

Safety and Efficacy of a New Regimen in Homogenizing and Brightening Skin Complexion Among Filipino Women

Truchuelo MT^{1*}, Gabriel MT², Chan HP², Chan GP³ and Vitale M⁴

¹Nuestra Señora de América Hospital and Clínica Grupo Pedro Jaén, Madrid, Spain

²Research Institute for Tropical Medicine, Department of Dermatology, Department of Health, Manila, Philippines

³Clinical trial management & Testing Associates, Inc., Alabang, Philippines

⁴Medical department of Industrial Farmaceutica Cantabria (IFC), Madrid, Spain

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*Corresponding author

Truchuelo MT, Nuestra Señora de América Hospital and Clínica Grupo Pedro Jaén, Madrid, Spain, Tel: +34606516077; Fax number: +34913735088; Email: mayttd@yahoo.es

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Abstract

Objectives: To evaluate the safety and efficacy of a new regime of treatment based on the combination of two technologies: Retinsphere® and Fernblock®, topical and oral respectively, in decreasing the visibility of skin blotches and creating a more even complexion. Secondly, the anti-aging effects were also assessed.

Methods: Randomized, double blind, vehicle-controlled study. 80 women aged between 35 and 55 years old were recruited and grouped in 4 arms of 20 subjects: (A) topical single treatment, (B) oral single treatment, (C) combined (oral and topical treatment) and (D) control. Efficacy was objectively evaluated with non-invasive devices (Chromameter®, Mexameter®, Tewameter®, Corneometer®, Triplesense®, SkinSys® and DermaLab®). Baseline values were compared to outcomes obtained at T30 and T90, and also each group (A, B and C) to control group (D). Subjective assessment was performed at the end of the study by the investigator as well as the patient, using a questionnaire especially developed for this purpose. Adverse events were monitored and followed up throughout the 90-day study period.

Results: Active regimens (A, B and C) were significantly better improving skin brightness, lightening and aging signs compared to control. They were also scored positively by the investigator and the patients regarding skin lightening, wrinkle correction and firmness. The overall safety evaluation revealed good tolerance for all regimens. Two patients of combined treatment arm (C) reported mild to moderate erythema and itchiness.

Conclusions: Globally, the outcomes obtained with the combined regime (C) were superior to single treatments separately (A and B). Furthermore, subjects that received combined regimen obtained faster results, reaching better scores within the first month. This could lead to improvement of the patient's adherence.

Introduction

Intrinsic aging is a genetically determined process that encompasses progressive physiological changes that lead to different signs such as loss of volume, which turns into wrinkling and laxity, pigmentary irregularities, actinic keratosis, telangiectasias and other dermatological disorders. These changes can be dramatically accelerated or amplified by personal and environmental conditions, mainly solar radiation, leading to extrinsic aging or photoaging [1,2].

UV exposure is the most important trigger in extrinsic aging: UVB radiation directly impacts several biomolecules and also DNA driving the DNA-strand breaks [3,4] that could lead to devastating consequences such as mutations and cancer. UVA radiation promotes Reactive Oxygen Species (ROS) formation, inducing oxidative stress and, subsequently, DNA damage, lipid peroxidation, protein glycation, activation of transcription factors, and activation of proteolytic enzymes such as collagenase, gelatinase and stromelysin-1, leading to degradation of both collagen and elastin [5], as well as other components of the dermal Extracellular Matrix (ECM) [1,3]. In addition, UV radiation has a deleterious action over the components of the skin immune system, inducing Langerhans cells depletion. Altogether, these effects may give rise to the carcinogenic process [1,4,5]. Accordingly, the mechanisms that should be implemented with the aim of preventing and reversing the damages associated with extrinsic aging are listed on Table 1.

Pigmentation disorders are related to both intrinsic and extrinsic aging, combining multiple factors such as sun exposure, skin injury, hormonal changes, lifestyle and air-pollution. Women and high skin phototypes are more often affected [6]. Conventional treatment includes photoprotection, topical drugs (tretinoin, hydroquinone) and cosmeceuticals. In addition, other technologies such as lasers or Intense Pulsed Light (IPL) are also indicated for pigmentation disorders, usually in combination with traditional treatment to obtain a synergic effect [7].

In the present work, the effects of a new regimen of products containing two different active ingredients have been studied. One of them is Retinsphere® Technology, a combination of two retinoids (retinol glycospheres and hydroxipinacolone retinoate), is formulated in a moisturizing

Table 1: Antiaging strategies.

Prevention of excessive ROS formation
Reinforcement of antioxidant mechanisms
Blockade and reduction of AGEs production
Induction ECM component synthesis
Decrease of undesirable pigment formation

system to reduce Transepidermal Water Loss (TEWL). This ingredient has proven lightening activity acting on the regulatory mechanisms of melanogenesis, acceleration of epidermal turnover and facilitating the penetration of other depigmenting agents included in the formula (Neoretin® Discrom control) [8]. This formula has demonstrated its efficacy, tolerability and safety in several previous studies [8-10].

