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ORIGINAL ARTICLE

Multicenter evaluation of a topical antioxidant serum

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Abstract

Background: A new antioxidant serum has been formulated with sodium ascorbate, a sodium salt of Vit C, which aims to address facial photodamage while maintaining a low irritation profile and preserving elastin. Detailed background science has been submitted in a previous publication. This open-label study was conducted to validate the science by demonstrating product efficacy and tolerability in patients with moderate to severe facial photodamage.

Methods: A multicenter, open-label clinical study was undertaken over 5 months from March 2023 to July 2023. Thirty six eligible participants (35 female, 1 male), aged 38–69 years, and Fitzpatrick skin types II–V were enrolled into and completed the study following 12 weeks of the topical antioxidant serum use twice daily, along with the following supporting products (gentle cleanser, moisturizer, and sunscreen for as needed use). Follow-up visits were conducted in Weeks 2, 4, 8, and 12. At every visit, participants were evaluated for facial photodamage severity and test product tolerability. Additionally, study participants underwent subject assessments and satisfaction questionnaires, investigator assessments, biopsy collection, and photography.

Results: Significant improvements in all evaluated facial photodamage parameters were observed at 12 weeks together with excellent tolerability and subject satisfaction persisting to Week 12 at study completion. Histology most notably revealed increased elastin fibers in 5 out of 5 post 12-week treatment biopsies on Movat staining, while Herovici stains revealed stimulation of collagen and early formation of new fibers.

Conclusion: A novel antioxidant serum has demonstrated to be safe and effective for addressing facial photodamage, while stimulating the production of both elastin and collagen in the extracellular matrix (ECM).

KEYWORDS
antioxidant, elastin, L-ascorbic acid

1 | INTRODUCTION

Stimulation of reactive oxygen species (ROS) in the skin results in "oxidative stress," a major contributor to skin aging. The commonest stressor and producer of ROS is ultraviolet (UV) radiation exposure. Due to the constant exposure of the skin to UV radiation, continuous production of intrinsic antioxidants is required to neutralize the resulting ROS. However, with time, aging, and external stresses, the antioxidants become depleted, and the skin loses this important protective system.[1] Thus, there is a need for topical antioxidant replacements to support the skin against ROS exposure and oxidative stress-induced damage.

Vitamin C (Vit C) is a well-known potent antioxidant that can neutralize and remove oxidants following UV radiation exposure. This activity appears to be of particular importance in the epidermis, where Vit C is concentrated in the skin.[2] Alastin antioxidant serum (C-Radical Defense, Alastin® Skincare, Inc., Carlsbad, CA) contains sodium ascorbate (SA), a Vit C sodium salt. Ex vivo tests have demonstrated substantial equivalence of this formulation compared to a leading brand. Further, in a different ex vivo model, the new formulation containing SA actually demonstrated an elastin conserving capacity, which contrasts the elastin damage induced by L-ascorbate forms of Vit C.[3–5] The sodium alternative has been shown to facilitate entry into the skin at much smaller concentrations, in turn allowing entry of active ascorbic acid into the cell.[5] Stability and lack of irritation make it an ideal candidate as an antioxidant constituent for use in all skin types. Additionally, Alastin antioxidant serum (C-Radical Defense) has been formulated with additional potent antioxidants from hydrophilic, hydrophobic (lipophilic), and enzymatic categories.[3] These include constituents such as ergothioneine, oleuropein, centella asiatica, bisabolol, lactoferrin, phytoene, and phytofluene to name a few.[3] These ingredients work synergistically to protect each of them from direct degradation, while collectively neutralizing incurred ROS.

Accordingly, this clinical trial was undertaken to assess the efficacy and tolerability of this new topical antioxidant formulation in individuals with moderate to severe facial photodamage over a 12-week treatment period, with particular interest in evaluating the effects on skin health and assessing histological changes related to elastin status within the extracellular matrix.

2 | MATERIALS AND METHODS

This multicenter prospective, open-label study was approved by WCG IRB (Puyallup, WA). This study was conducted under applicable regulations in accordance with the principles of Good Clinical Practice. Study participants were consented prior to any study procedures. Eligible subjects were men and women ages 35–70 years presenting with moderate to severe global facial photodamage per investigator clinical grading at screening and were Fitzpatrick skin types I–VI. Additionally, eligible subjects agreed to not use any new facial topical products, withhold all facial procedures and treatments, and minimize sun and artificial light exposure, including the use of tanning beds for the duration of the study. Male subjects agreed to refrain from mustache and/or beard growth while participating in the study, and to shave their face the morning of study visit days. Individuals were excluded if they were nursing, pregnant, or planning to become pregnant, had known allergies or sensitivities to any study product ingredients, had a current dermatologic disease of their facial skin (cancerous or pre-cancerous lesions, any history of facial psoriasis, eczema, seborrheic dermatosis, vitiligo, or any other inflammatory skin condition, active acne, facial excoriations) or a history of immunosuppression/immune deficiency disorders, undergone treatment for malignancy within 1 year or diagnosed with any malignancy in the past 5 years, current use of oral antioxidant supplements, underwent facial cosmetic surgery or an ablative laser resurfacing within 12 months prior to enrollment, received botulinum toxin and/or injectable filler treatments within 6 months of enrollment, or facial cosmetic procedures (non-ablative laser resurfacing, or IPL) within 3 months of enrollment, or chemical peel(s), microdermabrasion, or microneedling treatment(s) on their facial skin within 6 weeks of enrollment, used retin-A, Rx/OTC retinoid-containing products, cosmetic products with alpha- or beta-hydroxyl acids, Vit C, hyaluronic acid, or other antiaging ingredients on their facial skin within 2 weeks of enrollment, or had any planned surgeries or invasive medical procedures scheduled to occur will participating in the study. Additionally, individuals with a history of keloid formation were excluded from biopsy participation.

