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ORIGINAL ARTICLE

Confirmed efficacy of topical nifedipine in the treatment of facial wrinkles

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***Introduction*: Over the past two decades, there has been increasing demand for aesthetic procedures to reverse the effects of aging, particularly in the facial area. Recently, topical nifedipine has been proposed for its anti-wrinkle efficacy. *Objective*: To confirm the anti-wrinkle efficacy of a 0.5% nifedipine-based topical formulation. *Materials and methods*: A randomized study was conducted in 20 healthy female volunteers, aged between 45 and 60 years, with moderate to moderately severe facial wrinkles. 10 volunteers applied a 0.5% nifedipine cream and 10 volunteers applied a good moisturizer twice daily for 90 days. The aesthetic improvement was evaluated by a blinded investigator using the Wrinkle Severity Rating Scale (WSRS). Anti-wrinkles effectiveness was also objectively assessed by measuring transepidermal water loss (TEWL), moisture levels of the stratum corneum, skin viscoelasticity and skin folding capacity by instrumental analysis. *Results*: Post-treatment WSRS score was significantly lower than the baseline WSRS score only in the nifedipine group. The mean WSRS score at T_0 was 3.85 and at T_3 1.84 in the nifedipine group, while the mean WSRS score at T_0 was 3.78 and at T_3 3.36 in the control group. Corneometry showed significant increases in measures of skin hydration and TEWL values decreased in all the patients of both groups, indicating a trend toward improved integrity of skin. Dermolab® recorded significant increases in measures of skin hydration in the nifedipine group and a lower increase in the control group. The colorimetric evaluation showed that use of the tested product resulted in significant overall lightening of the skin during use compared with baseline, while the moisturizer didn't produce any change of skin lightening parameters. *Conclusion*: The tested topical preparation is effective in reducing facial wrinkles' depth and in increasing skin hydration and elasticity.**

Key words: nifedipine, calcium antagonists, facial wrinkles, skin aging

Introduction

Wrinkles are modifications of the skin associated with cutaneous ageing and develop preferentially on sun-exposed skin (1). Four main types of wrinkles can be recognized: atrophic crinkling rhytids, permanent elastotic creases, dynamic expression lines and gravitational folds. Each type usually develops on specific skin regions exhibiting distinct microanatomical characteristics (2).

The dynamic expression lines result from the facial muscles action. The mimetic muscles of face are striated muscles lying just underneath the skin to control facial expression. They generally originate on the bone, insert into the undersurface of the skin and interdigitate with the others, like an elastic sheet stretched over the facial bones. When these mimetic muscles contract, they pull and move the skin, causing wrinkling that is generally perpendicular to the direction of muscle contraction. Over time, the actions of the facial musculature, coupled with loss of skin elasticity and a reduction in subcutaneous tissue, result in the development of permanent facial lines. The dynamic expression lines are always oriented in a stereotyped pattern according to the forces imposed by facial muscles. The frown lines, the glabellar lines and the crow's feet wrinkles are typical examples (3).

Different strategies to inhibit mimetic muscles contraction have been researched in the last years. Calcium channel blocker, such as nifedipine, blocks the transmembrane influx of calcium ions into muscle cells inhibiting their contraction. With this in mind, topical use of nifedipine has been recently proposed for the treatment of facial wrinkles. The aim of this study was to confirm anti-wrinkle efficacy and tolerability of a 0.5% nifedipine-based topical formulation, demonstrated previously by Innocenti et al. (4).

Materials and methods

Participant selection

Twenty otherwise healthy Caucasian women, Fitzpatrick phototypes II–IV, aged between 45 and 60 years (mean ± SD = 52.7 ± 5.4 years), with moderate to moderately severe facial wrinkles were enrolled at the University of Naples "Federico II", Department of Systematic Pathology, Division of Clinical Dermatology.

Participants were excluded if they reported: (a) use of topical anti-aging products in the previous 3 months; (b) tretinoin and superficial peels in the previous 6 months; (c) history of fillers, botulinum toxin, medium-depth peel, ablative laser and surgical lifting in the previous 24 months; (d) pregnancy or breast-feeding; (e) exposure to artificial UV radiation; (f) dermatologic disease; (g) allergy to any ingredients in the cream; (h) hypotension. All patients signed a written informed consent, before entering into the study. The study was conducted in accordance with the guidelines for Good Clinical Practice and the principles established in the 1975 Declaration of Helsinki.