The second active ingredient involved in this study is Fernblock®, an extract of polypodium leucotomos (PLE) with proven safety and efficacy. It has clinically demonstrated its photoprotective, antioxidant and anti-inflammatory properties and has been used for years as a powerful skin photoimmunoprotector [11,12].

Daily oral intake of PLE provides significant photoprotection to subsequent exposure to artificial UV radiation by reducing the presence of several markers of UV injury [13]. It also acts as an adjuvant to sunscreen in the treatment and prevention of melasma [14,15]. In addition, PLE has demonstrated to be effective in decreasing skin alterations induced after irradiation with highenergy visible light and infrared radiation [16]. Fernblock® technology also exerts its antiaging properties maintaining the status of the ECM through the inhibition of metalloproteinases and the cyclooxygenase-2 [17,18]. Heliocare Purewhite® Radiance, is an oral formula used in this study containing Fernblock®, vitamin C, niacinamide, cystine and phenolic compounds. This association provides a synergic effect in order to achieve better whitening results.

Objectives

Primary objective

To evaluate the efficacy of a new combined regime based on a topical treatment with Retinsphere® (Neoretin® Discrom Control

Table 2: Description of eligibility criteria for the study.

Inclusion criteria	Exclusion criteria
Women 35 years old or older in good health at study entry Presenting skin blotches on the face due to natural aging or sun, and dull skin, showing other clinical signs of aging. Signed Informed Consent Form	Dietary supplements intake within the last 4 weeks that may interfere in general skin condition or on contraceptive pills within the last 3 months Currently on whitening /anti-aging cosmetic products on the face Currently pregnant, plans to get pregnant, or on breastfeeding

Table 3: Description of the four groups into which the subjects were randomly assigned.

Group	Regime	Regime description
Group A: 20 subjects	Topical	Day: Neoretin® Cream+ 1 capsule Heliocare® PWRPlacebo Night: Neoretin® Serum+ 1 capsule Heliocare® PWR Placebo
Group B: 20 subjects	Oral	Day: Neoretin® Cream vehicle + 1 capsule Heliocare® PWR Night: Neoretin® Serum vehicle + 1 capsule Heliocare® PWR
Group C: 20 subjects	Topical + Oral	Day: Neoretin® Cream+ 1 capsule Heliocare® PWR Night: Neoretin® Serum+ 1 capsule Heliocare® PWR
Group D (control): 20 subjects	Vehicle + Placebo	Day: Neoretin® Cream vehicle+ 1 capsule Heliocare® PWR Placebo Night: NeoRetin® Serum vehicle + 1 capsule Heliocare® PWR Placebo

Cream SPF 50 and Neoretin® Discrom Control Serum) and oral treatment based on Fernblock® (Heliocare Purewhite® Radiance) in reducing the visibility of skin blotches. To evaluate the safety of this regime by monitoring any adverse events occurring during the 90-day study period.

Secondary objective

To evaluate the anti-aging efficacy of the regime in skin hydration, skin moisturization, skin firmness, wrinkle reduction, skin smoothness and skin thickness.

Population, Materials and Methods

A prospective, randomized, double blind, placebo-controlled clinical trial designed to assess the efficacy of three different regimens of treatment at several time points: Baseline (T0), day 30 (T30) and day 90(T90) compared to control. 80 female subjects were recruited for this study following the eligibility criteria detailed in Table 2.

Treatment

The subjects were randomized into one of four groups (Table 3). Each group received a different regime combining Neoretin® Discrom Control Cream SPF 50, Neoretin® Discrom Control Serum or topical vehicles, and Heliocare Purewhite® Radiance or placebo capsules. NeoRetin® Discrom Control Cream SPF 50 (Neoretin® Cream) or vehicle SPF 50 were applied in the morning to a clean face and one Heliocare Purewhite® Radiance (Heliocare® PWR)/placebo capsule was orally taken. In the evening, NeoRetin® Discrom Control Serum (Neoretin® Serum) or vehicle were applied to a clean face, and one Heliocare® Purewhite®/placebo capsule was orally taken.

Neoretin Discrom Control cream combined Retin Sphere technology (0.1% Hydroxipinacolone retinoate and 1% retinol glycospheres) and a whitening Booster system composed of Kojic acid, Chroma Bright and Natriquest, a Moisturizing system composed of Hydracare, Hydromanil and Diffuporine peptide solution, Physavie and a broad spectrum sunscreen containing physical and chemical filters with SPF 50.

Neoretin® Discrom Control serum combined RetinSphere® technology with a Whithening booster system in this case, with different active ingredients (Niacinamide plus N-Acetyl Glucosamine, Kojic acid available in free form and in nanocapsules, Natriquest, Albatin plus Alistin), Moisturizing system (Hydracare, Hydromanil and Acetylhexapeptide, Portulaca extract and an finally Salicylic Acid).

Heliocare PureWhite combined Polypodium leucotomos extract (Fernblock® 240 mg) with other active ingredients such as: Pomegranate extract, Cystine, Vitamin C (Ascorbic acid) and Niacinamide (Vitamin B3).