Enrolled subjects completed up to six visits, including screening, baseline, and follow-up visits at Weeks 2, 4, 8, and 12. At the baseline visit, subjects were provided the antioxidant serum (C-Radical Defense, Alastin® Skincare, Inc., Carlsbad, CA) for twice daily use, and every subject was dispensed a cleanser (Gentle Cleanser, Alastin® Skincare, Inc., Carlsbad, CA and Cetaphil® Gentle Skin Cleanser, Galderma Laboratories, L.P, USA), sunscreen (SilkSHIELD SPF 30, Alastin® Skincare, Inc., Carlsbad, CA or HydraTint Pro Mineral Broad Spectrum Sunscreen SPF 36, Alastin® Skincare, Inc., Carlsbad, CA), and moisturizer (Ultra-Light Moisturizer, Alastin® Skincare, Inc.) as supporting skincare regimen products to use throughout the study. Incidence and severity of adverse events was assessed and collected at each visit.

2.1 | Photography

Standardized facial imaging, including red and brown channel, and UV filter photography was performed at each visit on clean skin, free of any study products, using a VISIA® imaging system (Canfield Scientific, Inc., Parsippany, NJ). Three different views were captured, including a frontal view and 45-degree angles on both the left and right sides of the face.

2.2 | Investigator assessments

At each visit, investigators assessed clinical grading of facial photodamage parameters (global photodamage, fine lines, coarse wrinkles, mottled hyperpigmentation, facial erythema, tactile roughness) using a 10-point modified Griffiths scale (0: none, 1–3: mild, 4–6: moderate, 7–9: severe). Additionally, at each visit, the study investigator evaluated test product tolerability to measure objective irritation parameters (dryness, scaling) incurred by subjects using a 4-point scale (0: none, 1: mild, 2: moderate, 3: severe).

Baseline patch testing was also performed to assess product related irritation and allergic reaction, in accordance with study enrollment criteria. This was performed via in-office application of the antioxidant serum test product to the center of the forehead at the baseline visit and was followed by a 15-min observation period. After the timed observation period, a final assessment for any presence and severity of study defined irritation parameters (erythema, burning, itching, edema) was measured using a 4-point scale (0: none, 1: mild, 2: moderate, 3: severe). Participants with any irritation parameters scored as a 2 (moderate) or 3 (severe) in severity were withdrawn from the study and an AE was captured.

2.3 | Subject assessments and questionnaires

Subject pre- and post-self-assessments were performed at baseline and after 12 weeks of antioxidant serum test product use to self-assess severity of facial photodamage parameters (fine lines, coarse wrinkles, hyperpigmentation, facial redness, skin roughness, uneven skin tone) using a 4-point scale (0: none, 1: mild, 2: moderate, 3: severe).

At each visit, subjects also self-evaluated and reported tolerability using a 4-point scale (0: none, 1: mild, 2: moderate, 3: severe) to assess subjective irritation parameters of facial skin (dryness, burning, stinging, itching). Additionally, subjects completed a questionnaire regarding the appearance of their skin by selecting 1 of 4 responses (very satisfied, satisfied, moderately satisfied, not satisfied), to the following statements: (1) made my skin look healthier, (2) made my facial skin tone look more even, (3) improved my facial skin texture, (4) increased the radiance (glow) and clarity of my facial skin, (5) reduced the appearance of fine lines and wrinkles on my face, (6) made my facial redness look less noticeable, (7) brown spots look less visible, (8) appear brighter, (9) facial skin feel firmer, (10) look more youthful, (11) improved the overall appearance, (12) The test product did not cause facial skin dryness, (13) skin irritation, (14) skin redness, and (15) overall satisfaction with product. At the final study visit (Week 12), Subjects were also asked (1) if they would continue using the antioxidant serum as part of their skincare regimen, and (2) if they would recommend the antioxidant serum to their family and friends by answering yes or no to each respective question.

