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Efficacy of a collagen hydrolysate and antioxidants-containing nutraceutical on metrics of skin health in Indian women

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Abstract

Background: The skin's aging process involves a decreased biosynthesis of extracellular matrix proteins (predominantly collagen) compounded by damage from environmental and intrinsic stressors. The Indian population is susceptible to skin damage given its geography and increasing urbanization or a genetic disposition. Previous studies have investigated nutrients such as collagen peptides, vitamins and phytonutrient-rich botanical extracts for their individual benefits on skin.

Aims: This study examined the collective effect of a proprietary blend of these nutrients (in Nutrova Collagen+Antioxidants; NCA) on skin parameters, which has not been previously studied, especially in an Indian context.

Patients/Methods: 34 healthy, Indian women (mean age = 39.5 years) were given a placebo daily for 30 days to establish a baseline, followed by NCA for two intervals of 30 days. 3D image reconstruction allowed the analysis of skin topography and blemishes. Instrumental measurements also included skin firmness, elasticity, hydration, and transepidermal water loss. Clinical evaluation was used to grade blemishes, wrinkles and periorbital hyperpigmentation.

Results: Based on instrumental evaluation, NCA significantly reduced wrinkle width, open pores, skin roughness, and the colour of hyperpigmented blemishes, while improving skin hydration, firmness and barrier function from baseline to Day 30 and Day 60. NCA also increased elasticity at Day 30. Clinical evaluation showed that periorbital hyperpigmentation and wrinkles reduced significantly.

Conclusion: NCA is effective for improving overall skin health in Indian women. These results show that targeted nutrient supplementation can improve skin health and further research over extended durations is merited.

KEYWORDS

collagen hydrolysate, grape seed extract, skin health, taurine, tomato lycopene

1 | INTRODUCTION

Human skin has a multilayered, composite structure of cells, and its extracellular matrix (ECM). An imbalance between the synthesis and degradation of ECM proteins (predominantly collagen) leads to hallmark age-related changes in skin, such as roughness and wrinkles. Antiaging treatments for skin aim at preserving and renewing this ECM protein structure and protecting it from oxidative damage. Herein, we study the effects of a nutraceutical product, called Nutrova Collagen + Antioxidants (NCA), which provides collagen hydrolysate (or collagen peptides; average size <5 kDa) and a proprietary blend of botanical extracts, vitamins, and taurine, in a cranberry flavored drink on skin health.

Collagen peptides are receiving increasing attention for their benefits on connective tissue. A number of studies have shown improvements in skin health parameters (such as hydration, elasticity, and texture) after daily ingestion of collagen peptides for 4-8 weeks.[1-4] Its oral consumption is thought to provide active dermal fibroblasts with the main amino acids (namely glycine/Gly, proline/Pro, and hydroxyproline/Hyp) involved in collagen synthesis. However, this may not be its only mode of action. Human studies show that hydrolyzed collagen, when administered orally, is absorbed in the small intestine as both, free amino acids and as oligopeptides (of molecular weight up to 8 kDa). Di- and tripeptides (such as Pro-Hyp and Gly-Pro-Hyp) are thought to exert physiological benefits by acting as ligands to receptors on fibroblasts.[5-7] Although the exact mechanism of these bioactive peptides is largely unclear, this outcome is likely, as in vitro studies have demonstrated, a range of effects on fibroblasts, including an increase in chemotaxis, proliferation, and production of other ECM structures such as elastin and hyaluronic acid.[8,9]

Given that these bioactive properties are observed, it is speculated that these peptides are able to initiate target gene transcription even in their soluble form, which lacks the structural function of undigested collagen. Pro-Hyp in particular has gained attention as recent reports indicate that they may be transported to the cytoplasm and act as a direct signal. As a breakdown product of ECM damage, Pro-Hyp is thought to initiate, at least in part, tissue repair by stimulating precursor cells and immune cells.[10] Zague et al suggested that the bioactivity of collagen peptides is not limited to stimulating collagen biosynthesis. They also decreased its enzymatic breakdown by inhibiting matrix metalloproteinase (MMP) 1 and 2 activity.[11] Furthermore, isotopic biodistribution studies in a mouse model have shown that, after oral administration, these peptides are in fact delivered to the skin,[12-14] where they can exert these effects.

