaureth-4 phosphate-based blend were supplemented with 0.10%, 0.25% or 0.50% (w/w) of freeze-dried *Aloe vera* extract and applied to the volar forearm of 20 female subjects. Skin conditions in terms of the water content of the stratum corneum and of transepidermal water loss (TEWL) (Corneometer™ CM 825 and Tewameter™ TM 210) were analysed before and after a single and 1- and 2-week period of daily application.
Results: After a single application, only formulations supplemented with 0.25% and 0.50% (w/w) of *Aloe vera* extract increased the water content of the stratum corneum, while after the 2-week period application, all formulations containing the extract (0.10%, 0.25% and 0.50%) had the same effect, in both cases as compared with the vehicle. TEWL was not modified after a single and after 1- and 2-week period of application, when compared with the vehicle.
Conclusion: Our results show that freeze-dried *Aloe vera* extract is a natural effective ingredient for improving skin hydration, possibly through a humectant mechanism. Consequently, it may be used in moisturizing cosmetic formulations and also as a complement in the treatment of dry skin.

Key words: *Aloe vera* extract – moisturizers – corneometer – TEWL – skin hydration

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The appearance and function of the skin are maintained by an important balance between the water content of the stratum corneum and skin surface lipids (1, 2). Exposure to external factors, i.e., air humidity, ultraviolet radiation, temperature, as well as endogenous factors, i.e. hormones (3–5), may disrupt this balance. In addition, frequent use of soaps, detergents and topical irritants such as alcohol and hot water can remove the skin surface lipids (6). When this balance is disrupted, a dermatological condition known as dry skin ensues, a phenomenon that is observed particularly in patients with atopic dermatitis, a chronic and pruritic form of dermatitis (7).

In these cases, effective cosmetic products must be used to improve skin hydration not only for aesthetics purposes but also to maintain the normal conditions of skin and to prevent dry skin alterations. The moisturizing effect of formulations may be influenced by many factors, such as type and concentration of the active substances used, as well as the composition of the vehicle (8, 9).

Medicinal plants of the lily family (Liliaceae), genus Aloe, have been used for the treatment of skin diseases for more than 2000 years (10). Among more than 360 Aloe species, *Aloe vera* (*Aloe barbadensis* Miller) has been the most popular in both folk and officinal medicine (11).

Aloe vera extracts are widely used in a variety of over-the-counter and dermatological products. Many studies report the effective use of this plant when applied topically for the treatment of

burns, sunburns, inflammatory skin disorders and wounds (12, 13). However, the action of *Aloe vera* as a moisturizing agent still mostly remains a popular concept (12), and it has been used in many moisturizing products, in different concentrations, an important factor in the efficacy and cost of a cosmetic product.

Consequently, clinical studies to evaluate the moisturizing effect of *Aloe vera* extracts scientifically are necessary to validate this claimed effect. Objective methodologies are considered appropriate to prove and to clarify the mechanisms of action of substances that improve skin hydration. Among these are non-invasive skin bioengineering techniques, which are often used as they allow evaluation of cosmetic products under actual conditions of use.

In the present study, we used skin bioengineering techniques to evaluate the effects of cosmetic formulations containing different concentrations of freeze-dried *Aloe vera* extract on skin hydration, after a single and a 1- and 2-week period of application

Methods

Formulations

The formulations studied (Table 1), containing 5% (w/w) of a trilaureth-4 phosphate-based blend (Hostacerin SAF™, Clariant, São Paulo, Brazil), were prepared in a Heidolph RZR 2021 shaker at approximately 625 r.p.m., and supplemented or not with 0.10%, 0.25% or 0.50% (w/w) of freeze-dried *Aloe vera* (*Aloe barbadensis* Miller) extract, a commercial 200:1 concentrate (ACTIValoe™ Aloe vera GEL FD200 ×, Aloecorp, Washington, DC, USA).

Study protocol

Approval for the study was obtained from the Faculty of Pharmaceutical Sciences of Ribeirão Preto – USP Ethics Committee (CEP/FCFRP 07/2001).

TABLE 1. Vehicle components

Components	Percentage of components (w/w)
Trilaureth-4 phosphate-based blend	5.0
Propyleneglycol	2.50
Glycerin 86%	2.50
Phenoxyethanol and parabens	0.80
Hydrogenated and etoxillated castor oil 40 OE	2.00
NaOH (sol 10%)	pH 5.5–6.0
Deionized water	87.2

Twenty healthy female subjects 20–45 years old, having skin Fitzpatrick types II and III, participated in this study after having given their informed consent. The exclusion criteria were as follows presence of, any dermatitis and/or other skin or allergic diseases, smokers and previous treatment of forearms' skin with cosmetic formulations such as moisturizers, sunscreens or anti-ageing cosmetics. During the test period, the subjects were allowed to wash normally, but were instructed not to use any other skin care products on their arms.

