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ORIGINAL CONTRIBUTIONS

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An open-label, investigator-initiated, single-center, prospective, pilot clinical study to evaluate the efficacy of a skin whitening serum applied twice daily combined with a spot-preventing SPF50+ sunscreen in healthy female subjects with melasma hyperpigmentation

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Abstract

Background: Melasma is a common skin disorder characterized by alterations in normal skin pigmentation. The objective was to evaluate the efficacy and safety of a skin whitening serum containing niacinamide, hydroxyphenoxy propionic acid, dipotassium glycyrrhizate, glycolic acid, and 4-n-butylresorcinol applied twice daily combined with a spot-preventing SPF50+ sunscreen for treatment of melasma.

Methods: Twelve healthy Caucasian women with melasma (Fitzpatrick skin types II–IV) were enrolled in this pilot clinical study. Efficacy evaluations were performed at baseline and weeks 4, 8, and 12 of treatment and included clinical and instrumental assessments.

Results: All endpoints for melasma hyperpigmentation showed a statistically significant improvement from baseline to the end of the study. There was only one dropout. No signs of irritation or discomfort were observed at baseline, w4, w8, or w12. An overall improvement in melasma was observed both clinically and on reflectance confocal microscopy (RCM).

Conclusion: This topical skin whitening serum had favorable outcomes for the treatment of melasma hyperpigmentation in adult women, as demonstrated on investigator and instrumental assessments. The results of this pilot study need to be confirmed in randomized, controlled studies with a larger sample size.

KEYWORDS
4-n-butylresorcinol, dipotassium glycyrrhizate, glycolic acid, melasma, niacinamide

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1 | INTRODUCTION

Melasma is a disorder of skin pigmentation characterized by the development of asymmetrical, hyperpigmented macules in sun-exposed areas, especially the upper lip, the cheeks, the forehead, and neck.[1] It is particularly common in women aged approximately 20–45 years old and in people of Hispanic, Oriental, and Indo-Chinese origin, or skin types IV through VI.[2] The development of this disorder may be attributed to sun exposure, genetic predisposition, pregnancy, oral contraceptives, and certain antiepileptic drugs. There are three main clinical patterns of melasma: centrofacial, malar, and mandibular; while four types exist based on Wood's light (320–400 nm) examination: epidermal, dermal, mixed, and intermediate.[3]

Treatment can be difficult as long-term therapy is often required and recurrence is common. The first line of treatment is the elimination of risk factors and topical treatment based on sun protection and products aimed at inhibiting melanocyte activity and melanin synthesis, disrupting melanin granules, and removing melanin. The second and third lines of treatment include chemical peels, lasers, and lights.[4] Patients with melasma hyperpigmentation are often enrolled in clinical studies to evaluate whitening or depigmenting products.

The aim of this study was to assess the efficacy and safety of a topical serum containing 4-n-butylresorcinol, hydroxyphenoxy propionic acid, niacinamide, glycolic acid, and dipotassium glycyrrhizate together with a spot-preventing SPF50+ sun protection (measured protection: UVB factor 100, UVA factor 61) containing hyaluronic acid and allantoin, in the treatment of melasma hyperpigmentation. Efficacy parameters included clinical grading, instrumental evaluations, self-assessment questionnaires, and subjective cutaneous tolerability assessment.

2 | MATERIALS AND METHODS

The study was conducted between January 2019 and March 2019 at the University of Naples "Federico II," Department of Clinical Medicine and Surgery, Section of Dermatology. Due to the cosmetic, rather than medicinal nature of the product, ethics committee approval was not required. Nonetheless, the study was conducted in accordance with the principles of good clinical practice and the Declaration of Helsinki and its updates.

The study involved 12 women aged 30–60, with skin phototypes II to IV (Fitzpatrick), who provided written informed consent for study participation and use of photographs.

The inclusion criteria were women aged ≥30 years with moderate hyperpigmentation or dark spots, moderate facial aging, good state of health, absence of skin disease other than melasma, absence of ongoing topical or pharmacological treatments, and no history of atopy. The exclusion criteria were pregnant or breastfeeding women, and allergies or sensitivity to cosmetic products.

Participants were instructed to apply the study product to the face twice daily (1–2 ml/d) for 3 months. Volunteers were asked to apply the spot-preventing SPF 50+ sunscreen in the morning and throughout the day. Other topical or systemic treatments for

TABLE 1 Investigator global assessment (IGA) of hyperpigmentation

Score	Grade	Definition
0	Clear	No brown spots or areas of discoloration
1	Almost Clear	Overall, there are a few brown spots with increased pigmentation; they are very small in size and only very slightly darker than surrounding skin
2	Mild	Several brown spots with increased pigmentation; they are small in size and slightly darker than surrounding skin
3	Moderate	Many brown spots with increased pigmentation; they are medium in size and much darker than surrounding skin
4	Severe	Many large brown spots with increased pigmentation; they are large in size and markedly darker than surrounding skin