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Table I. Wrinkle severity rating scale.

Score	Description
1	Absent: no visible fold; continuous skin line
2	Mild: shallow but visible fold with a slight indentation; minor facial feature; implant expected to produce a slight improvement in appearance
3	Moderate: moderately deep folds; clear facial feature visible at normal appearance but not when stretched. Excellent correction expected from injectable implant
4	Severe: very long and deep folds; prominent facial feature; less than 2-mm visible fold when stretched. Significant improvement expected from injectable implant
5	Extreme: extremely deep and long folds detrimental to facial appearance; 2- to 4-mm visible V-shaped fold when stretched. Unlikely to have satisfactory correction with injectable implant alone

Treatment protocol

Patients were assigned to two groups: 10 volunteers applied a commercially available cream (Antrox® cream) containing nifedipine at a concentration of 0.5%, hyaluronic acid, collagen and vitamin A and E (group A) and the other 10 volunteers applied a good moisturizer, containing hyaluronic acid, collagen and vitamin A and E (group B).

Patients of both groups were instructed to apply precisely measured amounts (0.1 g) of topical formulations on forehead, nose-geniene, periocular and perilabial wrinkles, respectively, twice daily (morning and night) for 3 months. Before the application of the products, the subjects were asked to wash the skin using the same mild facial nickel-tested cleanser. They were not allowed to use other cosmetic products during the period treatment.

Efficacy assessment

The evaluation of the effectiveness of the product was performed by clinical examination, photographic and instrumental documentation. All parameters were evaluated before the beginning of treatment (T_0), and 30 (T_1), 60 (T_2) and 90 (T_3) days later. Volunteers were also asked not to use make-up on the day of measurement. Wrinkles severity was evaluated first clinically by a blinded investigator, using the Wrinkle Severity Rating Scale (WSRS). The WSRS is a validated 5-point scale, based on the current appearance rather than a comparison with the pretreatment appearance. The grading varies from 1 indicating minimum severity to 5 indicating maximum severity (Table I). It is a valid instrument with good intraobserver and interobserver agreement (5–7). Photographic evaluation was performed by the means of a photographic digital camera (Sony DSC W180). Instrumental evaluation was performed using the following equipment:

(1) Corneometer CM 825 PC® (Courage and Khazaka Electronic GmbH, Cologne, Germany) was used to determine stratum corneum hydration (Figure 1) (8–10).
(2) Tewameter™ TM 210 (Courage and Khazaka Electronic GmbH) was used to determine the transepidermal water loss (TEWL). TEWL can be considered as an indicator of the integrity of the epidermal water diffusion barrier and was expressed in g/m^2/h (Figure 2) (11,12).
(3) Dermalab® (Cortex Technology, Hadsund, Denmark) was used to determine the viscoelastic properties of the skin. The instrument calculates the mechanical parameters of the skin based on the stress necessary to achieve a given deformation (Figure 3) (13,14).
(4) Spectrocolorimeter X-Rite 968® was used to determine the skin lightening, to evaluate according to the L*a*b* system, recommended by the Commission International de l'Eclairage (Figure 4) (15).

Statistical analysis

Descriptive analysis was created using median values and 95% confidence intervals (CI). The differences in the WSRS scores in the different time-points of each group were found using the Wilcoxon's test for non-parametric-dependent continuous variables. SPSS software (version 17.00, SPSS, Chicago, IL, USA) was used for the statistical analysis. A p-value (two-tailed) of <0.05 was considered to indicate statistical significance.

Results

Compliance to study design and treatment tolerability

The mean percentage of days in which the doses were taken as prescribed (regimen compliance) was 99.3% in group A and

94.6% in group B. As to tolerability, all volunteers did not report any adverse reaction, whereas one volunteer reported skin tightness perception of minimum intensity for few minutes after the cream application during the first 2 weeks of treatment. In order to exclude percutaneous absorption of the drug and its systemic effects, patients were asked to monitor blood pressure and heart rate three times a week: no changes have been recorded during the study.