Prior to all measurements, subjects remained in the room for at least 30 min in order to allow full skin adaptation to room temperature ($20 \pm 2\ ^{\circ}C$) and humidity (45–60%) (14). The forearm skin area of each volunteer was subdivided into two sites (36 cm^2). The formulations studied and the measurement sites were randomized between subjects.

All measurements were carried out according to the relevant guidelines (15, 16).

Effects after a single application

After the baseline measurements, 0.2 g of each formulation containing the three different concentrations of *Aloe vera* extract (0.1%, 0.25% and 0.5%, w/w) and the formulation without extract (vehicle) were applied on the different sites; 1, 2 and 3 h after application, new measurements were carried out.

Effects after 1- and 2-week period of daily applications

After the baseline measurements, the subjects applied 0.2 g of the formulations studied on their forearms, twice daily, in the morning and in the evening; 1 and 2 weeks after application, new measurements were carried out, 10–15 h after the last treatment, i.e., the formulations were applied in the evening and the measurements were taken the following day (8).

Instrumentation

The water content of the stratum corneum was measured with a skin capacitance meter (Corneometer™ CM 825, Courage & Khazaka Electronic GmbH, Cologne, Germany) (17, 18). The device determines the water content of the superficial epidermal layers down to a depth of about 0.1 mm and expresses the values in arbitrary

units (19). The average values of 20 measurements/site were used in subsequent calculations.

The transepidermal water loss (TEWL) was measured with an evaporimeter (Tewameter™ TM 210, Courage & Khazaka Electronic GmbH), and registered in g/m^2h during 2 min after probe equilibration on the skin for 30 s (20).

Statistical analysis

Non-parametric tests were selected for statistical analysis of the experimental data points, as they showed a non-Gaussian distribution. The paired Friedman test was used for comparison of multiple measured data points using statistical software, GMC. Differences were considered as statistically significant at $P<0.05$.

Results

Effects after a single application

Electrical measurements in the short-term study are reported in Figs 1 and 2.

Significant increases in the water content of stratum corneum readings ($P<0.001$) relative to baseline were observed 1, 2 and 3 h after application of all formulations studied (vehicle and vehicle containing *Aloe vera* extract) (Fig. 1).

However, when compared with the vehicle, only the formulation containing 0.50% of freeze-dried *Aloe vera* extract increased the water content of the stratum corneum ($P<0.01$) after 1 h (Fig. 1). After 2 and 3 h, formulations containing 0.25% ($P<0.01$) and 0.50% ($P<0.001$) of *Aloe vera* extract enhanced the water content of the stratum corneum when compared with the vehicle (Fig. 1).

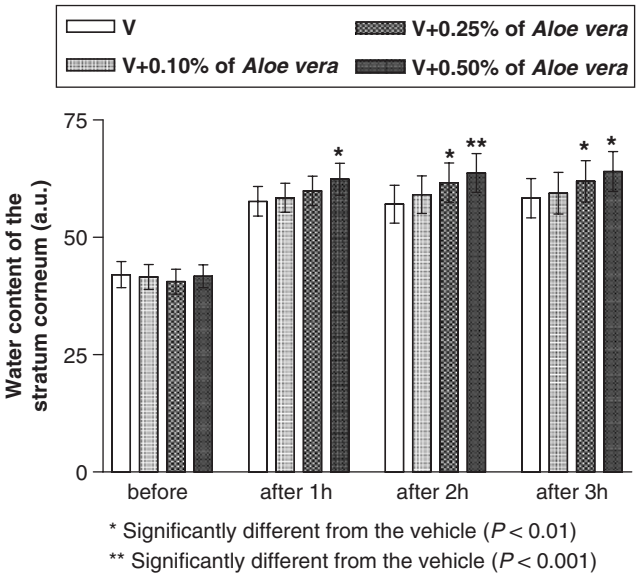

Fig. 1. Water content of the stratum corneum before (baseline values) and 1, 2 and 3 h after the application of the formulations: vehicle (V), V+0.10%, V+0.25% and V+0.50% of Aloe vera extract (Friedman's test, n = 20 subjects, mean ± SEM).