TABLE 2 Investigator global assessment (IGA) of aging

Score	Grade	Definition
0	None	No signs or symptoms present
1	Almost None	Very mild signs of facial aging and photodamage
2	Mild	Mild signs of facial aging and photodamage
3	Moderate	Moderate signs of facial aging and photodamage
4	Severe	Severe signs of facial aging and photodamage

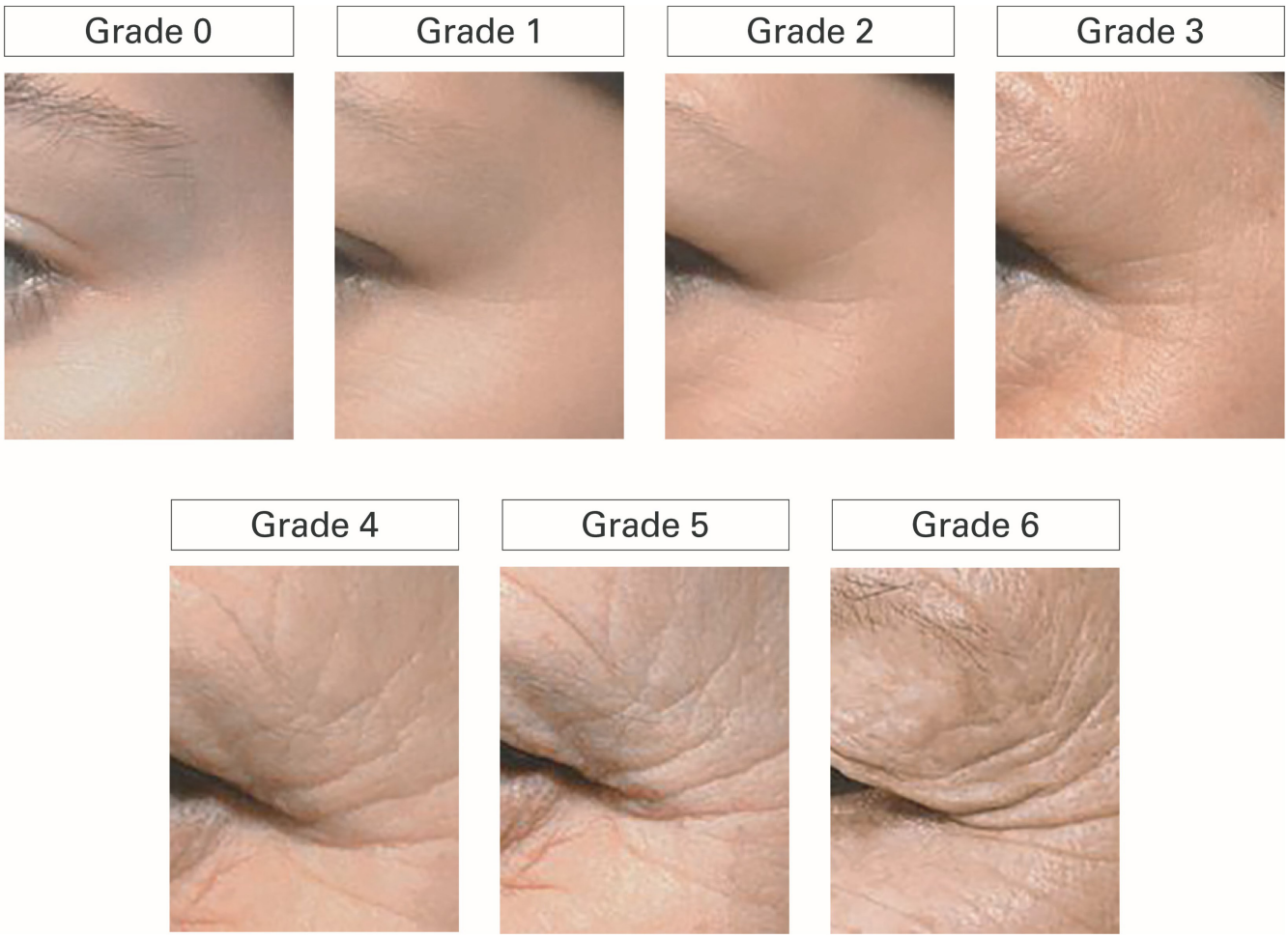


FIGURE 1 Bazin wrinkle scale: crow's feet

TABLE 3 GAIS and SGAIS: objective and subject assessment of improvement

Rating	Description of change
−1	Worse
0	No change
+1	Mild improvement
+2	Moderate improvement
+3	Significant improvement

melasma hyperpigmentation and other diseases were not permitted during the study period.