2.4 | Biopsies

Five participants consented to having 2-mm punch biopsies collected from the right preauricular area at baseline and post 12 weeks of study product application. Participants that elected to undergo biopsies were instructed to apply the topical antioxidant serum on the entire face, including the preauricular area, twice a day for the entire study duration. All biopsies were evaluated for histological changes pre (baseline) and post (12 weeks) of study antioxidant serum use by an independent dermatopathologist using the following stains; Movat, Herovici and H&E (hematoxylin and eosin).

Statistical analyses were performed using mean, standard deviation, paired *t*-tests for normally distributed variables to compare changes between time points, and Wilcoxon signed rank tests for non-normally distributed values. Values of $p < 0.05$ were considered statistically significant.

3 | RESULTS

3.1 | Demographics

Overall, 36 subjects completed the study. Mean age was 57.4 years (range: 38–69 years), and 97% ($n = 35$) were female and 3% ($n = 1$) were male. Three participants were terminated early for protocol noncompliance. The study subject Fitzpatrick skin type distribution included, 30.5% ($n = 11$) were Type II, 50% ($n = 18$) were Type III, 16.7% ($n = 6$) were Type IV, and 2.8% ($n = 1$) were Type V.

3.2 | Investigator clinical grading of photodamage

Statistically significant improvements were seen across all facial photodamage parameters evaluated from baseline at week 12 as measured by investigator clinical grading where a mean improvement was demonstrated in; global photodamage for 83.3% of subjects ($p < 0.0001$), fine lines for 61.1% of subjects ($p < 0.0001$), coarse wrinkles for 39% of subjects ($p = 0.0001$), mottled hyperpigmentation for 81% of subjects ($p < 0.0001$), facial erythema 83.3% of subjects ($p < 0.0001$), and tactile roughness for 92% of subjects ($p < 0.0001$; Figure 1).

3.3 | Subject assessments and questionnaires

At both the Weeks 8 and 12 follow-up visits, 100% of subjects reported overall satisfaction with the topical antioxidant serum. Further, as reported by the subject questionnaire at Week 12, 83% of subjects agreed their facial skin looked healthier and their skin tone more even, 75% agreed their facial skin texture improved, radiance and clarity of the skin increased, and their facial skin appeared brighter. Additionally, at Week 12, 100% of subjects agreed that the product did not cause facial skin irritation, 97% agreed that the product did not cause facial dryness or

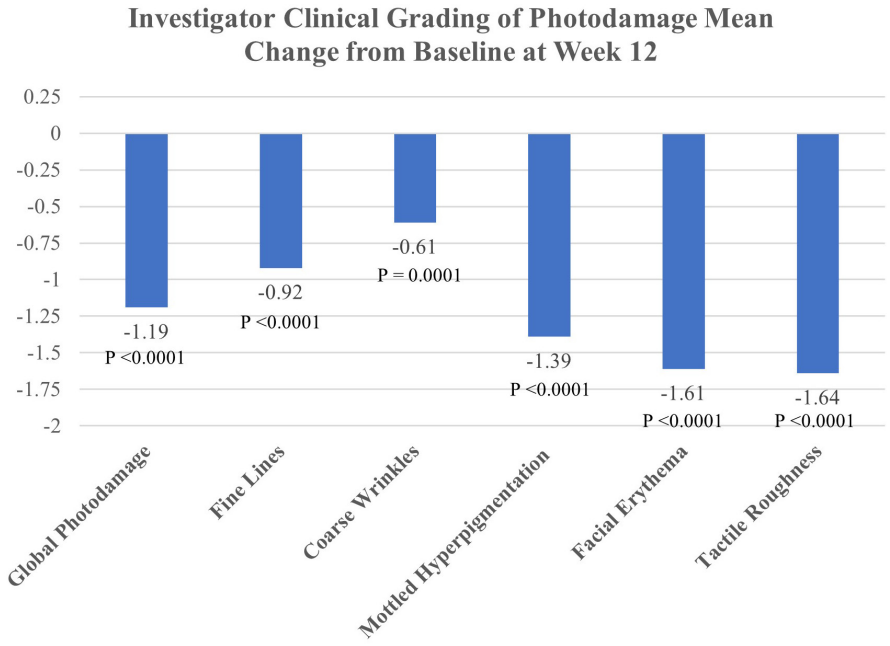

FIGURE 1 Investigator clinical grading of photodamage parameters using a modified Griffiths 10-point scale.

