

A Pilot Study to Evaluate the Efficacy and Tolerability of a Topical Combination Serum for the Improvement of Aged Skin

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ABSTRACT

Background. With intrinsic aging the epidermis becomes thinner and fine wrinkles appear. Extrinsic aging, or photoaging, is characterized by deep wrinkles, skin laxity, and hyperpigmentation.

Objective. To evaluate the efficacy and safety of a novel combination serum with retinol, hyaluronic acid, and trichloroacetic acid (Test Product) for the improvement of aged facial skin.

Methods. Female subjects (n=22) enrolled in the single-site, open-label, pilot study. Subjects had mild to moderate fine lines and wrinkles and mild to moderate photodamage. Subjects applied the Test Product, cleanser, and moisturizer-sunscreen combo once daily to the face during the 12-week study. Radiance, skin tone, skin smoothness, skin texture, red/blotchy, dryness, overall appearance, skin quality, and tolerance were evaluated at each visit.

Results. Skin radiance, tone, smoothness, texture, and overall appearance improved significantly with continued use of the Test Product up to 12 weeks. Subjective improvement was significant for texture, tone, glow, smoothness, youthful appearance, pores, and satisfaction. More than half of subjects showed improvement in skin elasticity, redness/broken capillaries, and dryness. The treatment was well tolerated.

Conclusions. The combination serum with retinol, hyaluronic acid, and trichloroacetic acid has been shown to improve aged facial skin and is well tolerated when used daily for up to 12 weeks.

Introduction

With aging, the natural rejuvenation process of human skin decelerates and the skin becomes thinner, drier, and less elastic.¹ Manifestations include wrinkles, discoloration, uneven texture, and alterations in pore size. Hyaluronic acid (HA) and collagen are also reduced in the dermal matrix.²

Intrinsic aging occurs with advancing age; the epidermis becomes thinner and fine wrinkles

appear. Extrinsic aging, or photoaging, is due to chronic sun exposure and is characterized by deep

wrinkles, skin laxity, and hyperpigmentation. The skin becomes coarse, the epidermis thickens and then becomes thinner. There is also laxity, sallowness, wrinkles, irregular hyperpigmentation, lentigines, and telangiectasias. With either type of aging, wrinkles and reduced elasticity are typical

manifestations and the result of progressive atrophy of the dermis.³

A youth-engaging skin serum (Y.E.S.S.®, Induction Therapies, Louisville, KY) is a novel collagen-building, tone-improving product with all-trans retinol, hyaluronic acid (HA), and trichloroacetic acid (TCA) designed for the treatment of skin aging, discoloration, and hydration. Squalene and shea butter are also present.

The primary objective of the present study was to evaluate the efficacy and safety of this Test Product for the improvement of (1) fine lines and wrinkles, (2) appearance of discoloration, and (3) uneven texture of facial skin after 12 weeks of use. The secondary objective was to assess the improvement of moisture level and pore size during the same period.

Methods

Subjects. Female subjects (n=22) aged 41 to 60 years (mean SD = 53.3 ± 5.4) and Fitzpatrick skin types II (n=12), III (n=8), and IV (n=2) enrolled in the single-site, open-label, pilot study. Subjects had mild to moderate fine lines and wrinkles and mild to moderate photodamage. Wrinkles were grade 2 (class I) to 6 (class II) on the Fitzpatrick Wrinkles scale and photodamage was Glogau grade II or III. Subjects of child-bearing potential provided negative urine pregnancy test results. All subjects were willing to remain on their current skin-care regimen and to not use products other than the dispensed skin cleanser, moisturizer/sunscreen, and Test Product, the Youth Engaging Skin Serum (Y.E.S.S.) of Induction Therapies, Louisville, KY, USA). All subjects provided written informed consent to participate in the study and to have facial photographs taken for potential use in media or scientific publications.

Subjects were excluded if they had excessive facial exposure to sunlight or UV light; recent history or active presence of any facial skin condition/disease which, in the opinion of the principal investigator (PI), might interfere with diagnosis or evaluation of study parameters (i.e. moderate to severe acne vulgaris, atopic dermatitis, psoriasis, rosacea, seborrheic dermatitis, excessive facial hair or coloration,

suspicious lesion); or invasive or non-invasive skin treatments, surgeries on the face, hair removal, light treatment, filler or toxins performed in the treatment area within the previous 3 months. Other criteria were history of allergy or hypersensitivity to any of the product ingredients; pregnancy, breast feeding, planning to become pregnant, or unwilling to use an accepted form of birth control; participation in a drug or other research study currently or within the previous 30 days; unwillingness to have facial photographs used for media or scientific publications; recent use of medication that may interfere with skin characteristics; and per the PI's discretion, any other mental or physical condition that might make it unsafe for the subject to participate in this study.