Clinical assessment of efficacy

WSRS comparison.

Group A (nifedipine): when WSRS scores at T_0 were compared with T_3 assessment scores, each post-treatment WSRS score was significantly lower than the baseline WSRS score; 1- to 3-point improvement occurred in all treated patients. More precisely, the mean WSRS score at T_1 was approximately 1.38 times lower than mean WSRS score at T_0 (2.79 vs. 3.85), at T_2 1.93 times lower than

Figure 5. Photographic comparison before and after the treatment.

Figure 6. Photographic comparison before and after the treatment.

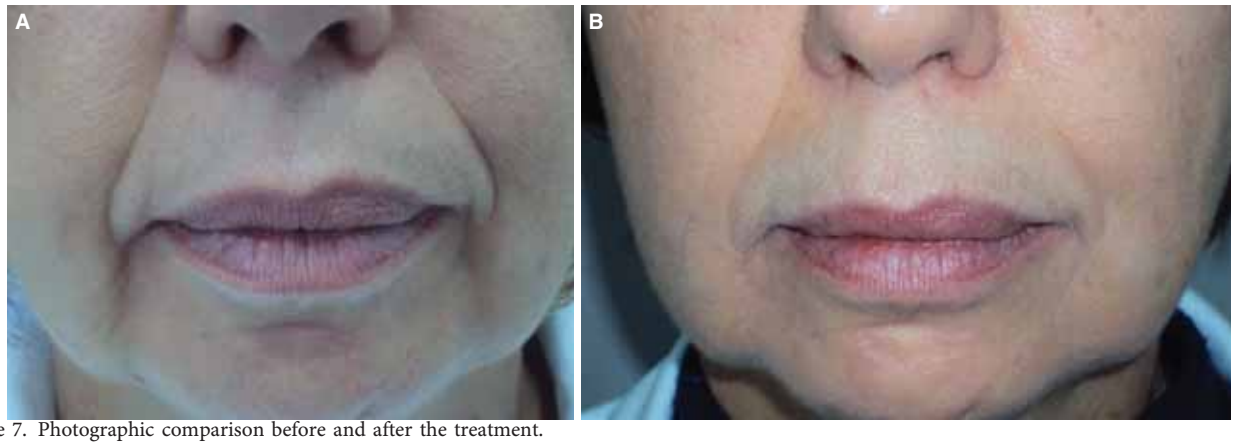


Figure 7. Photographic comparison before and after the treatment.

mean WSRS score at T_0 (1.99 vs. 3.85) and at T_3 2.09 times greater than mean WSRS score at T_0 (1.84 vs. 3.85). Clinical results are shown in Figures 5, 6 and 7.

Group B (moisturizer): the mean WSRS score at T_0 was 3.78, at T_1 3.41, at T_2 3.42 and at T_3 3.36.