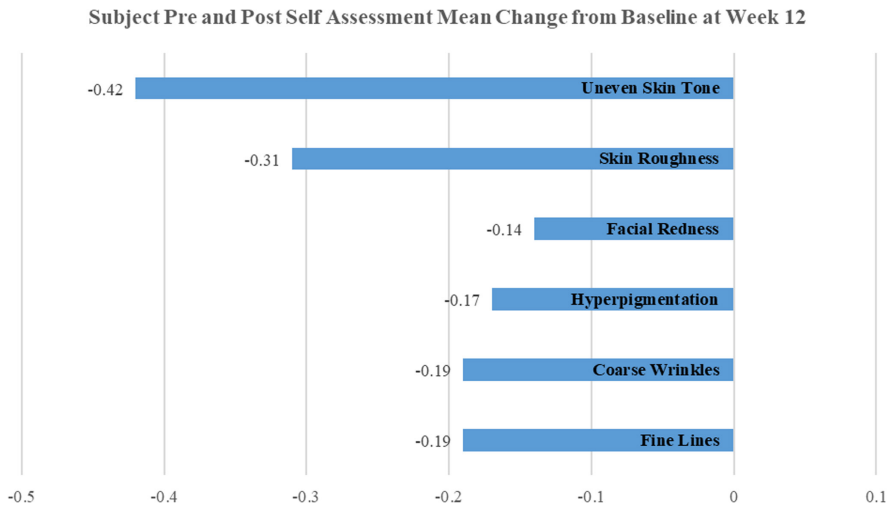

FIGURE 2 Subject Self-Assessment performed pre- and post 12 weeks use of study antioxidant serum. Improvement was noted for all parameters in Week 12 compared to baseline.

redness, 81% agreed the overall appearance of their facial skin improved, and 89% agreed that they would continue use of the antioxidant serum and would recommend it to their friends and family.

At 12 weeks post-topical antioxidant test serum use, compared to pre-study topical use at baseline, a mean improvement was demonstrated for all Subject Self-Assessment facial parameters evaluated. Overall, subjects reported a mean improvement of 32% in facial skin roughness ($p=0.0322$), 23% improvement in uneven skin tone ($p=0.0016$), 13% improvement in coarse wrinkles, 12% improvement in redness, 11% improvement in fine lines, and 9% improvement in hyperpigmentation (Figure 2).

3.4 | Tolerability assessments

Tolerability assessments were evaluated at baseline, prior to study topical use. At each follow up visit, the Investigator assessed parameters demonstrated statistically significant improvements at week 12 ($p<0.0001$), which trended from baseline. At Week 12, scaling improved 47.2% (baseline: 47.2%, Week 2: 33.3%, Week 4: 19.4%, Week 8: 6%, Week 12: 0%) and dryness improved 44% (baseline: 86%, Week 2: 47.2%, Week 4: 44.4%, Week 8: 42%, Week 12: 42%). Mean subject tolerability scores at Week 2 were burning (0, 0% change from baseline), stinging (0.03, 3% increase from baseline (1 patient)), itching (0.03, 0% change from baseline), and dryness (1.03, 23% decrease from baseline). The stinging and itching tolerability parameters all resolved by Week 4, and dryness reduced by 31% from baseline (pre-antioxidant serum use).

Tolerability parameters self-assessed by subjects demonstrated statistically significant improvements in dryness at Week 12 of 50% ($p<0.0001$), while burning, stinging, and itching completely resolved to 0% by Week 4, which trended through study completion at Week 12.

3.5 | Adverse events

One possibly related adverse event of mild right eye irritation was reported at one clinical site, in which the subject deviated from the study product usage instructions to avoid the eye area, and inadvertently test product was applied into the right eye with minor self-limiting irritation.

3.6 | Photography

Figures 3–5 (left, standard imaging) shows all participants demonstrate significant improvements as reflected in the clinical grading of the global photodamage parameter scores. Figure 3 shows study participant improvement in the global photodamage parameter scores (baseline: 7—severe, Week 12: 5—moderate) and mottled hyperpigmentation parameter scores (baseline: 7—severe, Week 12: 5—moderate). Figure 4 participant illustrates improvement in erythema as early as week 8 as demonstrated by standard imaging and red channel (baseline:5—moderate; week 8: 3—mild). Figure 5 participant illustrates improvement in erythema at 12 weeks as demonstrated by standard imaging and red channel (baseline: 4—moderate, week 12: 2—mild).

3.7 | Biopsies

Five female subjects, aged 46–63 years (mean age 55 years), of the following ethnicity, Hispanic or Latino: 1; non-Hispanic or Latino: 4, and with moderate (3 patients) to severe (2 patients) facial photodamage at baseline elected to have 2-mm punch biopsies collected from the right preauricular area, pre-application of antioxidant serum test product at baseline and after 12 weeks of twice daily use, including the preauricular areas. Because of the significance in