Table 4: Non-invasive bioengineering techniques used to obtain objective measurements of the changes occurring along the study.

Parameter	Technique / Device	Assessed area	Values obtained by the non-invasive equipments used
Skin brightness (glow value)	Chromameter® CR-10 (Konica Minolta)	Cheek area	Through standard or customized evaluation formulas it accurately identifies color characteristics in objects, determines color differences. CR-10 Plus has the ability to measure precise color difference expressed in L*a*b* and dE* or L*C*H* and dE*. L* values provide an objective measure of skin darkening. The results are shown by the Chromameter according to a color scale.
Skin lightening (melanin index)	Mexameter® MX18 (Courage + Khazaka, electronic GmbH)	Cheek area	Shows melanin and erythema values according to an arbitrary scale between 0 – 999. Based on previous studies, a reduction of at least 5 points is already significant. We obtained a decrease in melanin value >210 which was considered as lightening [18].
Skin hydration (TEWL index)	Tewameter® 300 (Courage + Khazaka, electronic GmbH)	Cheek area	The measured TEWL-value is expressed in g/h/m². The study results were between 3-25. A decrease in mean value signified improvement of skin hydration.
Skin moisturization (moisture index)	Corneometer® CM 820 (Courage + Khazaka, electronic GmbH)	Cheek area	Corneometer quantifies the degree of moisture/hydration state of the skin surface. It provides data on condition and type of skin as well as on the effects of drugs and cosmetics on the moisture contents of stratum corneum. The results are shown by the Corneometer according to an arbitrary scale between 0-130, where: < 30 very dry; >45: enough moisturization. The study results were between 35-80 [19]. An increased in the mean value signified increased moisture content.
Skin firmness (elasticity index)	TripleSense® TR-3 sensor scan device (Moritex Corporation)	Area below the cheek bones	The results are shown by the TripleSense according to an arbitrary scale of elasticity index between: 10 – 100; a 3 points increased in mean value signify improvement of skin elasticity. The results obtained in the study ranged between 70-95. Increments of 3 or more units were considered significant.
Wrinkle reduction (curvature index)	Aramo-SG instrument (SkinSys®) software	Crow's feet area	The results are shown by the SkinSys according to an arbitrary scale of the curvature index between: 010-100, a 3 points reduction in the mean values signify reduction in wrinkle depth. The results obtained in the study ranged between 65-95. Increments of 3 or more units were considered significant.
Skin smoothness (keratin index)	CoScan 3E (Skin Diagnosing Digital Camera System)	Crow's feet area	Analyze keratin through precise edge detection algorithm. The results are shown by the CoScan 3E according to an arbitrary scale of keratin values between: 010 -100, a 5 points reduction in the mean values signify skin smoothening efficacy. The results obtained in the study ranged between 10-50. Increments of 5 or more points were considered significant.
Skin thickness (collagen intensity)	DermaLab® Series, SkinLab USB (Cortex Technology)	Crow's feet area	Uses high-frequency (20 MHz) ultrasound systems. Ultrasound skin imaging is performed by firing an acoustic pulse into the skin and measuring the acoustic response from the skin which is picked up by an ultrasound transducer. The signals are then processed, and a cross-sectional image is produced which represents an intensity/amplitude analysis of these returned signals. The intensity of the received signal refers to a color scale, where dark colors represent areas of the skin with low reflection (i.e. none or small changes in density between the structures in the skin) and bright colors represent areas with strong reflections (i.e. significant changes in density between structures. Results are shown according to this color scale.

Due to ethical reasons, NeoRetin® Discrom Control Cream SPF 50 and its vehicle were formulated with the same UV filters to reach SPF 50.

Clinical assessment

The observation period was 90 days long. The instrumental multi-parametric approach was measured by non-invasive bioengineering techniques (Table 4) in each visit (T0, T30, T90) to ensure objectivity of the outcomes.

Subjective evaluations

To assess the satisfaction reached with the treatment both by the investigator and the patient, an Investigator Global Assessment (IGA) was performed at every visit (T0, T30 and T90) and Patient Global Assessment (PGA) was sought at the end of the study (Table 5).

Statistical Analysis

The study was a randomized controlled trial which compared four regimens through time to determine if the treatments promote any changes in the visibility of skin blotches and in the creation of a

more even complexion (primary objective), as well as on the efficacy of the products in promoting skin hydration, skin moisturization, skin firmness, wrinkle reduction, skin smoothening and thickening.

Quantitative variables

Differences of each parameter through time: differences in the quantitative parameters (glow value, melanin index, TEWL, moisture index, elasticity index, curvature index, keratin index and collagen intensity) through time (T0, T30 and T90) were evaluated by conducting a simple paired t-test. Mean differences during these comparison periods were calculated. The null hypothesis was that the means at every time point would be equal. Graphs show standard error of the mean.

Differences between groups: Secondly, differences between these outcome variables at every time point were calculated. As these variables do not follow a normal distribution, the selected tests were non-parametric test of Kruskal-Wallis for the intergroup comparison and the Mann-Whitney test for the comparison between the groups two by two.