The restorative effect of collagen peptides in NCA is complemented by the cytoprotective benefits of its other nutrients. The proprietary blend in NCA includes: (a) a tomato extract with lycopene and other carotenoids, which improve skin's appearance and are potent mitigators of UV-induced damage[15,16]; (b) a proanthocyanidin-rich grape seed extract, a strong antioxidant with potential benefits for melasma treatment[17,18]; (c) a green tea extract that's rich in catechins; (d) vitamin C, a cofactor for collagen synthesis; (e) vitamin E, a lipid-soluble antioxidant; and (f) taurine which plays a role in osmoregulation, and is therefore a key nutrient for skin hydration.[19,20]

There is extensive literature on orally administered botanical extracts for the benefits of skin health. The extracts added to NCA's unique formulation are very potent antioxidants and offer a range of distinctive benefits. Lycopene is thought to inhibit the production of MMPs and support collagen synthesis (as shown by an increase in procollagen I). As a nonprovitamin A carotenoid, lycopene has long been used for protection against UV light-induced erythema, further supported by the colorless carotenoids (phytofluene and phytoene) found in natural extracts.[21-23] In addition to UV protection, grape seed proanthocyanidins are both fat- and water-soluble, allowing them neutralize both intracellular and intercellular free radicals. In addition to an antioxidant capacity several-fold higher than vitamins C and E,[24] grape seed and green tea polyphenols have shown to improve vasculature and reduce inflammation, and can be used for related disorders such as atopic dermatitis.[25] Together their absorption spectra span the UVA and UVB range providing "broad spectrum" photoprotection. Coupled with the collagen peptides, and at clinically relevant doses, their potential synergistic effect on skin health may help address skin issues as a monotherapy as well as in conjunction with other topical and surgical treatments.

While the benefits of collagen peptides and a number of antioxidant compounds have been established for some metrics of skin health, they have not been looked at in the Indian context. The aim of this study was to establish the efficacy of NCA and its unique formulation, with regard to frequent dermatological complaints of an Indian population. Many parameters of skin health were investigated including hydration, transepidermal water loss (TEWL), biomechanical properties, wrinkle size, and other indicators of skin aging. Given the higher predisposition of hyperpigmentation, pores and scarring in the Indian cohort,[26,27] periorbital hyperpigmentation, dark pigmented spots, and pores were also examined. By protecting the preexisting skin structure and possibly eliciting a biological response from fibroblasts, NCA can therefore improve skin health for both cosmetic and therapeutic outcomes.

2 | MATERIALS AND METHODS

2.1 | Test materials and study design

NCA contains 5 g of fish collagen peptides, the dose that has been shown to improve skin parameters in clinical studies, with a proprietary blend of natural antioxidants (Table 1). The drink has been flavored with fructose, cranberry flavor, and citric acid to make it

TABLE 1 Key ingredients of placebo and NCA

Placebo	NCA
Fructose Citric acid Flavor (cranberry)	Marine Collagen Peptides Antioxidant blend (natural tomato extract, grape seed extract, green tea extract, vitamin C,and vitamin E) Fructose Taurine Citric acid Flavor (cranberry)

palatable and improve compliance. The placebo used in the study comprised of the nonactive ingredients (Table 1).

This observational study was carried out for three months where the test product was compared with initial state and a placebo. The placebo was dispensed for 30 days to establish a baseline (Day −30 to Day 0), after which the test product was given for a total of 60 days (Day 1 to Day 30 and Day 31 to Day 60; Figure 1). All measurements were taken at Day −30, Day 0, Day 30, and Day 60. Participants were blinded to the contents of the sachets as both, the placebo and the test product, were provided in nondescriptive packaging. Participants were instructed to dissolve the contents of a sachet in 100 mL of water (about half a cup), and consume daily, 30 minutes after dinner.

The study was conducted in accordance with Good Clinical Practice Guidelines (ICH-GCP) and Schedule Y of the Drugs and Cosmetics Act of India. The trial was approved by an independent Ethics Committee (Registration No.: ECR/245/Indt/MH/2015), and the study was registered with the Clinical Trial Registry of India (Registration No.: CTRI/2018/07/014808).

2.2 | Study participants

A total of 34 Indian women aged 35-45 years (mean age 39.5 years) with wrinkles (as per clinical evaluation described in section 2.3), having at least one hyperpigmented blemish (of 2 mm) and undereye dark circles completed the study. Inclusion criteria also required participants to be nonvegetarians or consented vegetarians. All participants signed an informed-consent form. They were informed orally and in writing of all study procedures, objectives, and risks.

Exclusion criteria were as follows: subjects (a) with any chronic illness or any disease; (b) with known food allergies; (c) likely to undergo surgery during the period of the study; (d) with excessive skin damage or sunburn/tan; (e) who reported substance abuse (alcohol, tobacco, drugs etc); (f) with chronic gastrointestinal issues; (g) who were pregnant or lactating; (h) with a body mass index of ≥30 kg/m^2; and (i) those with abnormal levels in blood and urine test parameters (Appendix A).