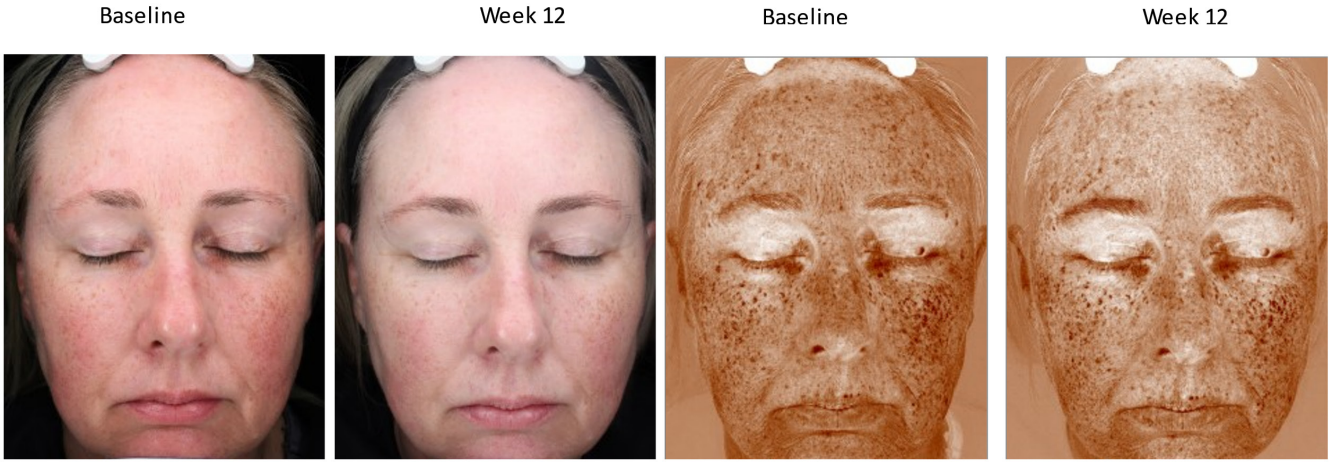

FIGURE 3 Female, age 46 years, non-Hispanic or Latino, Fitzpatrick skin type II. Photodamage clinical scoring at baseline (7: severe) compared to Week 12 (5: moderate). Standard imaging (left). Brown channel, (right) pre- and post 12 weeks of antioxidant serum use.

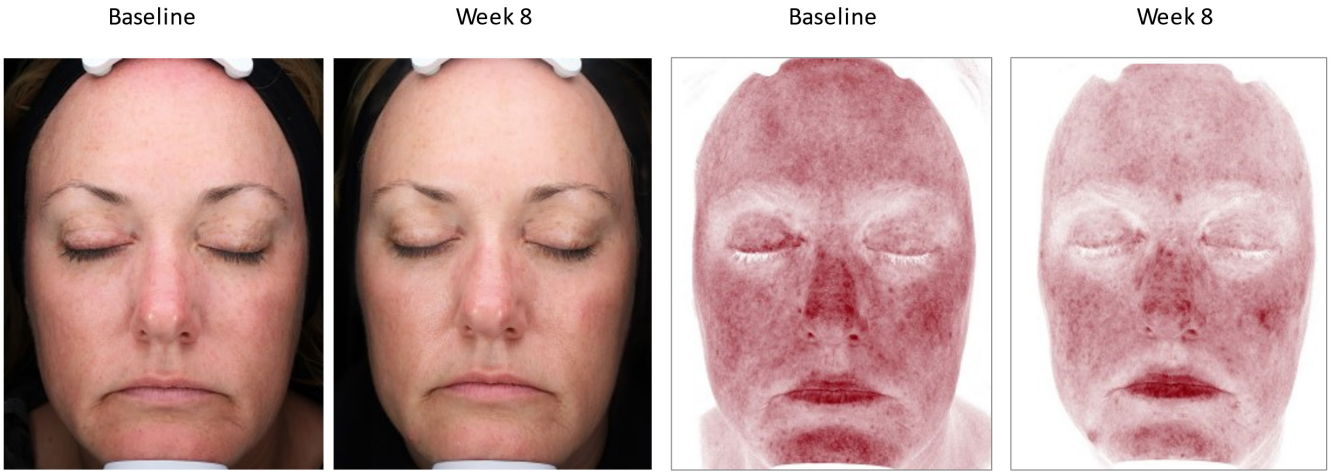

FIGURE 4 Female, age 45 years, non-Hispanic or Latino, Fitzpatrick skin type III. Photodamage clinical scoring at baseline (5: moderate) compared to Week 8 (3: mild). Standard Imaging (left). Red channel, (right) pre- and post 8 weeks of antioxidant serum use.