Procedure. Qualified subjects were evaluated at each visit by a trained evaluator. At the screening or baseline visit, informed consent, medical history and demographics, Fitzpatrick skin typing, inclusion/exclusion criteria, medications, wrinkles and photodamage, and pregnancy test results were evaluated. The schedule of other procedures is shown in Table 1.

Product Distribution. Test product, cleanser, moisturizer, and sunscreen were given to subjects at baseline. The trained evaluator instructed each subject on their proper use and how to record product application, new medications, and adverse events in their diary to ensure compliance. Products dispensed included the Test Product, cleanser (IT Cleanse), and moisturizer-sunscreen combo (Block IT-SPF30). Subjects were instructed to apply all products once daily, each evening, during the study.

Photography. Full-face, right side (45°), and left side (45°) images were obtained from each subject at each visit using a Visia CR facial imaging system (Canfield Scientific, Parsippany, NJ, USA). Subject wore no makeup and removed all jewelry for photographs.

Assessments.

Wrinkles. Wrinkles were evaluated at screening, using the Fitzpatrick Wrinkle Scale below. Qualified subjects were between grade 2 and grade 6.

Score	Degree of Elastosis
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I (grades 1-3)	Mild: fine texture changes with subtly accentuated skin lines
II (grades 4-6)	Moderate: distinct popular elastosis (individual papules with yellow translucency under direct lighting) and dyschromia
III (grades 7-9)	Severe: multipapular and confluent elastosis (thickened, yellow and pallid) approaching or consistent with cutis rhomboidalis

Photodamage. Photodamage was evaluated at screening, using the Glogau scale below.

Type	Characteristics
I – No wrinkles	Minimal or no discoloration or wrinkling, no keratoses
II – Wrinkles in motion	Wrinkling in skin with movement, slight lines near mouth and eyes, no keratoses
III – Wrinkles at rest	Visible wrinkles all the time, noticeable discoloration, visible keratoses
IV – Only wrinkles	Wrinkles throughout, grey or yellow discoloration of skin, history of skin cancer

Investigator Objective Assessments for Full Face. Radiance, skin tone, skin smoothness, skin texture, red/blotchy, dryness, and overall appearance were graded (0-4) at each visit. Low values indicated improvement except for radiance, in which 0 denoted dull skin and 4 indicated skin that glowed.

Subjective assessments for full face. Using a 0 to 10 severity scale, subjects completed the Subjective Skin Quality Questionnaire at baseline (before product application) and at the end of study (week 12).

Subjects were asked to rate fine lines and wrinkles, elasticity, texture, tone, redness and broken blood capillaries, glow, smoothness, youthful appearance, dryness, discoloration, pores, and satisfaction with the look of their skin.

Tolerability for full face. The investigator objectively rated erythema, edema, dryness, and peeling of the full face, using a 0 to 4 (minimal to

severe) scale, at each visit. Subjects rated stinging, tingling, itching, and burning, using the same scale, at each visit.

Global Aesthetic Improvement Scale (GAIS).

Using a 5-point scale for improvement (none, minimal, mild, moderate, marked) the investigator qualitatively assessed improvement at the end of the study.

Data analysis. Since assessment scales had a small range and data were in small whole numbers, non-parametric statistics were used to analyze data, using $p = 0.05$ as the cutoff value for significance. For multiple comparisons, the Bonferroni correction was used to adjust the cutoff value. For example, if the median scores at 4, 8, and 12 weeks were each compared to the median baseline score, the cut-off value ($p = 0.05$) was divided by three, the number of non-independent comparisons. In this case, the corrected p value is $0.05/3 = 0.017$.

Results

Twenty-one subjects completed the study. One subject withdrew consent due to a scheduling conflict.