Subjects who had used a supplement containing collagen peptide in the past 6 months or those who had taken high doses of vitamin C (≥500 mg a day) or any other antioxidant product in the past 1 month and those who were on any other experimental investigational study within the last 1 month were also excluded from the study.

To minimize confounding the results of the study, participants were asked to refrain from using any supplement, topical sunscreen, or antiaging creams/serums or undergoing any skin treatments, masks, or massages. They were reminded of these guidelines at every visit and were also asked to maintain their general daily routine. A total of 34 participants completed the study, as 36 were recruited and 2 were lost to follow-up. Each participant washed their face and was then allowed to acclimate for 1 hour (temperature: 20-22°C; humidity: 40%-60%) prior to taking measurements.

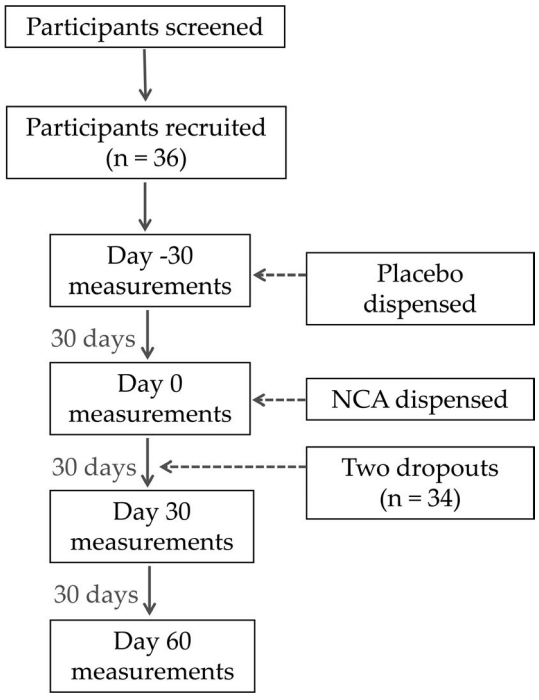


FIGURE 1 Flow chart of the study. Baseline results of the interventional study were established with 30 days of placebo consumption, followed by 60 days of NCA consumption. Of the thirty-six participants recruited, thirty-four completed the study, and two were lost to follow-up

2.3 | Clinical evaluation

Clinical evaluation was done for wrinkles in the lateral canthal region near the eyes ("crow's feet"), hyperpigmentation blemishes and periorbital hyperpigmentation ("dark circles"). Wrinkles were graded from 0 to 7 using the Jin Ho Chung Scale, where 0 represented no wrinkles and 7 indicated severe wrinkles. One hyperpigmented blemish (of ≥2 mm) was selected and evaluated on a 5-point scale adapted from the Melasma Area and Severity Index (MASI; proposed by Kimbrough-Green et al[28]), where 0 indicated normal skin color and 4 indicated severe pigmentation. Finally, undereye dark circle evaluation was done for four zones (median and lateral for both eyes) and graded on a bespoke scale of 0-5 (0 = no dark circles and 5 = blueish black dark circles). The scores in the four regions were added to make a maximum possible score of 20.

2.4 | 3D imaging

The Antera 3D® (Miravex Limited) camera comes equipped with light-emitting diodes of different wavelengths (455-625 nm). The skin is illuminated from different directions for computer-aided reconstruction of its surface. Its Spot-On™ function ensures the image of the same test area is captured. This instrument, with a field of view of 56 × 56 mm, was used to make several measurements:

2.4.1 | Skin topography

The Antera 3D® software quantifies skin texture by measuring its deviation from its ideal form, a parameter that is a product of blemishes, scars, pores, and wrinkles. Skin topography data allowed for the quantification of skin texture/roughness (arbitrary units; au), along with depth and width of the chosen crow's feet wrinkles (μm).

Skin pores, which can be defined as openings of the skin to the surface, were also analyzed as enlarged/open pores are of cosmetic concern. Facial pore volume was acquired from 3D imaging followed by calculating skin's Porosity Index (equation a), which is a function of depth, size, and density of pores in the selected area (of 532.01 mm^2). These parameters of skin pores have been previously shown to be correlated with visual assessment of skin pores.[29,30]

$$\text{(a) Porosity Index} = (\text{total pore volume}/\text{total selected area}) \times 1000$$

2.4.2 | Colorimetric measurements

Colorimetric measurements were made using the Commission Internationale del'Eclairage's *L*a*b** colorimetric model (CIE *L*a*b**). Luminescence (represented by *L**) was measured for the chosen blemish. Melanin concentration in the blemish was determined by mathematical correlation of blemish color with known spectral absorption of melanin.