Clinical and instrumental assessments included, at baseline and weeks 4, 8, and 12 of treatment: (a) standardized clinical photography by Visia™ photo imaging system, (b) melasma area and severity index (mMASI),[1] (c) investigator global assessment (IGA) of hyperpigmentation (Table 1), (d) investigator global assessment (IGA) of photodamage (Table 2) which qualitatively measures the degree of improvement after therapeutic intervention,[5] (e) crow's feet evaluation according to Bazin' scale (Figure 1),[6] to evaluate potential anti-aging properties of the serum, and (f) skin quality assessment—investigator & subjective; and at weeks 4, 8, and 12 of treatment only: (g) global aesthetic improvement (GAIS) (Table 3), (h) subject global aesthetic improvement (SGAIS) (Table 3), and (i) subjective questionnaire on cosmetic qualities and treatment satisfaction (1 = very satisfied, 2 = satisfied, 3 = dissatisfied, 4 = very dissatisfied).

The total mMASI score was calculated using the following formula (darkness (D) + homogeneity (H)) × area (A) of involvement (total scoring from 0 to 48 points). A was rated on a scale of 0–6 (0: no involvement; 1 = <10% involvement; 2 = 10–29% involvement; 3 = 30–49% involvement; 4 = 50–69% involvement; 5 = 70–89% involvement; 6 = 90–100% involvement). D and H were rated on a scale of 0–4 (0 = absent; 1 = slight; 2 = mild; 3 = marked; and 4 = maximum). A factor was assigned to each area (forehead 0.3; right malar 0.3, left malar 0.3, chin 0.1).[5]

Skin quality assessment, investigator and subjective, included grading for radiance, smoothness, pigmentation, erythema, pore

FIGURE 2 mMASI

size, skin clarity, skin brightness, skin tone, luminosity, and skin complexion (total score from 1 to 10).

In vivo RCM was performed using a VivaScope® 3000 (Caliber Imaging and Diagnostics). This non-invasive imaging modality