Table 5: Scales for the subjective evaluations by the investigator and the patient.

Subjective evaluation by investigator	Self- assessment evaluation
Appearance of skin blotches(1-4 scale) 1: No variation 2: Slight improvement 3: Moderate improvement 4: Remarkable improvement Clinical classification of skin wrinkles(7-step scale) 0: No wrinkle 0,5: Very shallow yet visible wrinkles 1: Fine wrinkle, slight indentation 1,5: Visible wrinkles and clear wrinkles 2: Moderate wrinkles, clear and visible 2,5: Prominent and visible wrinkles 3: Deep wrinkles Clinical classification of skin firmness (1-4 scale) 1: Non-toned 2: Insufficiently toned 3: Toned 4: Well-toned Changes on T30 and T90 compared to T0 (1-4 scale): 1: No variation 2: Slight improvement 3: Moderate improvement 4: Remarkable improvement.	A questionnaire with 4 items (scored from 1, totally disagree; to 5, totally agree) and a fifth question regarding the overall result (scored from 1, not at all; to 10, very much) was completed by each patient. Would you say that your skin is brighter after the treatment? Do you find your skin color is more even? Do you feel that your skin has brightened up following the treatment? Have you noted a reduction of your skin blotches? How satisfied were you with the overall results?

Subjective variables

They were evaluated according to an ordinal evaluation scale. A one-sample Kolmogorov-Smirnov test was performed to assess normality, and as it was significant in most cases, a non-parametric Wilcoxon test was subsequently performed.

Statistical Product and Service Solution (SPSS v17.0.1) was used for the data analysis. The statistics included descriptive and bivariate analysis. In all conditions, a significance level of 5% ($p < 0.05$) was considered.

Results

80 women aged between 35 and 55 years old were enrolled in the study. Each subject was randomized to one of the four experimental groups (Regimens A, B, C and D, control). Three subjects dropped out because of adverse events. Two of them were related to the treatment and occurred in subjects under the combined regime (C). They both experienced a reaction characterized by erythematous patches on the cheeks accompanied skin tightening that lasted for 3 days. The third subject dropped out due to clinical symptoms unrelated to the

treatment. The remaining 77 participants did not experience side effects and were included in the final data analysis.

Table 6 describes the results obtained with each instrumental technique at every time point (T30 and T90) compared to baseline within each group. In regimens A, B and C, all the values were statistically better compared to baseline (at T90 significance was $p \leq 0.001$ for each parameter in all groups). With the aim of simplifying, these data are not shown in the table. Most parameters of the control group, regime (D), did not reach statistical significance. However, it must be highlighted that it was found a -1.1% significant worsening of skin brightness and of -2.4% skin lightening at T30 and T90, respectively, compared to baseline ($p \leq 0.001$).

When comparing the results between different regimens, the three active groups showed better outcomes than the control group. Topical treatment (regime A) obtained the best results compared to control at T90 regarding the increase of skin lightening (+19.0%), moisturization (+21.7%), wrinkle reduction (+10.0%, $p \leq 0.01$), and skin smoothness (+32.6%, $p \leq 0.05$). Oral treatment (B) was especially effective in the amelioration of skin brightness (+9.9%), wrinkle

Table 6: Percentage of improvement of the different variables in T30 and T90 compared to T0 in each group. Statistical significances of the variations at each time point in each group compared to control group (regime D) are shown as * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. Statistical significances of the changes experienced in group C (combined treatment) compared to regimens A and B are shown as * $p \leq 0.05$.

PARAMETER	A – TOPICAL		B - ORAL		C-COMBINED		D-CONTROL	
	T30	T90	T30	T90	T30	T90	T30	T90
Brightness	6.3%	8.9%**	3.0%*	9.9%**	6.8%*	11.6%***	-1.1%	0.5%
Lightening	13.8%*	19.0%**	4.6%*	15.9%*	7.7%*	15.1%**	-0.5%	-2.4%
Hydration (TEWL)	-7.7%	-10.2%	-5.3%	-11.8%*	-6.8%	-14.2%**	5.0%	6.5%
Moisturization	11.7%*	21.7%**	12.1%*	27.6%*	14.8%*	22.5%**	2.7%	6.6%
Firmness	0.2%	1.8%	1.6%	4.6%*	2.9%	4.9%*	-0.3%	-1.4%
Wrinkle reduction	-3.0%*	-10.0%**	-3.9%*	-9.3%**	-7.5%*	-9.4%**	-1.3%	-1.2%
Skin smoothness	17.6	32.6*	16.1%	32.1%***	18.8%*	26.8%***	4.5%	-1.3%
Skin thickness	13.7%	30.7%*	22.3%	24.2%*	6.9%	18.8%**	-0.9%	-2.3%