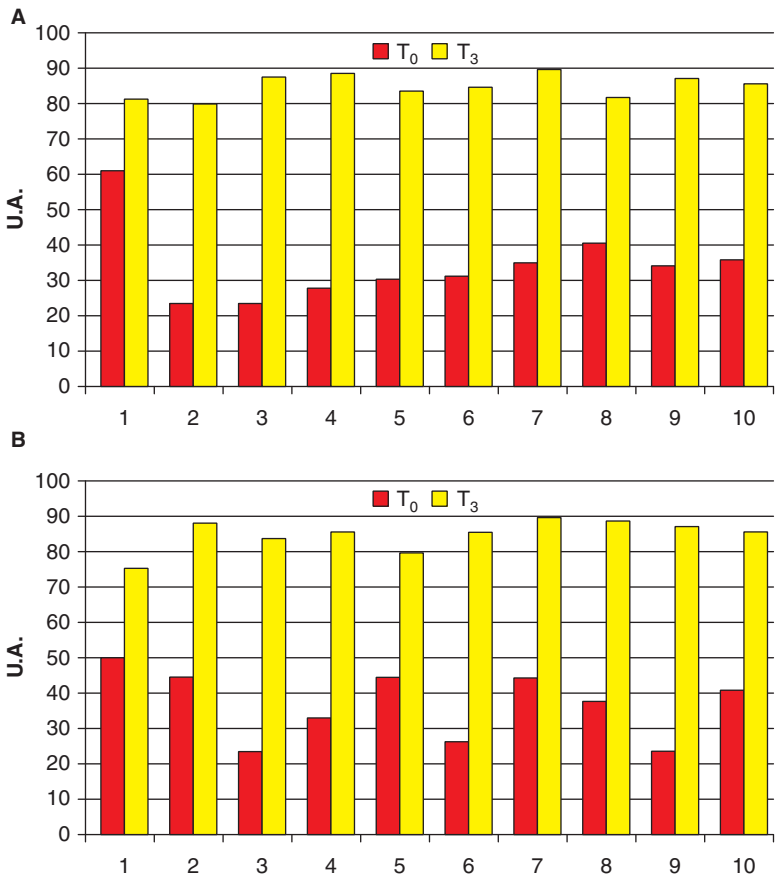
Instrumental assessment of efficacy

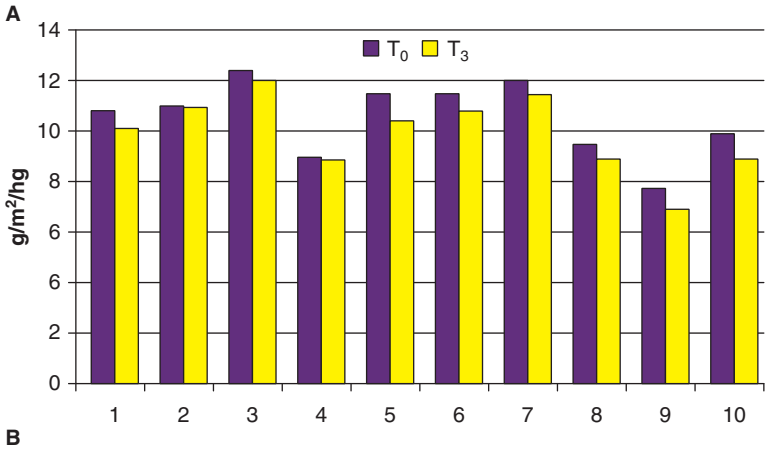
Skin hydration evaluation.
Corneometry showed significant ($p \leq 0.05$) increases in measures of skin hydration in both groups as shown in details in Figure 8A and B.

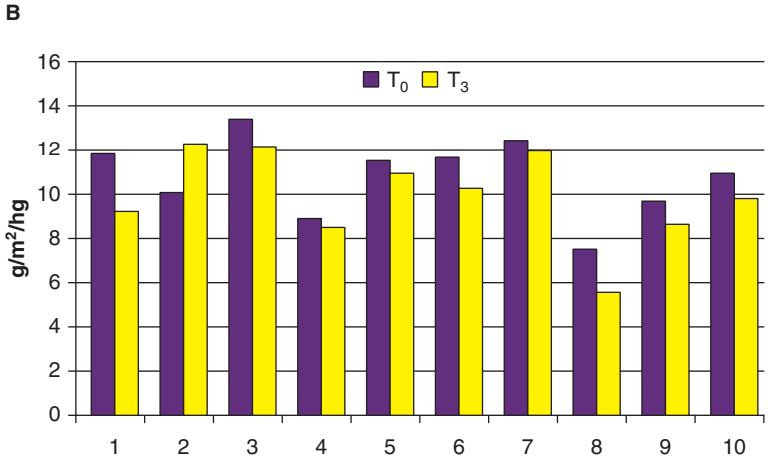
Barrier function evaluation.
Barrier function was analyzed by examining the effect of product use and of moisturizer on TEWL measurements. Changes in TEWL are shown in Figure 9. These values decreased in all 20 patients, indicating a trend toward improved integrity of skin.