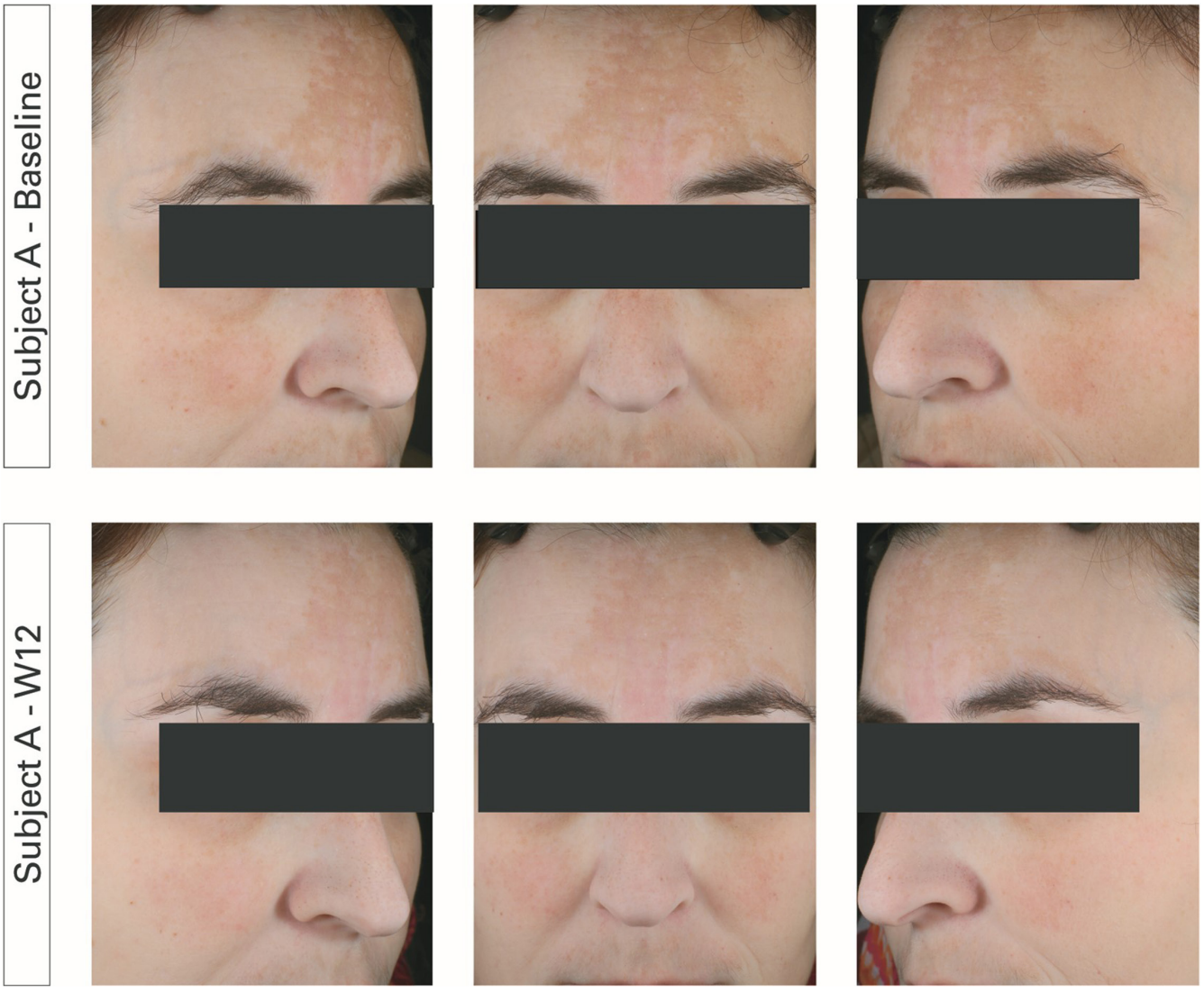


FIGURE 3 Clinical photographs

FIGURE 4 IGA hyperpigmentation

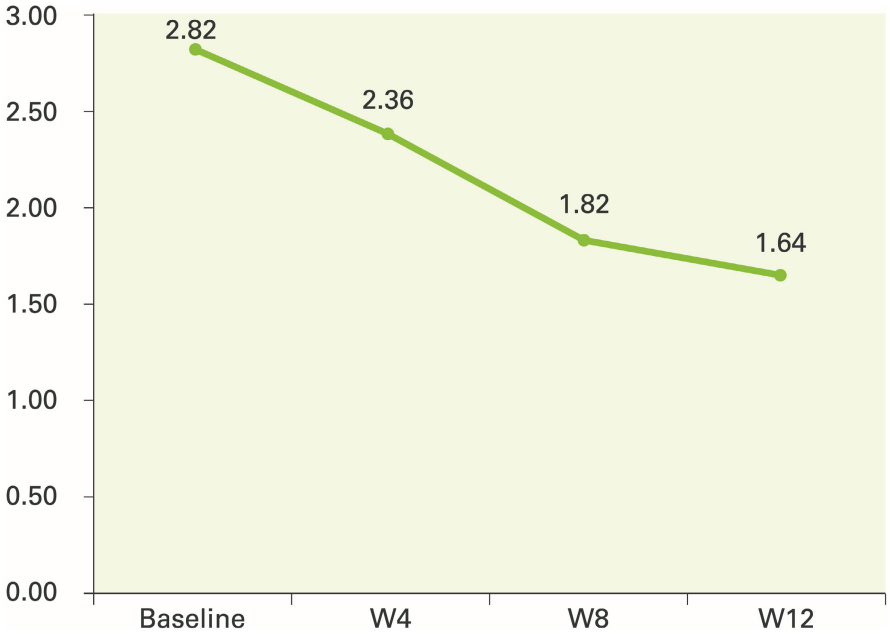
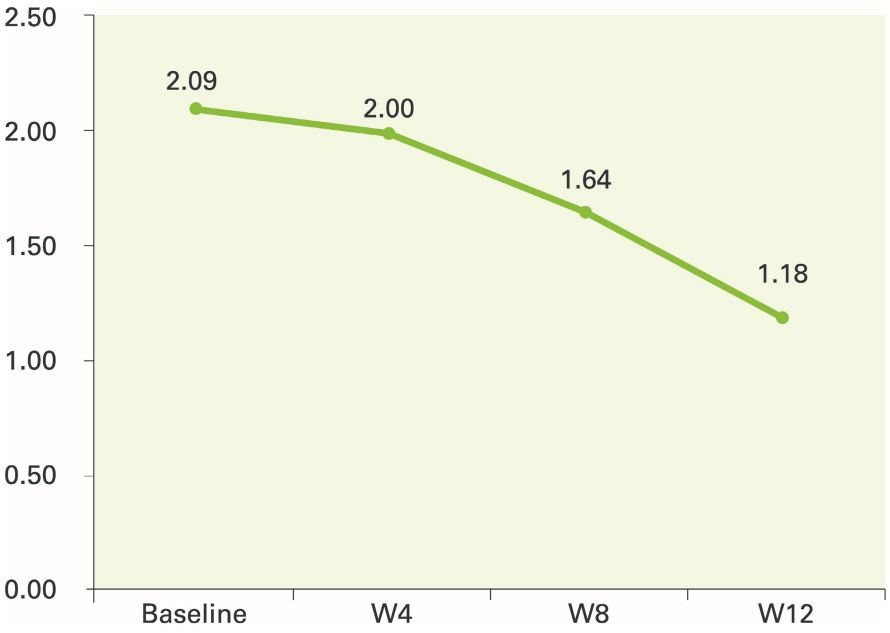


FIGURE 5 IGA photodamage



provides real-time images at a nearly cellular resolution level (1). Typically, RCM of epidermal melasma shows a significantly increased hyper-refractile cobblestone pattern at the level of the basal cell layer and an inflammatory infiltrate. Dendritic cells and melanophages at the level of the dermo-epidermal junction can also be observed, especially in mixed or dermal melasma.[1]

2.1 | Statistical analysis

Results were presented as mean ±standard deviation. The comparisons between time points were carried out using paired t test for continuous data and chi-square test for categorical data. Statistical significance was set at p-values <0.05.