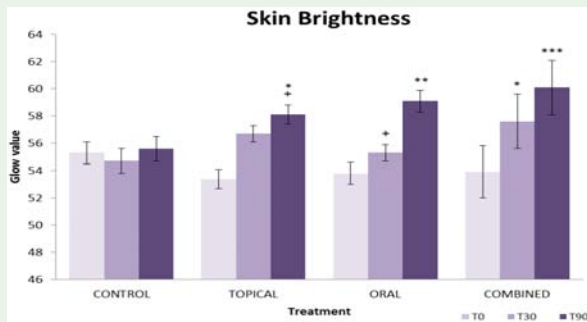


Figure 1: Improvement of skin brightness (Glow Value) achieved with the different treatments. Statistical significances of the changes experienced at each time point in each group compared to control group are shown as * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. Statistical significances of the changes experienced by group C (combined treatment) compared to topical and oral regimens are shown as * $p \leq 0.05$.

reduction (+9.3%) (both, $p \leq 0.01$), and skin smoothness (+32.1%, $p \leq 0.001$). Statistical significances for all the parameters are shown in Table 6.

Combined treatment (C) deserves a special mention. In general, the subjects with this regimen reached higher percentages of improvement in most of the studied parameters compared to single treatments. In particular, the combined treatment was better than monotherapy improving skin brightness (+11.6%), hydration (+14.2%) and firmness (+4.9%). In addition, regime (C) showed better outcomes in most parameters at T30 than the other regimens.

Objective parameters

The analysis of **pigmentation parameters** of each group yielded the following results:

Skin brightness: A significant increase ($p \leq 0.05$) of skin brightness was achieved in all groups with active ingredients vs control after 90 days of treatment, reaching the best improvement of skin brightening (+11.6%, $p \leq 0.001$) with the combined regimen (C). The three regimens were also better than control at T30, however this improvement was only statistically significant in the combined

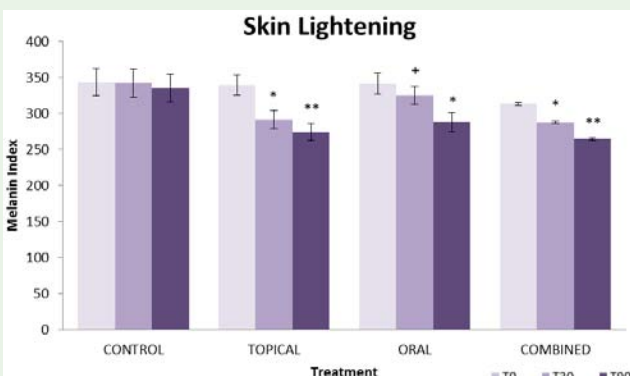


Figure 2: Improvement of skin lightening (melanin index) achieved with the different treatments. Statistical significances of the changes experienced at each time point in each group compared to control group (D) are shown as * $p \leq 0.05$; ** $p \leq 0.01$. Statistical significances of the changes experienced by combined treatment (C) compared to regimen (B) are shown as * $p \leq 0.05$.

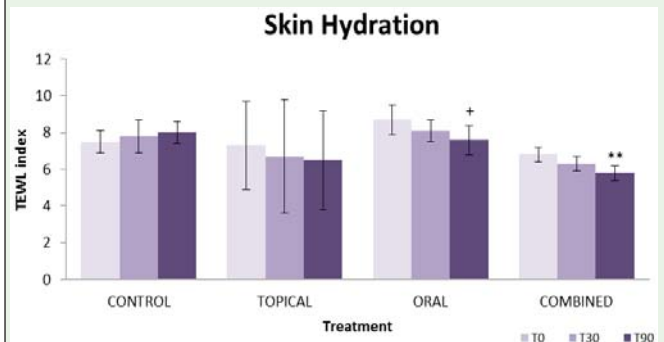


Figure 3: Improvement of skin hydration (TEWL index) achieved with the different treatments. Statistical significances of the changes experienced at each time point in each group compared to control group (D) are shown as ** $p \leq 0.01$. Statistical significances of the changes experienced by combined treatment arm (C) compared to regimen B are shown as * $p \leq 0.05$.

regime ($p \leq 0.05$). In addition, combined regime was significantly better than oral treatment at T30 ($p \leq 0.05$) and the topical treatment at T90 ($p \leq 0.05$) (Figure 1).

Skin lightening: The three active regimens achieved significant improvement of skin lightening at T90 ($p \leq 0.01$ for regimens A and C) compared to control. Combined treatment promoted a faster reduction of the melanin index than the oral treatment alone as soon as T30 ($p \leq 0.05$). Interestingly, topical regime alone (A) reached the best results, even when compared with the combined regime, although this difference did not reach statistical significance (Figure 2).

Globally, the melanin content of skin blotches reached a reduction of 16.6% in groups (A), (B) and (C). The pigmentation on topical regime (A) decreased 13.8% at T30 and 19.0% at T90. Oral regime (B), decreased 4.6% at T30 and 15.9% at T90. Combined regime obtained a mean reduction of 7.7% at T30 and 15.1% at T90.

The evaluation of anti-aging parameters was included as a secondary objective, yielding the following results.