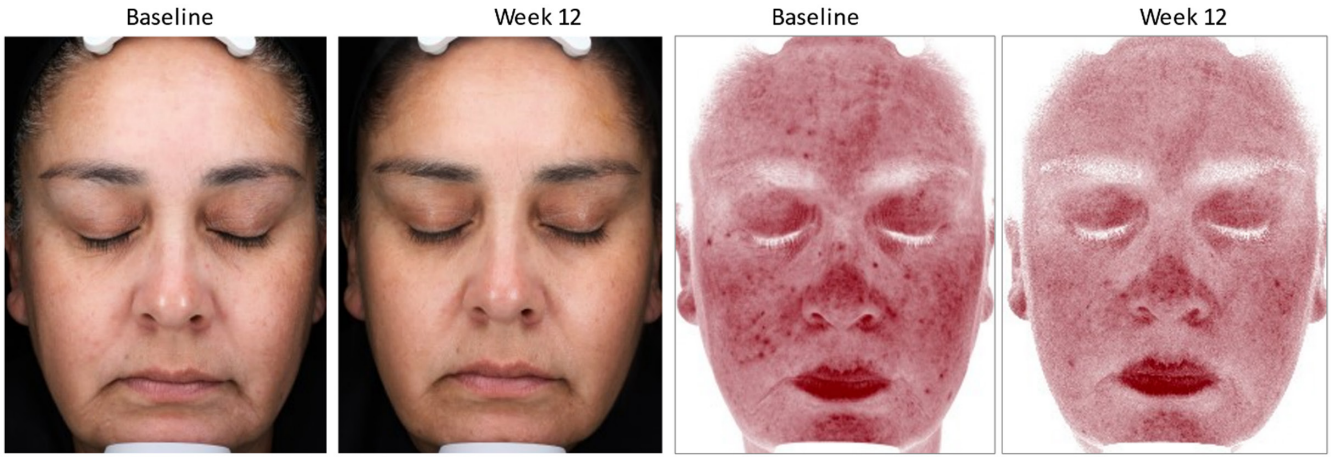

FIGURE 5 Female, age 48 yrs, Hispanic, Latino, Fitzpatrick skin type IV. Photodamage clinical scoring at baseline (6:moderate) compared to week 12 (4: moderate). Red Channel (right) scoring (baseline 4: moderate, week 12 2—mild).

this situation the histology focused on elastin production and conservation. Movat staining revealed an increase in elastin production in all five participants beyond what was anticipated (Figures 6A2–E2). Figure 6 (A—Movat 10× magnification) (A2) at Week 12 shows an increase in elastin fibers which also appears thickened compared to baseline (A1). Figure 6 (B—Movat 40× magnification) (B2) shows increased elastin post-treatment, where elastin fibers appear plumper, and fine elastin fibers appear to be present in the superficial reticular and papillary dermis, which are not identified in baseline biopsy (B1). Figure 6 (C—Movat 40× magnification) (C2) demonstrates an increase in elastin at Week 12. Figure 6 (D—Movat 10× magnification) (D2) shows increased, plump and more diffuse elastin fibers at Week 12. Figure 6 (E—Movat 10× magnification) (E2) shows an increased elastin fibers and a healthier dermoepidermal junction (DEJ) at Week 12. Additionally, Figure 7 (A2) H&E (40× magnification) staining shows an increase in collagen in the papillary dermis at week 12. Increased collagen also appears to have pushed down the solar elastosis at Week 12. Figure 7 (B2) Herovici (40× magnification) staining highlights the transition of old mature collagen to fine collagen fibers at Week 12 consistent with new collagen formation.

4 | DISCUSSION

An antioxidant serum has traditionally been considered an essential part of any skin health or anti-aging regimen. With constant production of ROS and a decreasing capacity to quench these free radicals as we age and are exposed to photodamage, the benefit of an added topical source of skin protection is an advantage and defense.[1] The overwhelming association of antioxidant activity has always been one with Vit C, this agent being the original validated constituent that was recognized as a key player in antioxidant therapy.[2]

In recent investigative studies, Hinek et al. revealed that traditional ascorbic acid used in 99% of market formulations can be detrimental and destructive to elastin. In contrast, the sodium salt of ascorbate in low concentrations, can be protective to elastin production.[5] Using these studies as the background to new investigations, a novel formulation was developed using this sodium salt of ascorbate and multiple other antioxidant constituents.[3] This clinical study was therefore undertaken to further validate the previous in vitro and ex vivo studies that represent the preclinical work conducted on this product.[3]

To that end, two important outcomes were sought. First, the general efficacy, tolerability, and skin health outcomes resulting from twice daily use for 12 weeks, and second, the validation of this new science examining the histologic effects of the formulation on elastin conservation and possible stimulation.

Thirty six subjects (male and female) of all skin types and at two centers in the United States completed the study. Investigator assessments using a modified Griffiths 10-point scale at 12 weeks across all photodamage parameters—global health, fine lines, coarse wrinkles, mottled pigmentation, erythema, tactile roughness—all showed highly statistically significant improvements from baseline. Patient assessments matched those of the investigators, with 100% of subjects reporting overall satisfaction with the topical antioxidant serum and excellent tolerability within the first 4 weeks of use, where subject evaluated stinging and itching completely resolved by Week 4, and dryness decreased 31% from baseline. The formulation is non-tacky with no odor and subjects were universally pleased with the application and performance of the formulation. Clinical photographs bore out the clinical assessments with marked changes of improvements observed in general skin health, glow/radiance, redness, and mottled hyperpigmentation.