3 | RESULTS

Eleven patients completed the study and one dropped out, due to unavailability. The mMASI score decreased significantly from 21.73 ± 12.15 at baseline to 7.55 ± 5.09 at the end of the study ($p = 0.0002$) (Figure 2, change in mMASI). Of note, a statistically significant reduction in the mean mMASI score was also observed at W4 (14.00 ± 7.71 vs 21.73 ± 12.15 at baseline, $p < 0.0013$) and W8 (11.27 ± 6.08 vs 21.73 ± 12.15, $p < 0.0054$). The difference between W12 and baseline was 14.18 points (Figure 3).

Figure 4 (IGA hyperpigmentation) shows the distribution of IGA pigmentation score. We observed a statistically significant improvement at W8 (1.82 ± 0.60, $p < 0.0016$) and W12 (1.64 ± 0.50, $p < 0.001$) vs baseline (2.82 ± 0.87). Likewise, a statistically significant

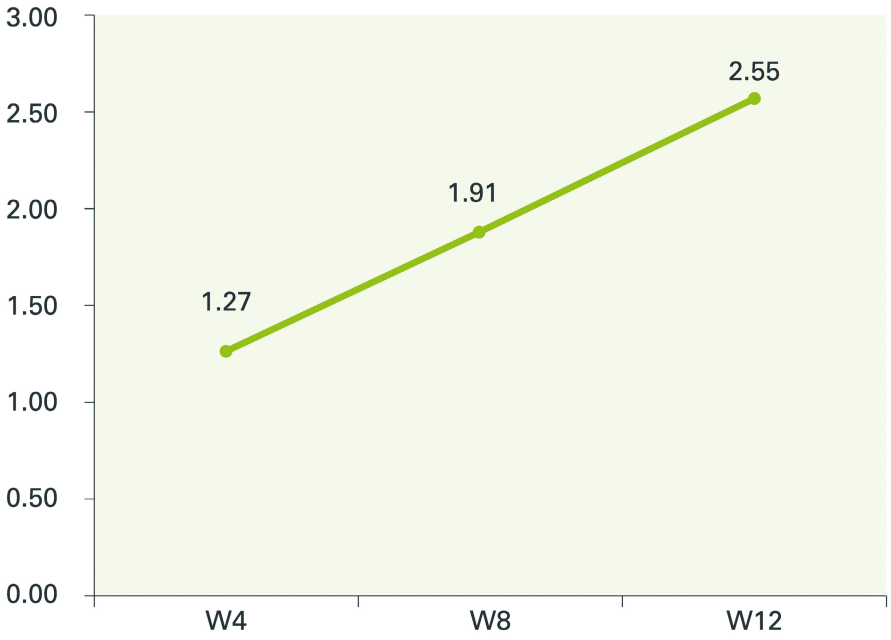


FIGURE 6 GAIS.

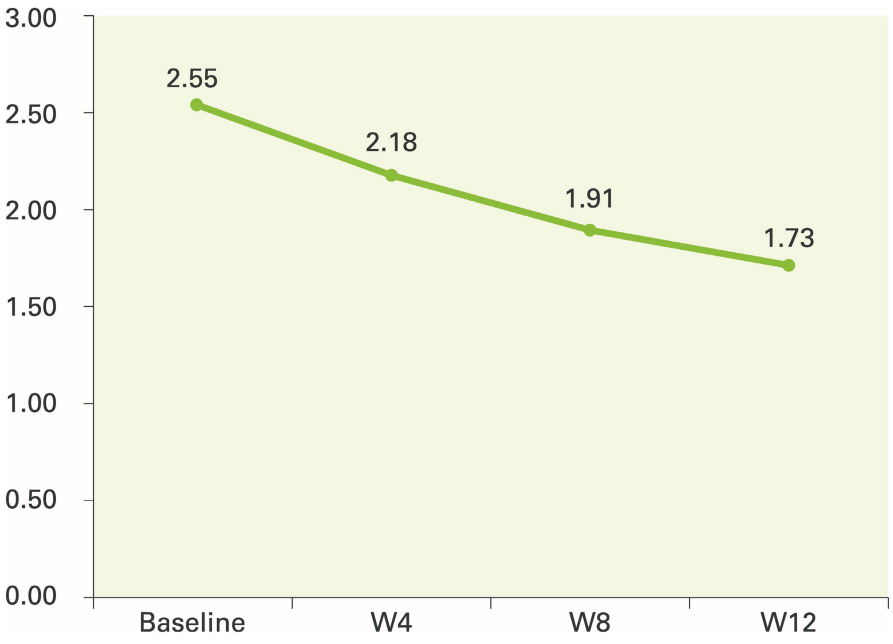


FIGURE 7 Bazin wrinkle scale

improvement at W8 (1.64 ± 0.50, $p < 0.0162$) and W12 (1.18 ± 0.40, $p < 0.0016$) vs baseline (2.09 ± 0.83) was observed for IGA photodamage (Figure 5).