2.5 | Measurement of skin hydration

The MoistureMeter SC Compact (Delfin Technologies) exploits the electrical properties of skin's layers to measure the hydration of its outermost layer, the stratum corneum. Measured in arbitrary units, the reading on the instrument is positively correlated to the moisture content in the stratum corneum. Dry skin typically shows a reading of ≤20, while well-hydrated skin is ≥40. Triplicate measurements were made on the cheek. Bespoke stencils were prepared for each participant to keep the site of measurement constant.

2.6 | Measurement of skin barrier properties

The epidermal lipids maintain a certain amount of water in the stratum corneum, which represents the barrier properties of the skin. The ECM proteins complement it with their water holding capacity. The passive loss of water from the skin or transepidermal water loss (TEWL), when heightened, suggests that the lipid barrier or the underlying protein structure is compromised.

TEWL can be measured by the VapoMeter (Delfin Technologies), which has a closed cylindrical chamber containing sensors for relative humidity (RH%) and temperature (°C). Once the device is placed on the skin, there is a linear increase of RH% in the chamber, which allows the calculation of TEWL. Values of ambient RH and temperature are recorded prior to skin contact. The chamber was passively ventilated between the triplicate measurements. Reduction in the VapoMeter readings indicates improvement in skin barrier properties.

2.7 | Measurement of skin biomechanical properties

The Cutometer® is a measuring device that helps to estimate the degree of firmness and elasticity of the skin using suction which deforms the skin mechanically. Suction causes the skin to be drawn into the aperture of the probe (probe size = 2.5 cm in diameter) and after a defined time released again. Skin firmness is measured by the skin's ability to resist the negative pressure created by the suction, while skin elasticity is measured by skin's ability to return into its original form, data of which are displayed as curves.

Cutometer® measurements were carried out on the cheeks using bespoke stencils to maintain the same area for measurement, and the following data from the curves were recorded in triplicates:

2.7.1 | Skin firmness

Referred to as R0, this is the highest point of the first curve and represents the passive behavior of the skin to force. The closer the value is to 0, the more firm the skin.

2.7.2 | Skin elasticity

Referred to as R7, this gives an indication of the elasticity of the skin. The closer the value is to 1 (100%) the more elastic the skin.

2.8 | Statistical analysis

Comparisons between variables were performed using a paired, Student's *t* test (for parametric data), Wilcoxon rank-signed test, and chi-square test (for nonparametric data). Statistical software SPSS (SPSS Inc; version 10.0) was used, and differences were interpreted at a 95% level of significance.

3 | RESULTS

Day −30 served as the baseline for the placebo, while Day 0 served as the baseline for Day 30 and Day 60 of consuming NCA. The changes in parameters were presented as means of differences (± SEM) for each time point from their respective baselines. Raw data of measurements are presented in Supplementary Materials (Table S1-S4).

3.1 | Effect of NCA on skin topography

Skin texture is a product of blemishes, scars, pores, and wrinkles, and is measured by deviations of the skin's surface from its ideal form. Based on this deviation, there was a statistically significant reduction in skin roughness with NCA in 30 days of consuming NCA (Figure 2). More specifically, wrinkle width in the crow's feet area reduced at these time points as well (Figure 3), but wrinkle depth remained unaffected (Table S1) as per the instrumental analysis. The improvement in wrinkles, however, was reflected in the clinical evaluation score of wrinkles only at Day 60. Pore volume and Porosity Index significantly worsened after 30 days of placebo consumption, while these parameters saw a significant improvement after NCA consumption (Figure 4).

3.2 | Effect of NCA on hyperpigmentation

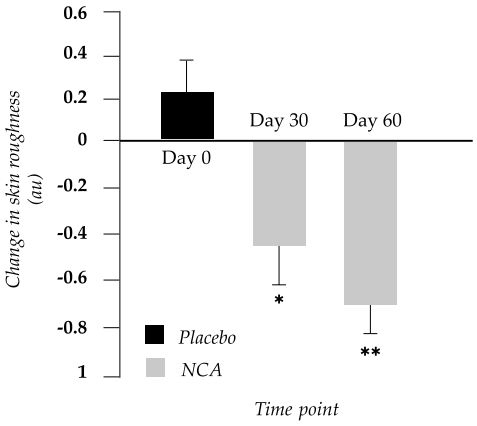
Hyperpigmented blemishes or "spots" were assessed clinically and instrumentally and did not show any change with placebo. While clinical grading did not show a difference in the intensity of spots with NCA, their colorimetry showed that there was a statistically significant reduction in their melanin level, and an increase in their luminosity from Day 30 onwards (Figure 5A,B). Clinical grading of periorbital hyperpigmentation was significantly reduced at Day 60 of NCA (Figure 5C).