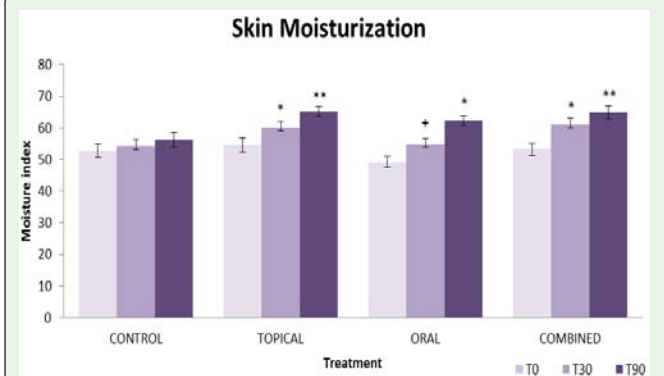


Figure 4: Improvement of skin moisturization (moisture index) achieved with the different treatments. Statistical significances of the changes experienced at each time point in each group compared to control group (D) are shown as * $p \leq 0.05$; ** $p \leq 0.01$. Statistical significances of the changes experienced by combined treatment (C) compared to regimen (B) are shown as * $p \leq 0.05$.

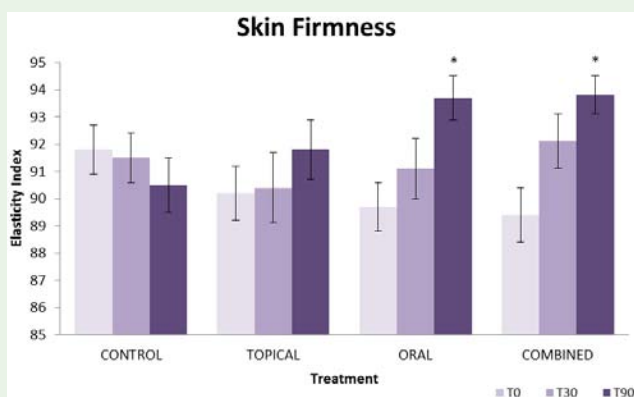


Figure 5: Improvement of skin firmness (elasticity index) achieved with the different treatments. Statistical significances of the changes experienced at each time point in each group compared to control group (D) are shown as * $p \leq 0.05$.

Skin hydration: Regimens (A), (B) and (C) showed an improvement of hydration, in correlation with a significant decrease of TEWL, which only reached statistical significance with the combined treatment (C) at T90 (14.2%, $p \leq 0.01$) (Figure 3).

Skin moisturization: A significant increase of cutaneous moisturization was reached in all the active groups at T90 compared to control group ($p \leq 0.01$ for regimens A and C). Furthermore, regimens (A) and (C) showed a significant increase of moisturization as soon as T30 ($p \leq 0.01$ vs control). In fact, regime (C) gave the best results at T30 with a moisturization improvement of 14.8%. Interestingly, the best results were obtained with regime B at T90, which ameliorated moisturization an average 27.6% ($p \leq 0.01$ vs control. No statistical differences vs other groups) (Figure 4).

Skin firmness: This parameter showed the slightest improvement of all the evaluated signs of aging. In fact, in the control group (D) it was observed a statistical significant worsening compared to baseline. However, the oral and combined regimen (B and C) showed significant improvement in the elasticity index compared to baseline and also to the control group at T90 ($p \leq 0.01$). Combined regime also provided a quicker response than mono therapy regimens, showing a greater improvement at T30, although it did not reach statistical significance (Figure 5).

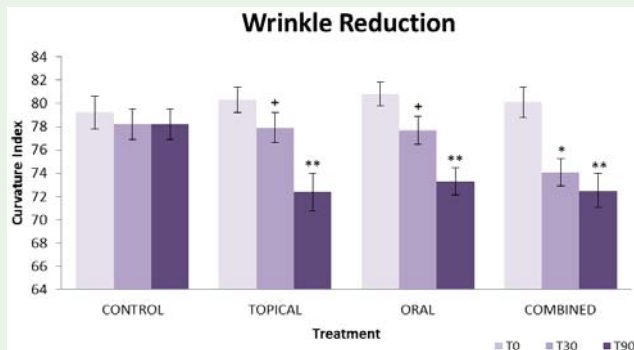


Figure 6: Wrinkle reduction achieved with the different treatments. Statistical significances of the changes experienced at each time point in each group compared to control group (regime D) are shown as * $p \leq 0.05$; ** $p \leq 0.01$. Statistical significances of the changes experienced by combined treatment arm (C) compared to regimens (A) and (B) are shown as * $p \leq 0.05$.