The results of GAIS revealed a significant improvement from baseline at W4, W8, and W12 (1.27 ± 0.47, 1.91 ± 0.70, and 2.55 ± 0.52, respectively, $p < 0.0001$ for all) (Figure 6). At W12, the mean score was 2.55 out of a maximum score of 3; 45.5% of the subjects had a moderate improvement, and 54.5% had a significant improvement (the maximum score). All subjects had some improvement even at W4 of treatment.

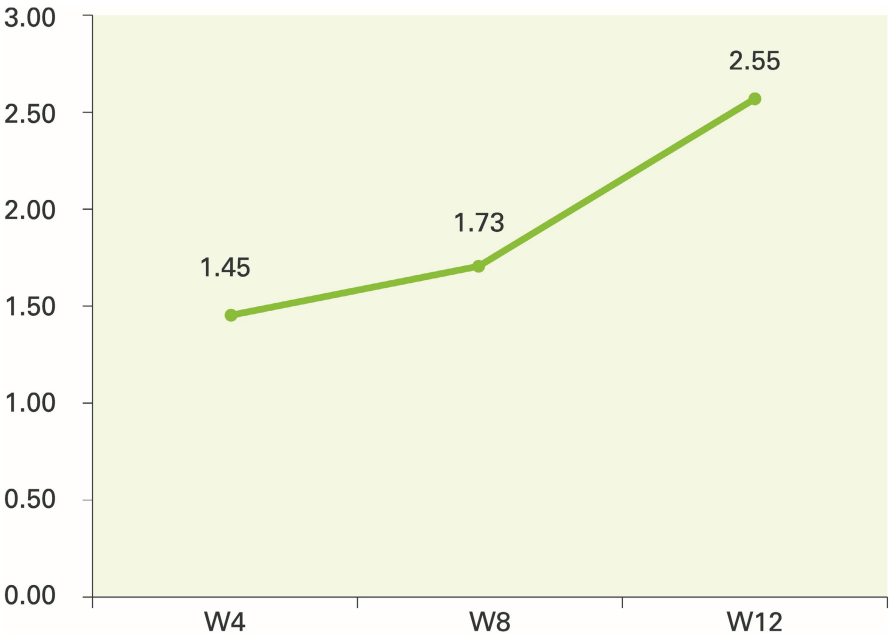
Figure 7 shows the distribution of Bazin wrinkle scale. The results at W4, W8, and W12 (2.18 ± 0.75, 1.91 ± 0.83, and 1.73 ± 0.90, respectively) were statistically significantly different ($p = 0.0379$, $p = 0.0300$, and $p = 0.0082$, respectively) compared with the assessment at baseline (2.55 ± 1.04).

SGAIS revealed a significant improvement at W4, W8, and W12 (1.45 ± 0.93, $p = 0.004$; 1.73 ± 0.47, $p < 0.001$; 2.55 ± 0.69, $p < 0.001$). The distribution of results is shown in Figure 8. At W12, 63.6% of the subjects noted a marked improvement, giving the maximum score (significant improvement).

In addition, all skin quality parameters assessed by the investigator and subjects showed a marked trend of improvement from baseline to week 12 (Figure 9).

An overall improvement in melasma hyperpigmentation was noticed both clinically and on reflectance confocal microscopy (RCM). The improvement in melasma could be detected on RCM with a

FIGURE 8 SGAIS



reduction of inflammatory infiltrate from baseline (inflammatory cells could be recognize in Figure 10A by green arrow) to the end of treatment (Figure 10C).

Moreover, a reduction in melanophages and dendritic cells in the papillary dermis was also detected (Figure 10).

The treatment satisfaction scale indicated that at W8, 100% were satisfied and at W12 72.7% were very satisfied with the treatment (Figure 11).

No adverse events, signs of intolerability, or reported sensations of discomfort were observed for any of the subjects.

4 | DISCUSSION

Melasma (also known as chloasma or the pregnancy mask) is a benign, acquired disorder of pigmentation. Diverse etiological factors can lead to increased melanogenetic effects resulting in symmetrical hyperpigmentation. To date, melasma and hyperpigmentation treatment remains a challenge because of its chronicity and recurrence rate. Several therapeutic modalities for therapy have been proposed, including topical and oral agents, chemical peels, and laser.

In this study, the serum containing 4-n-butylresorcinol, hydroxyphenoxy propionic acid, niacinamide, glycolic acid, and dipotassium glycyrrhizate along with sun protection has been shown by both clinical and objective instrumental assessments to produce significant improvement in hyperpigmentation disorders after 12 weeks of treatment.