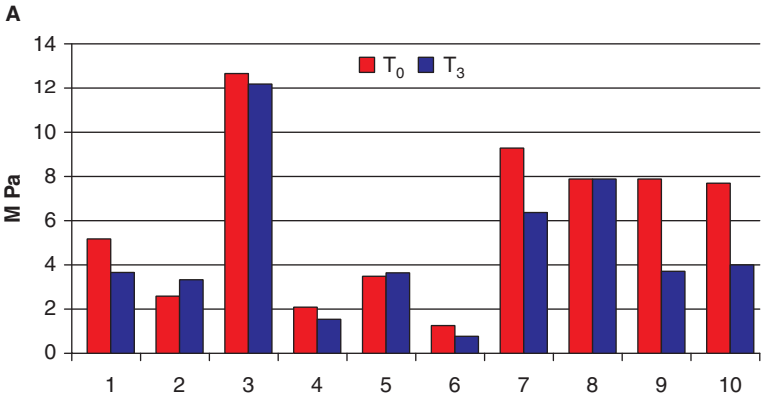
Viscoelastic properties of the skin.
Dermolab® showed significant ($p \leq 0.05$) increases in measures of skin hydration in group A and a lower increase in group B as shown in details in Figure 10A and B. The reduced viscosity of the interstitial fluid results from increased amounts of water in the epidermis.

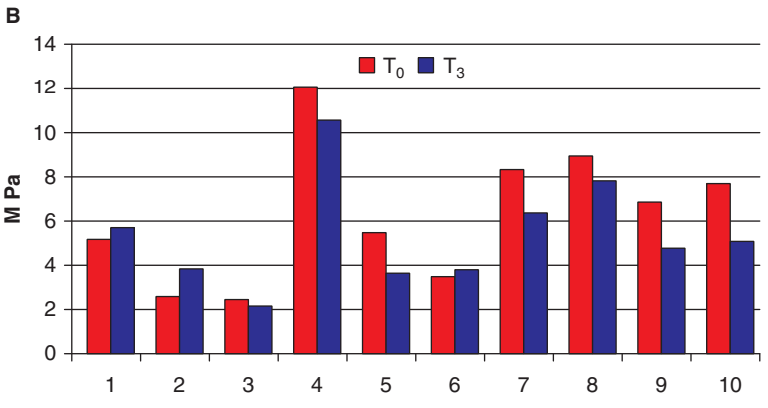
Skin lightening evaluation.
The colorimeter evaluation data of the skin before, during and after the treatment in groups A and B are shown in Figure 11A and B. In particular, a mean increase of the brightness index (L^*) of 6.32 was recorded in the nifedipine group and a mean increase

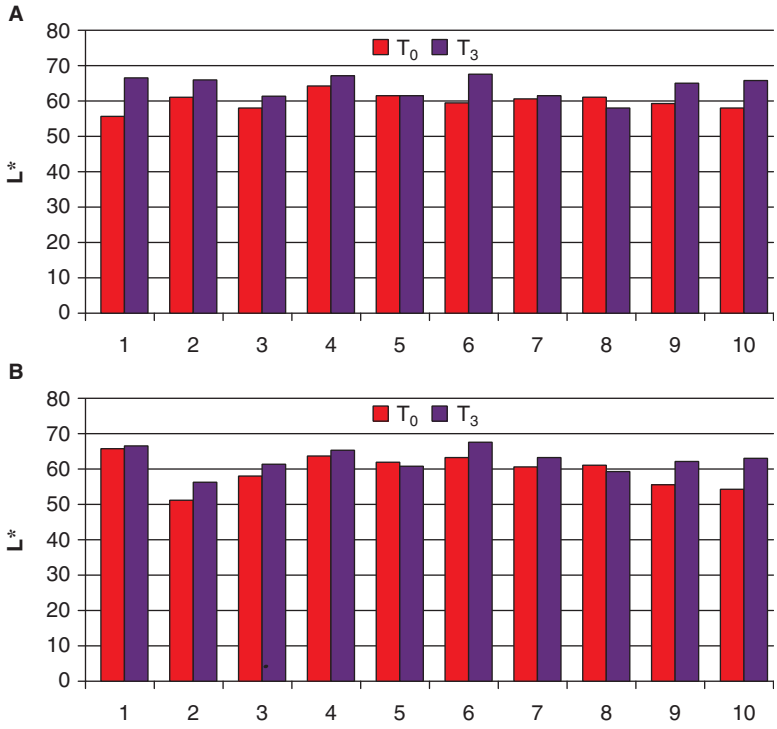












of the same index (L^*) of 3.72 in the moisturizer group. Analysis of L^* values showed that use of the tested product resulted in significant overall lightening of the skin during use compared with baseline and the control group. The absence of depigmenting agents in the tested product seemed to suggest a possible role of nifedipine in increasing skin lightening.