One of the most remarkable outcomes observed in this study is related to the histologic findings. Stimulation of collagen and early formation of new fibers was demonstrated and is typified in the biopsy Herovici stains portrayed above (Figure 7B). The collagen response seen in all patients was anticipated; however, the real question we sought to address with biopsy involved the elastin status. Reports in previous studies[5] and ex vivo studies

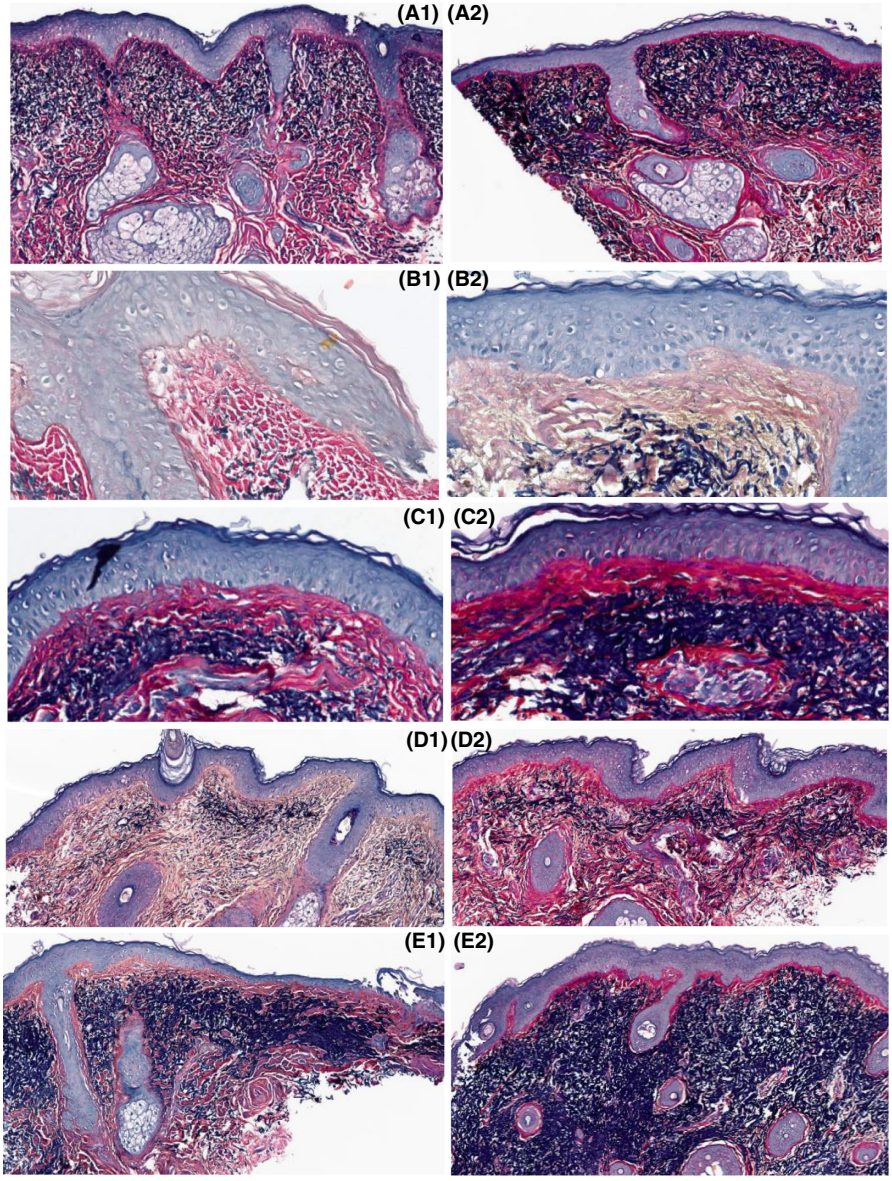

FIGURE 6 Movat stain—pre (left; A1–E1) and post 12 weeks of application (right; A2–E2)—5 participant biopsies. (A—Movat 10× magnification) Female age 46, Fitzpatrick skin type II. (B—Movat 40× magnification) Female age 63, Fitzpatrick skin type III. (C—Movat 40× magnification) Female age 56, Fitzpatrick skin type IV. (D—Movat 10× magnification) Female age 48, Fitzpatrick skin type IV. (E—Movat 10× magnification) Female age 62, Fitzpatrick skin type III.

conducted on this formulation[3] have demonstrated diminished elastin levels following L-ascorbate use, and conserved elastin following the use of the formulation described above. It was not clear if this was a protection effect of the formulation or whether it indeed stimulated elastin formation. Remarkably in the five biopsy patients, every biopsy, 100%, showed significant increased elastin levels, plumper fibers and an increased distribution (Figures 6A2–E2). This is a very significant new advance in regenerative antioxidant applications. This was a little unanticipated as the initial thought was that this sodium salt would be elastin "conserving". The biopsy results suggest that the compound is in fact elastin "stimulating" and thus further studies assessing changes in skin elasticity related to this increased elastin are warranted and will be interesting.