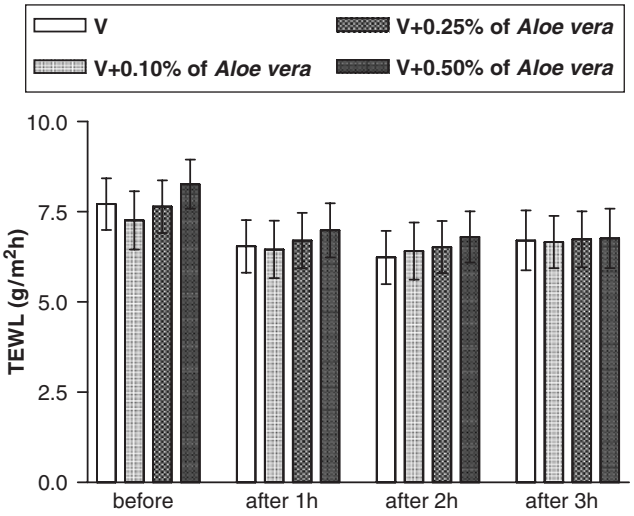

Fig. 2. Transepidermal water loss before (baseline values) and 1, 2 and 3 h after the application of the formulations: vehicle (V), V+0.10%, V+0.25% and V+0.50% of Aloe vera extract (Friedman's test, n = 20 subjects, mean ± SEM).

One, 2 and 3 h after the application, all formulations studied reduced TEWL values significantly ($P<0.05$) when compared with baseline values (Fig. 2).

Nevertheless, when compared with the vehicle, TEWL values were not modified after a single application of the *Aloe vera* extract-supplemented formulations, which means that the skin barrier function was not altered by this extract (Fig. 2).

Effects after 1- and 2-week period of daily applications

All participants reported strict compliance with the instructions. Electrical measurements in the long-term study are reported in Figs 3 and 4.

Significant increases in the water content of stratum corneum readings ($P<0.001$) relative to baseline were observed 1 and 2 weeks after the application of all formulations studied (vehicle and formulations containing *Aloe vera* extract) (Fig. 3).

When compared with the vehicle, all formulations containing freeze-dried *Aloe vera* extract (0.10%, 0.25% and 0.50% w/w) increased the water content of the stratum corneum ($P<0.01$), after a 1-week period of application, but they were not statistically different among themselves (Fig. 3).

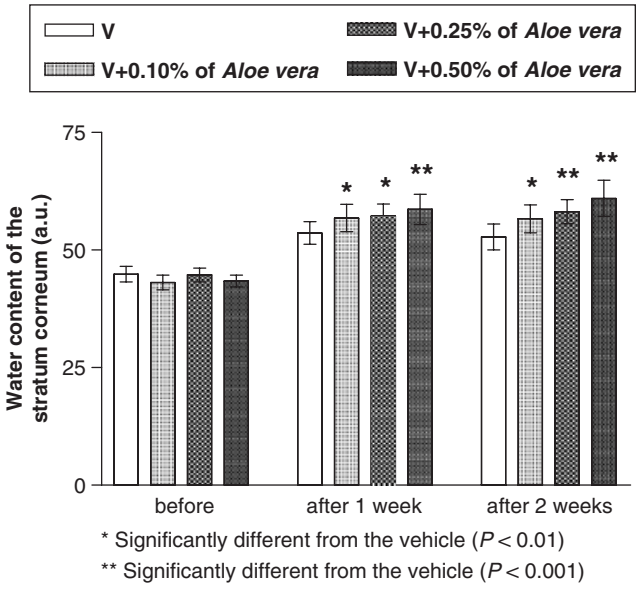

Fig. 3. Water content of the stratum corneum before (baseline values) and 1 and 2 weeks after the application of the formulations: vehicle (V), V+0.10%, V+0.25% and V+0.50% of Aloe vera extract (Friedman's test, n = 20 subjects, mean ± SEM).

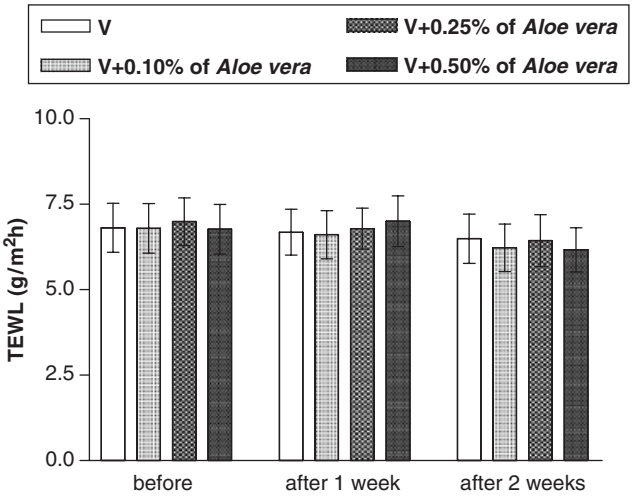

Fig. 4. Transepidermal water loss before (baseline values) and 1 and 2 weeks after the application of the formulations: vehicle (V), V+0.10%, V+0.25% and V+0.50% of Aloe vera extract (Friedman's test, n = 20 subjects, mean ± SEM).