3.3 | Effect of NCA on skin hydration and barrier properties

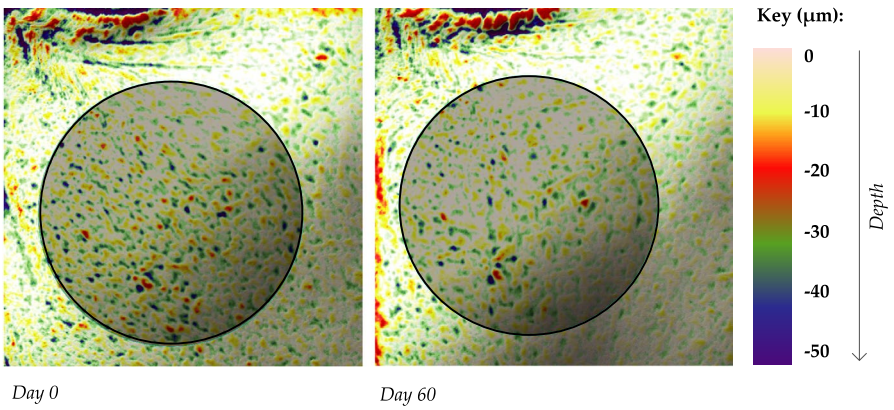
Skin hydration increased and TEWL reduced significantly after 60 days of NCA from baseline (Day 0) as shown by Figure 6. No significant change was seen with placebo or at Day 30 of NCA from their respective baselines.

3.4 | Effect of NCA on skin biomechanical properties

Skin firmness and elasticity were unaffected after 30 days of placebo consumption (at Day 0), but showed a significant improvement after 30 days of NCA consumption (Figure 7A,B). After 60 days of NCA consumption, however, only the improvement in firmness was statistically significant.