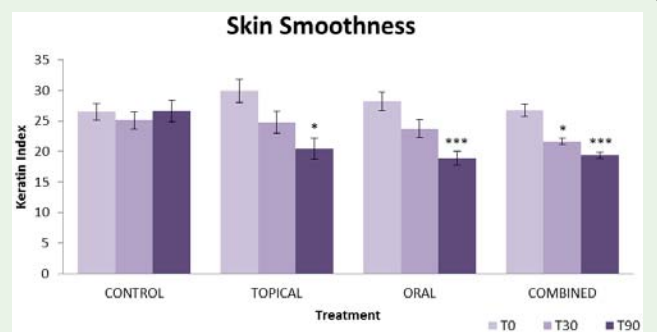


Figure 7: Improvement of skin smoothness (keratin index) achieved with the different treatments. Statistical significances of the changes experienced at each time point in each group compared to control group (regime D) are shown as * $p \leq 0.05$; *** $p \leq 0.001$.

Wrinkle reduction: Subjects treated with any of the active regimens (A, B, C) showed a significant reduction of wrinkles at T90 compared to control group ($p \leq 0.01$), as demonstrated by the decrease of the curvature index. The combined therapy (C) showed a significant reduction of wrinkles compared to control after only 30 days of treatment ($p \leq 0.05$ vs control), which even showed statistical difference with regimens A and B (Figure 6).

Skin smoothness: The entire active regimens diminished the keratin index at T90, demonstrating a recovery of skin smoothness. This was already visible and statistically significant at T30 in the combined treatment group (C), when there was an improvement of 18.8% compared to baseline ($p \leq 0.05$) (Figure 7).

Skin thickness: The measurement of collagen intensity showed that all the active regimens provided a significant increase of dermal collagen at T90, improving skin thickness and quality. The best outcomes were obtained with the topical treatment, which achieved a 30.7% increase of skin thickness ($p \leq 0.01$ vs control), while the oral regime resulted in the fastest results (22.3% improvement after only 30 days) (Figure 8).

Subjective parameters

Area of involvement of skin blotches: Evolution of the area of skin blotches between baseline and the end of the treatment was positively scored by the investigators in those subjects with regimens (A), (B)

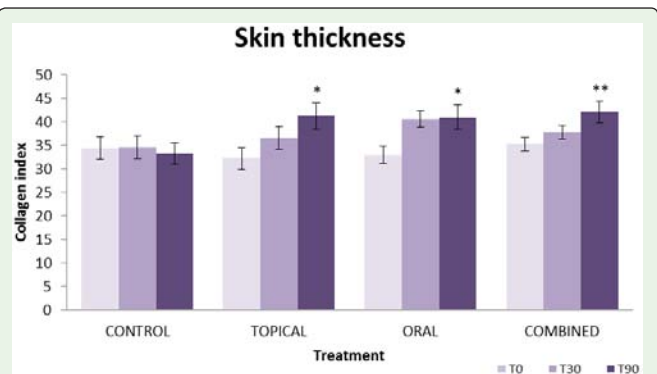


Figure 8: Improvement of skin thickness (collagen intensity) achieved with the different treatments. Statistical significances of the changes experienced at each time point in each group compared to control group (regime D) are shown as * $p \leq 0.05$; ** $p \leq 0.01$.



Figure 9: Global improvement of the area of involvement and intensity of skin blotches and complexion after 90 days of treatment. 75% of the patients of the active regimens compared to just 25% of the patients of the control group showed a clinical improvement of their skin condition.

and (C), ($p \leq 0.05$ at T30 and $p \leq 0.001$ at T90). Control group (D) experienced a significant worsening of skin blotches at T30 ($p = 0.048$), which persisted at T90.

Intensity of skin blotches: Subjective assessment of the intensity of skin blotches between baseline and the end of the study was positively scored in all regimens except control group. In the same line as the area of involvement, intensity of skin blotches was reduced in regimens (A), (B) and (C), reaching a statistical significance for topical and combined treatment at T30 ($p \leq 0.05$), and at T90 in all groups ($p \leq 0.01$). Again, regime (D) showed a non-significant worsening at both time points.

Improvement of aging signs: There was observed a high number of subjects with improvement of wrinkle reduction at T90 under the combined regime (94%), followed by oral treatment (80%) and topical treatment (78%). Almost 52% of subjects with topical treatment (A) and 45% under oral treatment (B) reached an improvement of skin firmness at T90 according to the investigator, which was detected in 100% of the subjects under combined treatment (C).

Global Improvement: Active treatment regimens (A, B, and C) showed a statistically significant global improvement at T90, (topical and combined, $p \leq 0.05$; oral, $p \leq 0.01$). According to these results, 75% of the patients under active treatment regimens showed a significant reduction of the affected area compared to the control group ($p = 0.000$). Regarding the intensity of the pigmentation, 35% of the patients belonging to the treated groups improved, compared to 0% in the control group within 30 days ($p = 0.002$). After 90 days of treatment, it was observed an improvement of skin pigmentation in 60% of patients ($p = 0.000$) (Figure 9).

Thus, the results of the objective measurements assessing the efficacy of the active regimens correlates perfectly with investigator and patient self-perception.

The degree of improvement perceived by the patient based on the questionnaire was satisfactory in 73% of patients under topical

treatment (A), 60% under oral treatment (B) and up to 95% of subjects under the combined regimen (C).