4-n-butylresorcinol (4nBR) inhibits melanogenesis by enhancing proteolytic degradation of tyrosinase and inhibiting the activity of tyrosinase and tyrosinase-related protein-1 (TRP-1).[7-10] Hydroxyphenoxy propionic acid inhibits melanin production and blocks the transfer of melanosomes to keratinocytes, reducing hyperpigmentation.[11] Niacinamide has been demonstrated to be effective in reducing hyperpigmentation, including melasma, also by suppressing melanin transfer from melanocytes to keratinocytes.[12,13] Glycolic acid is the a-hydroxy acid (AHA) with the smallest molecular size and is able to penetrate the skin, decreasing the stratum corneum barrier, promoting cell proliferation, and stimulating collagen production.[14] Thanks to its exfoliating action, GA allows the penetration of depigmenting products. Dipotassium glycyrrhizate is the dipotassium salt of glycyrrhizin which is a key component in licorice root extract with anti-inflammatory and skin soothing properties.[14-16]

To our knowledge, this is the first report of therapy with 4-n-butylresorcinol, hydroxyphenoxy propionic acid, niacinamide, glycolic acid, and dipotassium glycyrrhizate. Clinical and instrumental evaluation illustrate the effectiveness of this combination which can be used as an effective and safe treatment for melasma hyperpigmentation.

This topical formulation was demonstrated to be effective through multiple clinical and instrumental assessments (VISIA, MASI, IGA of photodamage and hyperpigmentation, GAIS, Bazin wrinkle scale, skin quality assessment, and RCM). The topical whitening serum was well tolerated and gave excellent cosmetic acceptability results in over 90% of the population. The spot-preventing sunscreen SPF50+ was also well tolerated.

5 | CONCLUSION

In this pilot clinical study, the skin whitening serum has shown a rapid clinical efficacy in reducing melasma hyperpigmentation, with good tolerability. These promising results need to be confirmed in randomized, controlled studies with a large sample size.