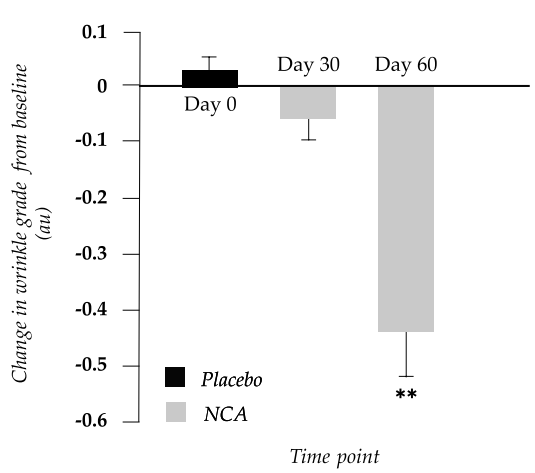


(A) **Skin texture**

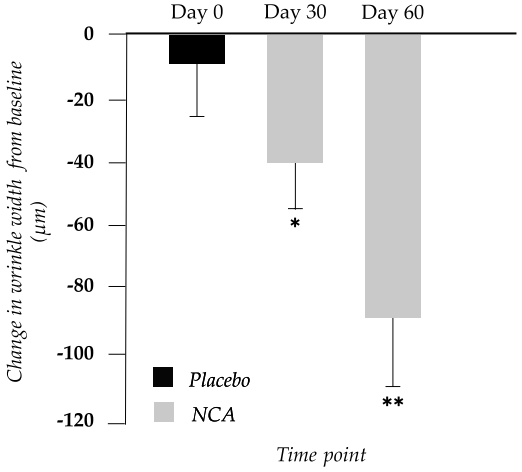


(B) **Representative 3D images of skin pores**

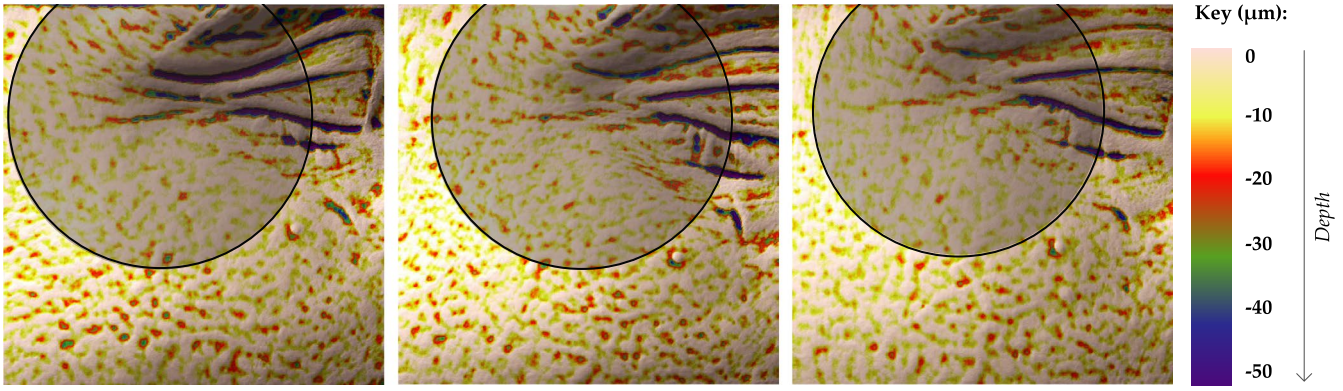
FIGURE 2 Skin texture measured using Antera 3D® imaging. The changes in skin roughness from baseline (A) are presented as mean differences ± SEM, which showed a significant reduction after NCA consumption, whereas no change was seen with placebo (at Day 0). Statistical significance (Student's *t* test) is indicated with * ($P \leq .05$) and ** ($P \leq .001$). The reduced roughness of skin has been demonstrated (B) as well, showing fewer and smaller blue/purple deep depressions



(A) **Clinical assessment of wrinkles**



(B) **3D imaging of wrinkle width**



(C) **Representative 3D images of wrinkles**

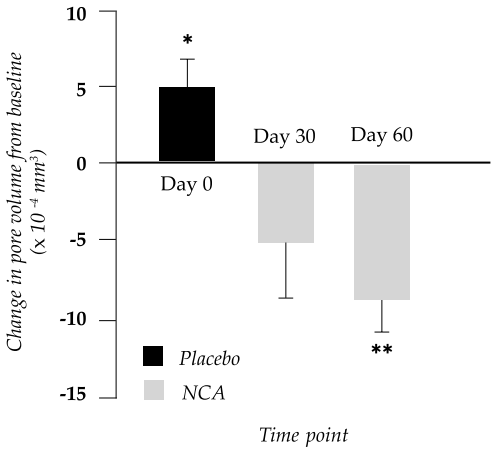
FIGURE 3 Changes in crow's feet wrinkles by (A) clinical grading and (B) 3D imaging (width) from baseline after 30 days of placebo consumption (Day 0), and 30 and 60 days of NCA consumption (Day 30 and Day 60, respectively). Changes are presented as mean differences ± SEM. Statistical significance with Student's *t* test is indicated with * ($P \leq .05$) and ** ($P \leq .001$). The reduction in wrinkles on Day 30 of consuming NCA compared to placebo is evident in the visual representation of its 3D images (C), with a further, significant improvement at Day 60

4 | DISCUSSION

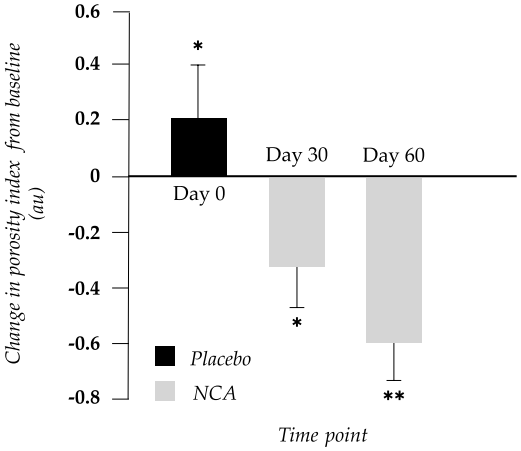
NCA showed an improvement in several parameters of skin health as discussed herein. The placebo on the other hand did not show any improvements in skin parameters as it lacked the ingredients known to be active in NCA.