Discussion

The well-established principle for skin protection against photo aging begins by avoiding excessive sun exposure and a routine use of suitable sunscreen in order to prevent the damage caused by UVB, UVA and visible light as many studies have demonstrated so far. Hyper pigmentation, a more prevalent disorder in women and high skin phototypes appears in exposed areas of the face and body and may affect the individual's self-perception in a negative manner [20].

The present study has demonstrated the primary outcome of skin brightening and lightening attributed to the active treatment (topical, oral or combined) and also the secondary one, related to its antiaging benefits.

All active treatments induced statistical improvement of skin brightening and lightening parameters compared to control at T90. However, only the combined treatment reached statistical improvement of both at T30. This faster response could be explained by the synergic effects of both treatments which would be acting through different pathways leading to the homogenization of skin pigmentation.

Neoretin® has demonstrated in previous studies to improve pigmentation disorders as it showed a significant MASI decrease compared to vehicle, after 30 and 90 days of treatment [8].

Notably, the split-face placebo-controlled study of Truchuelo et al. performed in 30 Caucasian women with melasma showed a statistically significant MASI improvement of 70% after 3 months of treatment on the treated side compared to the control side, which is equivalent to the percentage of improvement described with hydroquinone [8,9]. Cameli et al. also demonstrated the beneficial effects of this ingredient in the correction of antiaging and pigmentary disorders [10].

In previous studies, Fernblock®, the oral treatment, demonstrated to reduce the pigmentation after solar exposure by showing a statistical increase of the Minimum Pigmentary Dose (MPD) [21]. These previous data are in accordance with the results showed in the present study.

Although it has been described that sunscreen controls skin pigmentation [22], our data showed that in the control group, which applied a vehicle formulated with SPF 50, none of the pigmentation parameters improved, supporting the need of active ingredients to improve these parameters.

According to the rest of anti-aging parameters assessed, we should highlight the beneficial effects over skin hydration in all three regimens of treatment (A, B and C). However, it only reached statistical significance with the combined regimen at T90. Control group experienced worsening in TEWL parameter. Altogether would suggest that the active ingredients could be helpful in decreasing the TEWL and so, increasing skin hydration. The complexity of the skin barrier and the intrinsic mechanisms would explain the need of combining different treatments (oral and topical) for a long period (90 days) to improve significantly the TEWL. The antiaging and photoprotective properties of Fernblock® [17,18], would prevent skin barrier degradation so a functional stratum corneum will avoid water loss. In addition, Fernblock® also inhibits dermal degradation; therefore a certain amount of water will be correctly aggregated by GAGs. The moisturizing components of neoretin help in this process.

Skin moisturization improved in all three active treatments, reaching a significant and fast improvement in just 30 days of treatment (topical and combined treatments). Even the oral regimen group reached significant improvement after 90 days of treatment. The control group did not show significant improvement of the moisturization parameter. Regarding these results, skin moisturization would be more responsive to treatment than skin hydration.

Regarding firmness evaluation, significant improvement was reached with the oral and combined regimens at T90. No significant improvement was reached with the topical regimen alone, suggesting the importance of the oral treatment acting at dermal level, as firmness is correlated with collagen and elastic fibers maintenance. As expected, the changes in skin firmness took three months, suggesting that it could be necessary to stimulate the mechanisms of synthesis and other indirect processes to obtain measurable benefits in this parameter. Fernblock® has demonstrated to be able to induce the synthesis of collagen (I, II and V) [23], stimulate TIMPS 1 and 2 in fibroblasts and inhibit MMPs (1,2 and 3) [24,25]. Fernblock® also promotes the expression of elastin in dermal fibroblasts and keratinocytes [24,25]. It also exerts high antioxidant activity that regulates extracellular matrix proteins [25-27].

About the evaluation of wrinkles, significant improvement with all the three active regimens was observed. This improvement was statistically faster with the combined regimen at T30 compared to placebo, topical or oral regimen alone. These results would suggest the synergic effect of the technologies presented in the oral and the topical formulations stimulating the epidermal and dermal renewal that would lead to the wrinkle appearance improvement within just one month [8, 25-27].

The collagen index (skin thickness) also reached significant improvement on T90 with all three regimens of active treatment, and a non-significant worsening in the control group. This would be directly correlated with the improvement of firmness.

The skin smoothness reached significant improvement on T90 with all three active regimens, but faster improvement was observed with the combined treatment. Once again, the synergic action provides obvious benefits on the skin surface [8,28].

According to the subjective evaluation performed by the investigator to assess the global improvement, all parameters were improvement after treatment. Based on the patient's evaluation, the combined treatment reached the best results, followed by topical and oral regimens.

Conclusions

Significant improvement of skin brightness, lightening, moisturization, smoothness, thickness and wrinkle reduction was achieved after topical, oral and combined treatment based on Retinsphere® and Fernblock® technologies in Filipino women.

The combined regimen (topical and oral), reached a faster improvement in most of the assessed parameters than monotherapy. In addition to its efficacy, this regimen was safe and well-tolerated.

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