From the scientific studies thus far, it would appear that the negative effects of L-ascorbic acid on elastin and the inhibition of elastin deposition relate to a possible destabilization of tropoelastin mRNA.[5,6] In contrast, however, the SA included in this formulation stimulates elastogenesis due to the intracellular influx of non-oxidized ascorbate anions which is facilitated by the sodium-dependent ascorbate transporter, causing a reduction of intracellular ROS, activation of c-Src tyrosine kinase and the enhancement of IGF-1-induced phosphorylation of the IGF-1 receptor, ultimately triggering the elastogenic signaling pathway.[5] IGF-1 is a growth factor, promoting ECM production in skin, but an alternative elastogenic pathway induced by IGF-1, has also been identified and appears to be very relevant to the changes observed in this study.[5,7] The interesting findings of work by researchers such as Hinek etal suggest that "L-ascorbic acid may destabilize tropoelastin mRNA and cause overwhelmed hydroxylation on prolyl/lysyl residues of tropoelastin molecules, thereby promoting their intracellular accumulation and inhibiting their secretion".[5] This does bring into question potential deleterious effects of some forms of Vit C used over many years.

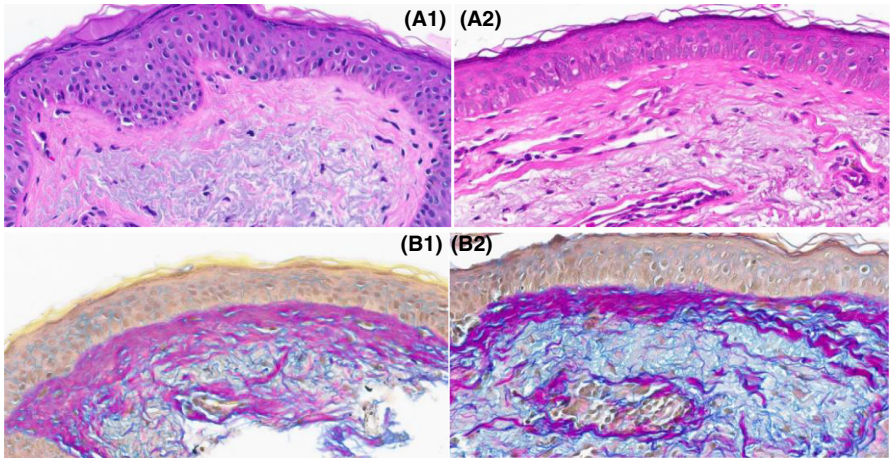

FIGURE 7 H&E (A) and Herovici (B) stains—pre (left; A1–B1) and post 12 weeks of application (right; A2–B2)—biopsy from one female age 56, Fitzpatrick type IV. (A2) H&E staining shows an increase in collagen in the papillary dermis at Week 12. Increased collagen also appears to have pushed down the solar elastosis at Week 12. Figure 7 (B2) Herovici staining highlights the collagen deposition (mucopolysaccharide deposition, fine blue fibers) at Week 12. The Herovici stain demonstrates the formation of new mucopolysaccharides which form early on in new collagen formation with fine fibers of collagen and mucopolysaccharide creating a web-like appearance. The transition from the old mature to new early fibers is observed by a change from the magenta pink to new blue/purple colors together with a fine web of fibers.

5 | CONCLUSION

A novel antioxidant serum has been formulated with SA and other antioxidant constituents. The sodium salt of Vit C aims to address facial photodamage while maintaining a low irritation profile and preserving elastin. Detailed background science has been submitted in a previous publication. This multicenter, open-label study was conducted to validate the science by demonstrating product efficacy and tolerability in patients with moderate to severe facial photodamage. Results of the study demonstrate excellent efficacy and tolerability of the product as shown in the investigator and subject assessment analysis. In addition, histologic evidence of significant and marked elastin stimulation in 100% of subject biopsies points to a new mode of action of this variation of Vit C. Overall, the formulation proved to be effective in improving global skin health, fine lines, coarse wrinkles, tactile roughness, erythema, and mottled hyperpigmentation, with the added benefit of stimulating new collagen and elastin in the ECM.

AUTHOR CONTRIBUTIONS

Alan D. Widgerow: developed the science, analysis, paper writing. Amir Moradi: study investigator, paper writing contribution. Faiza Shafiq: study design, sub-investigator, paper writing. Lora Colvan: study design. Jeannette Poehler: clinical research coordinator. Tiffany Robison: study design and management, data analysis, paper writing.

FUNDING INFORMATION

Funding applied for these studies was from Alastin Skincare, a Galderma company.

CONFLICT OF INTEREST STATEMENT

Dr Alan Widgerow is Chief Scientific Officer of Galderma. Dr Faiza Shafiq is Director Clinical Research Alastin, a Galderma company. Tiffany Robison is Clinical Research Manager, Alastin Skincare, Inc., a Galderma company. Lora Colvan is R&D Consultant, Alastin Skincare, Inc., a Galderma company. Alastin Skincare Inc (Carlsbad CA), a Galderma company, funded research into development of this product. This paper reports results of clinical studies of an anti-oxidant product.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received.

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