4.1 | Skin roughness and wrinkles

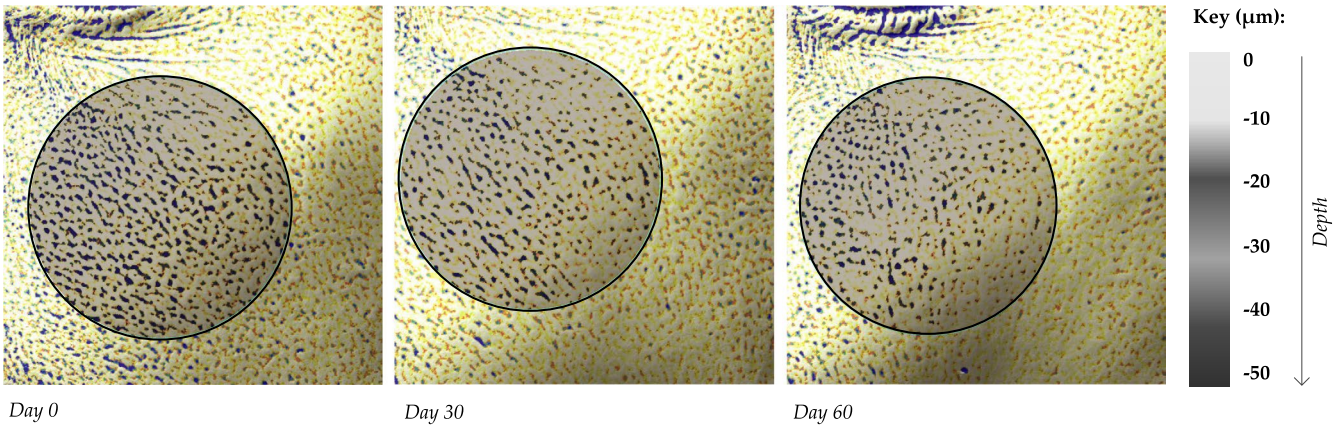
Antera 3D® has been previously validated to assess wrinkle severity and improvement.[29] Its 3D image analysis revealed that wrinkle width was significantly and consistently reduced over time with NCA consumption. This was also visually apparent, as shown by the significant reduction in wrinkles by clinical evaluation. Wrinkle depth, however, was not significantly affected by NCA. The depth of the wrinkles in wrinkle-prone areas of skin has been reported by previous studies to be associated with the underlying ligaments (retinacula cutis) structure and thickness. Tsukahara, K *et al* suggest that the reduced retinacula cutis density leads to the loss of supporting force to the dermis.[31] By extension, the building of dermal ECM would therefore lower the width of the wrinkles, and leave their depth unaffected, as was the result observed here.



(A) **Skin pore volume**



(B) **Porosity index**



(C) **Representative 3D images of skin pores**

FIGURE 4 Skin pores were assessed via two parameters using Antera 3D® imaging (A) skin pore volume and (B) Porosity Index, both of which worsened with placebo and showed improvements with NCA. Changes in these are presented as mean differences ± SEM, and statistical significance (Student's *t* test) is indicated with * ($P \leq .05$) and ** ($P \leq .001$). The images (C) display the difference in pores on Day 0 (before NCA) and Day 60 of consuming NCA

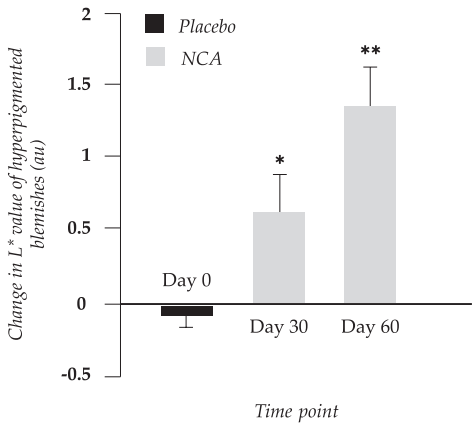
4.2 | Skin pores volume and porosity index

Enlarged skin pores, often reported in oily and acne-prone skin, can be considered a sign of aging as they have been shown to increase with age (in the Indian population) and linked to age-related loss of skin elasticity.[32] It is likely that invaginations of hair follicles appear bigger when their structural integrity is compromised. In this study, skin pore volume and Porosity Index worsened with placebo consumption. This may be due to skin damage as participants were restricted from using topical sunscreen and antiaging serums/creams for the duration of the study.

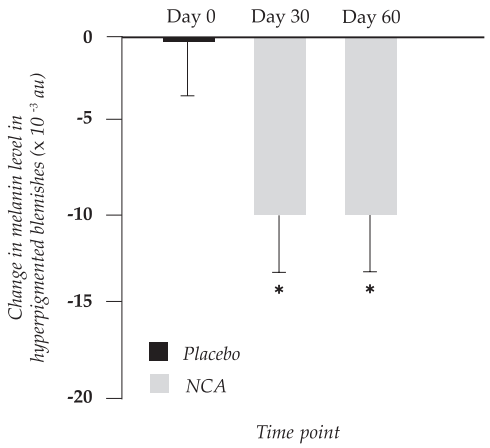
Skin pore volume was significantly reduced after 60 days of NCA consumption. Porosity Index was significantly reduced on Day 30 and Day 60 of NCA intake. The latter takes into account the density of open pores (and not just the size) in the selected area of the cheek as well. This suggests that the number of open pores may have started to reduce in size from 30 days of NCA consumption.