Similarly, the results obtained after a 2-week period application showed that all formulations containing *Aloe vera* extract produced a significant increase in skin hydration when compared with the vehicle ($P < 0.01$). However, when these formulations were compared with each other, the water content of the stratum corneum values obtained with the formulation containing 0.50% of *Aloe vera* extract was significantly higher ($P < 0.05$) (Fig. 3).

TEWL did not change when compared with baseline values 1 and 2 weeks after application for all *Aloe vera* concentrations and for the vehicle (Fig. 4). The different results in relation to the single-application study were probably because of measurements taken shortly after the application of the formulations (1, 2 and 3 h after the application), which were altered by the greasy film formed by the vehicle lipophylic components. In the long-term study, the TEWL measurements carried out 10–15 h after the treatment were not disturbed by the lipophylic components of the vehicle, which had already been removed.

In addition, when compared with the vehicle, formulations supplemented with *Aloe vera* extract did not change TEWL values as well, which means that the presence of *Aloe vera* in the formulations did not alter skin barrier function (Fig. 4).

Discussion

Studies of skin hydration have been performed mainly using short-term studies, where the measurements are carried out between 1 and 8 h after the application of the product, as it is possible to attain improved skin moisture shortly after a single application. Nevertheless, long-term studies are important to assess the maintenance and enhancement of this effect.

The moisturizers may act by an occlusive mechanism, impairing evaporation of skin moisture by forming an epicutaneous greasy film that prevents water loss, as is the case with oils and lipids, or as humectants, i.e., glycerin, urea, sodium pyrrolidone carboxylic acid, which act by attracting water from the other layers of the epidermis to the stratum corneum (5, 21). Consequently, studies with moisturizing products should evaluate the increase in the water content of the stratum corneum and also the decrease in TEWL, in order to determine their mechanism of action.

Our results showed that the freeze-dried *Aloe vera* extract studied improved skin moisture by a humectant mechanism, since when compared with the vehicle, the treatment with supplemented formulations significantly increased the water content of the stratum corneum but did not change the TEWL. This result probably occurred because the freeze-dried *Aloe vera* extract has a rich composition in hygroscope mono- and polysaccharides (22) and in the amino acids histidine, arginine, threonine, serine, glycine and alanine,

which may improve water retention in the stratum corneum (23).

As similar results of skin hydration were obtained after a single and 1- and 2-week period of application, the long-term results can be predicted by the single-application data. This is in agreement with the report by Li et al. (9), who found a linear correlation between changes in the electrical measurements after 1 h and the change in skin dryness grade after 1-week period of application of a glycerin lotion. As a result, these authors suggested that a single application could accurately predict results of long-term (2-week) studies with multiple applications. However, we concluded that it is very important to undertake both studies, as our findings showed that the concentration of *Aloe vera* extract also influences the improvement of skin hydration. Lower concentrations of this extract, like 0.10%, only lead to a significant increase in the water content of the stratum corneum in the long-term application.

Thus, we suggest that the daily use of moisturizers containing *Aloe vera* extract is important to maintain a humectant effect on the skin, which is usually immediate.

The presence of *Aloe vera* in the formulations did not alter the skin barrier function, as TEWL values were not changed when compared with the vehicle. Consequently, *Aloe vera* does not have an occlusive property. The only effect on TEWL values was because of the formation of a greasy film by the lipophylic components of the vehicle, which was observed only for a few hours (up to 3 h).

This study constitutes an objective evaluation of the moisturizing effect of cosmetic formulations containing *Aloe vera* extract, and contributes to the elucidation of its mechanism of action. In addition, the results showed that the formulations studied caused an immediate hydration effect, which was maintained after 1 and 2 weeks with daily applications.

Conclusion

Formulations containing different concentrations of freeze-dried *Aloe vera* extract showed efficacy in improving skin moisture by a humectant mechanism, when evaluated in short- and long-term application studies. After a single application, only formulations supplemented with concentrations above 0.25% improved the water content of the stratum corneum. After 1- and 2-week period of application, all the concentrations were significantly effective.

Thus, freeze-dried *Aloe vera* extract is a natural effective ingredient for improving skin hydration, which can be used in moisturizing cosmetic formulations and also to complement the treatment of dry skin.

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