Investigator's objective assessments

The investigator's objective assessment results are shown in Table 2. The scores at 4, 8, and 12 weeks were compared to the scores at baseline using the paired Wilcoxon signed rank test. After applying the Bonferroni correction, improvements compared to baseline were significant at all time points for radiance, tone, smoothness, texture, and overall appearance. For red/blotchy, 4-week improvement was not significant, 8-week improvement was of borderline significance (0.0171), and 12-week improvement was significant ($p = 0.0017$). Improvement in dryness was significant at 4 weeks and non-significant at 8 and 12 weeks due to low values (0 and 1) throughout the study for all subjects.

The percentage of subjects improved increased steadily from 4 weeks to 12 weeks for all parameters except for dryness. At 12 weeks, smoothness, texture, and overall appearance

showed the highest percentage of subjects improved at 90.5%.

Median percent improvement scores compared to baseline ranged from 33% to 50% at 4, 8, and 12 weeks for radiance, tone, smoothness, texture, and overall appearance. Values were more variable for red/blotchy and consistently 0 for dryness, the latter due to the small range of scores (0 to 1). Percent improvement for 4, 8, and 12 weeks compared to baseline was calculated by the following formula, using 12-weeks as an example.

Percent improvement = $([12\text{-week score} - \text{baseline score}] / \text{baseline score}) \times 100$

Investigator tolerability

For erythema, edema, dryness, and peeling, grades were generally low (0-2) and varied randomly between baseline and 12 weeks. All scores were 0 at baseline and at 12 weeks, except for a single subject with a grade of 1 for dryness at 12 weeks. Five subjects were graded 2 at 4 weeks and all decreased to 0 or 1 at 8 weeks and 12 weeks. These adverse events were study-related and were all mild to moderate, temporary, and resolved without treatment.

Subjective tolerability

All subjects were graded 0 at baseline and at 12 weeks for stinging, tingling, itching, and burning. Three subjects were graded 1 or 2 at 4 weeks and all decreased to 0 or 1 at 8 weeks and to 0 at 12 weeks. These adverse events were study-related and were all mild to moderate, temporary, and resolved without treatment.

Subjective skin quality

The subjective skin quality assessment results are shown in Table 3. For each question, the subject grades at 12 weeks were compared to the subject grades at baseline by the paired Wilcoxon signed rank test. Since this was a single comparison, the Bonferroni correction was not applied. Lower median grades at 12 weeks indicated improvement over baseline for all questions except 10 (discoloration) and 11 (pores).

Improvements at 12 weeks were significant for texture ($p = 0.0013$), tone ($p = 0.0002$), glow ($p = 0.0013$), smoothness ($p = 0.0031$), youthful appearance ($p = 0.0026$), pores ($p = 0.0038$), and satisfaction ($p = 0.0002$). The percentage of subjects improved for these parameters ranged from 57.1% to 85.7%. For the remaining skin parameters, percentages of subjects improved at 12 weeks ranged from 38.1% (lines and wrinkles, discoloration) to 61.9% (redness and broken capillaries).

Global Aesthetic Improvement Scale (GAIS)

Mild to moderate improvement was observed in 76.2% of subjects.

The median improvement was 3.0 (mild) with a minimum value of 1 (none) and maximum value of 4 (moderate).

Discussion

As stated earlier, the Test Product is a combination of all-trans retinol, HA, and TCA for the treatment of skin aging, discoloration, and hydration. Squalene and shea butter are also present. All-trans retinol (1%) supports collagen with little or no irritation and is encapsulated for stability and skin penetration. TCA improves the appearance of skin discoloration, pore size, and uneven texture. HA restores and improves skin moisture.

The findings of the present study support these expected clinical benefits and tolerability. The Investigator's objective assessments show that use of the Test Product as in the present study provides significant improvement in radiance, tone, smoothness, texture, and overall appearance and that improvement increases with continued use up to 12 weeks. These results are paralleled by the steady increase in the percentage of subjects improved during the study period. Improvement in red/blotchy became significant at 8 weeks and remained significant at 12 weeks with 61.9% of subjects improved.

Subjective skin quality assessments addressed changes in the same skin parameters (texture, tone, radiance [glow], smoothness, dryness, discoloration) with consideration also given to pores and overall satisfaction. As with the Investigator's objective assessments,

improvement at 12 weeks was significant for texture, tone, glow [radiance], and smoothness. Improvement was also significant for youthful appearance, pores, and satisfaction. More than half of subjects showed improvement in skin elasticity, redness/broken capillaries, and dryness, although the 12-week scores for these parameters did not achieve significance.