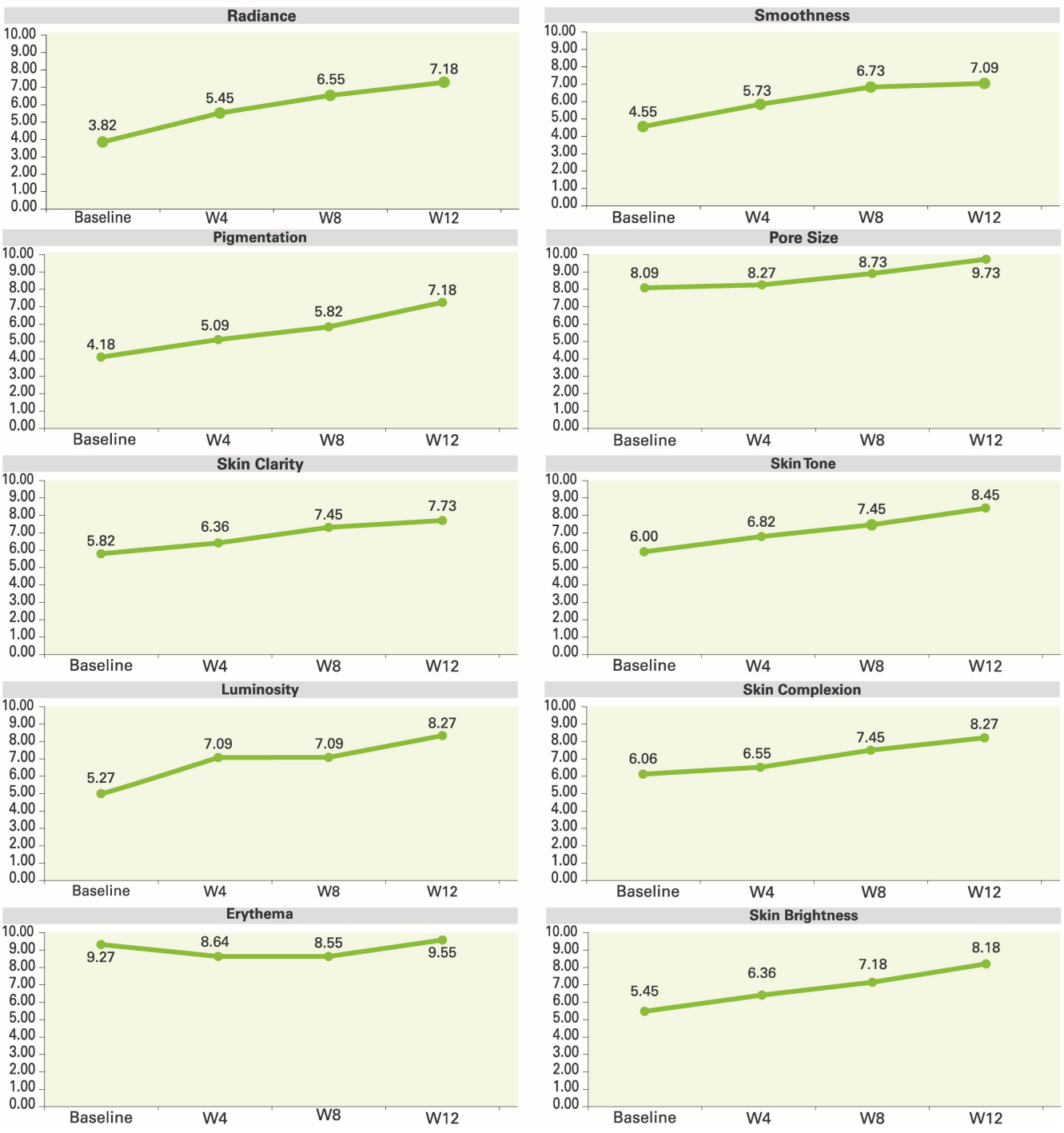


FIGURE 9 Skin quality parameters

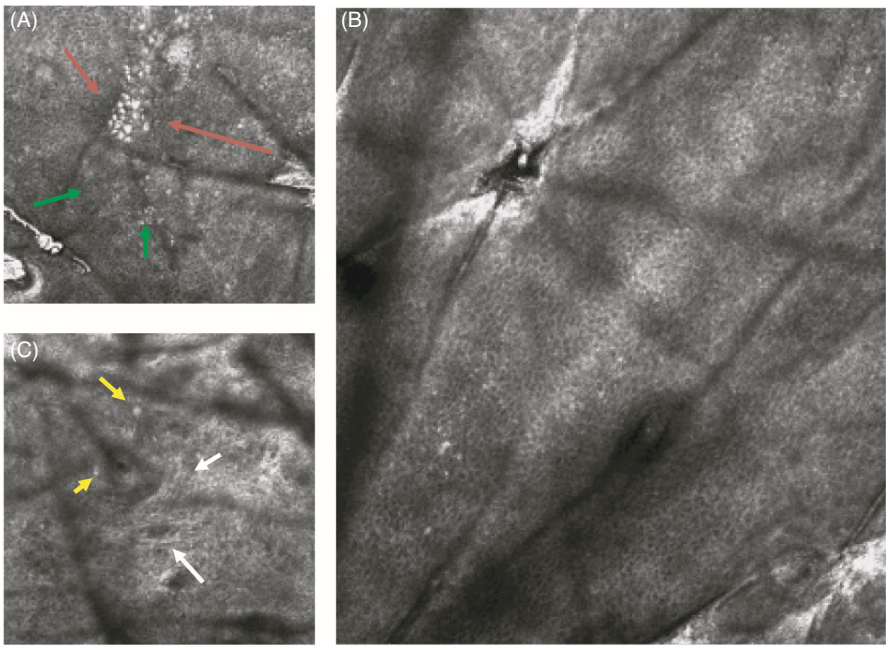


FIGURE 10 RCM evaluation at baseline and at W12

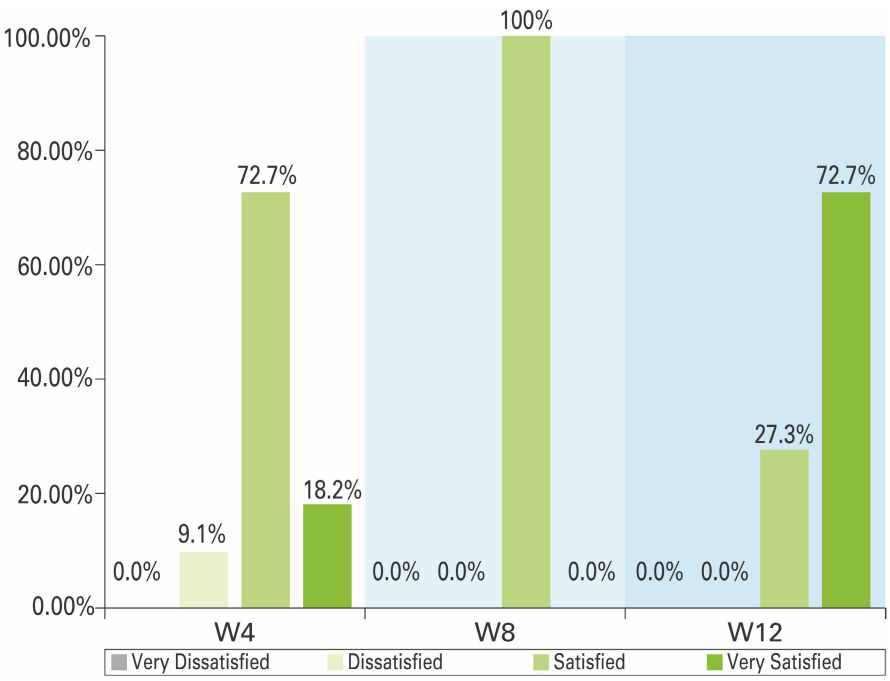


FIGURE 11 Treatment satisfaction scale

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AUTHOR CONTRIBUTIONS

MC, MF, and GF none declared; CG is employed by ISDIN.

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