Correlation between demographic data and obtained results

The correlation between volunteers' demographic data and obtained results showed that both clinical and instrumental improvement were independent of age and was the result of individual skin reactions to the cream.

Self-satisfaction assessment after the treatment

As regards participant self-satisfaction assessment in group A, at T_1 42.8% of the participants were satisfied and 14.3% very satisfied, at T_2 64.2% satisfied and 21.4% very satisfied and at T_3 57.1% satisfied and 35.7% very satisfied with the treatment effects. As regards participant self-satisfaction assessment in group B, at T_1 38.9% of the participants were satisfied and 6.4% very satisfied, at T_2 46.2% satisfied and 12.4% very satisfied and at T_3 49.7% satisfied and 21.1% very satisfied with the treatment effects.

Discussion

The tested cream improves appearance of facial wrinkles when used at least for 30 days. This improvement is associated with the objective improvement of several skin parameters such as hydration of stratum corneum and elasticity of skin.

The main structural changes resulting from skin aging process are characterized by a reduction in collagen and elastin and a loss in hydration. Reactive oxygen species (ROS) induced a cascade of biochemical reactions within the skin, which results in the production of matrix metalloproteinases (MMPs) and pro-inflammatory cytokines. MMPs decrease collagen formation and enhance collagen degradation, contributing to the breakdown of the dermal matrix. Pro-inflammatory cytokines lead to the degradation of elastin and also cause the production of additional ROS.

Moreover, over time, chronic contraction of subcutaneous mimetic muscles also contributes to cutaneous aging process (16). Le Louarn et al. have proposed their own theory of facial aging based on mimetic muscle activity. Specific facial muscles, called ''age marker fascicules'', through their contractions eject their underlying deep fat pads into superficial locations as they themselves flatten and shorten, developing increased muscle tone at rest and diminished amplitude of contraction over time. Only then, gravity begins its work on these superficial ectopic fat deposits of age, abetting the structural aging as recognized in the jowl, the nasolabial and marionette lines, the tear trough and the upper orbital hollow (17,18).

Over the past two decades, there has been increasing demand for aesthetic procedures to reverse the effects of aging, particularly in the facial area. Two different strategies have therefore been developed to attenuate expression wrinkles. The rationale for investigating the lifting properties of topical nifedipine consists of its relaxing effects on mimetic muscles. Nifedipine, a dihydropyridine-type calcium channel blocker, acts in the muscles by blocking the calcium channels that play a key role in the contraction of the sarcomere. For the same reason, topical nifedipine has also been successfully used for healing chronic anal fissures by the reduction of internal anal sphincter hypertonia. Oral assumption of nifedipine is well known to produce a rapid decrease of blood pressure and tachycardia. Anyway, previous studies have demonstrated that the poor percutaneous penetration of nifedipine limits its systemic absorption and this, combined with its rapid metabolism in the skin helps prevent accumulation of the drug in the plasma (19,20). As a proof of that, no significant change in blood pressure and heart rate were recorded during this study.

Conclusions

Despite the small number of volunteers, the results of this study are remarkable. The obtained results showed a significant reduction in wrinkle depth and an increase of skin hydration and elasticity after only 30 days of treatment with the tested topical preparation, based on nifedipine in association with other active principles with eudermal and emollient properties which act with a marked synergistic and co-adjuvant action. Further in-depth studies are needed to evaluate the anti-aging effects of topical nifedipine over longer treatment period and its possible role not only in skin rejuvenation, but also in the prevention of cutaneous aging.

Declaration of interest: The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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