Finally, the investigator and subjective tolerability data showed that the procedure was well tolerated by all subjects. Clinical examples are shown in Figures 1-3.

All-trans retinol is a precursor for the synthesis of endogenous retinal and retinoic acid (tretinoin). Retinol's potential for the treatment of photoaging was explored in a series of studies which showed that topical retinol had beneficial effects similar to tretinoin but with minimal erythema and skin irritation. Subsequent studies showed that compared to tretinoin, retinol produced less transepidermal water loss, erythema, and scaling, and it stimulated collagen biosynthesis in photoaged skin. Retinol also improved fine wrinkles, reduced collagenase, and lowered gelatinase expression while increasing fibroblast growth and collagen synthesis in tissue specimens. These and other findings led to the conclusion that retinol should be effective in the treatment of aging and photoaging.¹

In human skin, HA is a key contributor to water retention and mechanical support.² Found at the periphery and interfaces of collagen and elastin fibers, HA helps to hold collagen and elastin in a proper configuration. In aged skin, these connections with HA are absent, which may lead to the disorganized collagen and elastin fibers and the formation of fine lines, wrinkles, and nasolabial folds.⁴

HA is well known for its ability to hold a large amount of moisture. As skin ages, the amount of HA decreases to approximately 5% of baseline. HA is also a scavenger of free radicals and has anti-inflammatory properties. With these specific properties, HA should be a prime component of cosmetic products.⁴

For skin aging, trichloroacetic acid (TCA) has been considered a gold standard, as it is stable,

does not cause systemic toxicity, and does not need to be neutralized when applied to skin.⁵ The main indications for TCA peels are melasma, photoaging, acne scars, under-eye circles, frictional melanosis, actinic keratosis, actinic cheilitis, xanthelasma, flat warts, seborrheic keratosis, acanthosis nigrans.⁶ Side effects include redness, herpes, hyperpigmentation, and lentigines.⁷ These side effects were not observed in the present study.

Squalene, the main component of skin surface polyunsaturated lipids, offers advantages for the skin as an emollient and antioxidant, and for hydration. As in the Y.E.S.S. product, squalene is also used as a material in topically applied vehicles such as lipid emulsions and nanostructured lipid carriers.⁸ Shea butter is used in the cosmetic industry due to its high percentage of compounds (triterpenes, tocopherol, phenols, and sterols) which possesses potent anti-inflammatory and antioxidant properties.⁹

The results of the present study show that when applied daily for up to 12 weeks, the Test Product, cleanser, and moisturizer-sunscreen combo improves many of the manifestations of aged skin with high subject satisfaction and minimal adverse effects.

As for limitations, a placebo-controlled clinical trial with a larger sample size should be performed to verify the results of this pilot study. The future study should include skin biopsy results to further define the observed clinical effects of the Test Product.

Conclusions

The combination serum with retinol, hyaluronic acid, and trichloroacetic acid has been shown to improve aged facial skin and is well tolerated when used daily for up to 12 weeks.

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Tables

Table 1. Schedule of study procedures

Procedure	Baseline	Week 4 ¹	Week 8 ¹	Week 12 ¹
Dispense products	D			
Dispense/Reconcile/Collect diary	D	R	R	C
Adverse event query	x	x	x	x
Medication query	x	x	x	x
Photographs	x	x	x	x
Investigator objective assessment ²	x	x	x	x
Subject skin quality assessment	x			x
Tolerability assessments ³	x	x	x	x
Investigator GAIS ⁴				x

¹ ±3 days

²Radiance, fine lines/wrinkles, smoothness, firmness, and overall appearance.

³Objective (erythema, edema, dryness, peeling) and subjective (stinging, tingling, itching, burning).

⁴Global Aesthetic Improvement Scale.

Table 2. Investigator's objective assessments

Clinical Parameter	P value (Wilcoxon test) ¹ (4, 8, 12 wk vs. Baseline)			Subjects improved (%)			Median Improvement (%) (min. max)		
	4 wk	8 wk	12 wk	4 wk	8 wk	12 wk	4 wk	8 wk	12 wk
Radiance	0.0002	0.0001	<0.0001	61.9	66.7	85.7	50.0 (0, 200)	50.0 (0, 200)	50.0 (0, 200)
Tone	0.0005	<0.0001	<0.0001	57.1	76.2	85.7	33.0 (0, 50)	33.0 (0, 67)	50.0 (0, 67)
Smoothness	0.0005	0.0005	<0.0001	57.1	57.1	90.5	33.0 (0, 50)	33.0 (0, 50)	50.0 (0, 67)
Texture	0.0002	<0.0001	<0.0001	61.9	76.2	90.5	33.0 (0, 50)	50.0 (0, 67)	50.0 (0, 100)
Red/ Blotchy	0.0977 (ns)	0.0171	0.0017	38.1	52.4	61.9	0.0 (0, 100)	41.5 (-100, 100)	83.5 (0, 100)
Dryness	0.0156	0.5000	1.0000 (ns)	0	0	0	0 (0,0)	0 (0,0)	0 (0,0)
Overall	0.0020	0.0001	<0.0001	47.6	66.7	90.5	0 (0,50)	33.0 (0, 50)	50.0 (0, 67)

¹p = 0.017 as cut-off value for significance.

Table 3. Subjective skin quality assessments

Question	Baseline grade (median, min, max)	12 Wk grade (median, min, max)	Median % diff (min, max)	P value (Baseline vs. 12 Wk)	Subjects improved (%)
1 (lines and wrinkles)	5.0 (2,9)	5.0 (1,8)	0.0 (-40, 80)	0.1230	38.1
2 (elasticity)	5.0 (2,9)	5.0 (2,9)	12.0 (-100, 67)	0.4413	52.4
3 (texture)	5.0 (2,10)	4.0 (1,10)	33 (-100, 71)	0.0013	71.4
4 (tone)	7.0 (4,10)	5.0 (1,9)	25.0 (-20, 89)	0.0002	71.4
5 (redness, broken capillaries)	5.0 (2,9)	4.0 (2,8)	17.0 (-200, 71)	0.1084	61.9
6 (glow)	6.0 (3,9)	5.0 (1,9)	20.0 (-100,75)	0.0013	76.2
7 (smoothness)	5.0 (2,10)	4.0 (1,10)	17.0 (-67,75)	0.0031	57.1
8 (youthful appearance)	7.0 (3,9)	5.0 (2,7.33)	14.0 (-20,60)	0.0026	66.7
9 (dryness)	5.0 (3,8)	4.0 (2,8)	14.0 (-100, 75)	0.2522	52.4
10 (discoloration)	5.0 (3,8)	5.0 (2,9)	0.0 (-60,167)	0.5619	38.1
11 (pores)	4.0 (1,7)	6.0 (2,9)	40.0 (-40,400)	0.0038	66.7
12 (satisfaction)	7.0 (3,10)	5.0 (0,9)	29.0 (-67,100)	0.0002	85.7

Figures



Figure 1. A 60-year-old non-Hispanic Caucasian female of Fitzpatrick skin type 2 before (A) and after 12 weeks of treatment (B) with the Test Product. At the end of study, the subject's baseline investigator's objective assessments showed improvement in radiance, skin tone, smoothness, texture, red/blotchiness, and overall assessments. The subject's self-assessed skin quality improved in elasticity, smoothness, evenness, redness, radiance, softness, youthfulness, hydration, and pores. The investigator's aesthetic improvement assessment was 3 (mild). The subject was very satisfied with the treatment.



Figure 2. A 52-year-old non-Hispanic Caucasian female of Fitzpatrick skin type 2 before (A) and after 12 weeks of treatment (B) with the Test Product. At the end of study, the subject's baseline investigator's objective assessments showed improvement and smoothness and texture. The subject's self-assessed skin quality improved in fine lines and wrinkles, elasticity, smoothness, evenness, redness, radiance, youthfulness, and pores. The investigator's aesthetic improvement assessment was 2 (minimal). The subject was satisfied to very satisfied with the treatment.



Figure 3. A 43-year-old non-Hispanic Caucasian female of Fitzpatrick skin type 2 before (A) and after 12 weeks of treatment (B) with the Test Product. At the end of study, the subject's baseline investigator's objective assessments showed improvement and smoothness and texture. The subject's self-assessed skin quality improved in fine lines and wrinkles, elasticity, smoothness, evenness, redness, radiance, softness, youthfulness, hydration, and pores. The investigator's aesthetic improvement assessment was 4 (moderate). The subject was very satisfied with the treatment.