4.3 | Firmness and elasticity

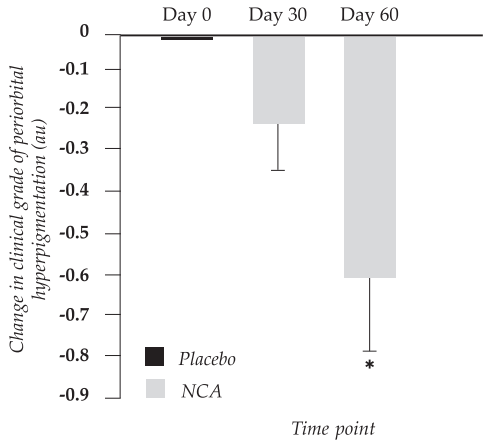
After 60 days of consuming NCA, there was no significant change in the mean elasticity from baseline (Day 0). There was, however, a significant improvement in elasticity at Day 30. A similar trend was observed in firmness as well—while both Day 30 and Day 60 showed a significant improvement from baseline, the improvement of Day 30 was more pronounced than that of Day 60. This seemingly conflicting outcome may have occurred due to the heterogenous nature of biological tissue.



(A) L^* value of hyperpigmented blemishes



(B) Melanin in hyperpigmented blemishes



(C) Periorbital hyperpigmentation score

FIGURE 5 Effects of placebo and NCA on hyperpigmentation. The colorimetry analysis of the Antera 3D® imaging software allowed the monitoring of changes in (A) the L^* value of hyperpigmented blemishes and (B) the melanin level in hyperpigmented blemishes from baseline. Changes in periorbital hyperpigmentation from baseline were assessed via clinical grading scores (C). The data are presented as mean differences ± SEM, and statistical significance (*t* test) is indicated with * ($P \leq .05$) and ** ($P \leq .001$)

Suction techniques make the assumption that skin is an isotropic elastic membrane. Skin is like a composite material, and its mechanical changes involve dynamic interactions of its elastic (proteins), viscous (water), and bioactive (cells and cytokines) components, making it highly anisotropic, viscoelastic, and simultaneously adaptable in nature.[33,34] The network of collagen predominantly provides firmness while that of elastin provides elasticity for all layers of the skin. Firmness and elasticity, however, depend on the same interconnected network and are therefore intrinsically related. Furthermore, collagen peptide consumption has also shown to promote elastin production in vitro and inhibit the release of matrix metalloproteinase-3 (MMP3), which degrades elastin.[35] This is likely the reason why a similar trend of improvement and a slight decline can be seen in the results of both firmness and elasticity. The orientation and fiber free length changes that occur due to newly synthesized ECM proteins, seasonal changes, as well as variance between people can further compound the complexity.[36]

While improvements in elasticity were seen on Day 30, a significant improvement was not achieved at Day 60 of consuming NCA. A longer study duration could show improvements in skin elasticity not observed in this study. These improvements were seen in previous studies at the given 5 g dose (and lesser) of low molecular weight collagen peptides.[37] Skin parameters such as skin pores and skin hydration, which were improved in this study, have been associated with an increase in skin elasticity.[30,36] These observations, in addition to the complexities of biomechanical testing on skin, highlight the short duration as a limitation of this study.

4.4 | Hyperpigmentation

Hyperpigmentation issues are very prevalent in the Indian context, especially in women. For example, hyperpigmented spots affect about 70% of women above the age of 30 years (to varying degrees), and periorbital hyperpigmentation is a concern in over 80% of women over the age of 35 years.[26]

In this study, the clinical evaluation did not show a significant decrease in hyperpigmentation spots. However, the melanin level of spots showed a significant reduction when measured using 3D imaging (on Day 30). Furthermore, according to the significantly increasing *L** value, the blemishes showed a progressive lightening on Day 30 and Day 60 of consuming NCA. It is not clear whether collagen peptides are accelerating epidermal turnover, which is lightening the blemishes, or they are interfering with one or more of the steps involved in the melanogenic pathway, melanin transfer to keratinocytes or simply degrading melanin. A reduction in hyperpigmentation may not have been apparent by visual inspection, but was seen by sensitive instrumental analyses. Given that studies on oral and topical depigmenting agents typically show results only after 4-12 weeks, the 60-day duration of the study could have been limiting with regard to clinical grading of hyperpigmentation.[38,39]

The reduction in pigmentation (shown by instrumental evaluation) may also be a result of NCA's antioxidants blend, as inflammation and photodamage can cause or worsen hyperpigmentation. We have already discussed the protective benefits of natural extracts from tomatoes, grapes, and green tea against UV-induced skin damage and their synergistic ability to provide UV protection. Further to this, the nutrients in NCA may have other mechanisms of action as well—for example, vitamin C and polyphenols from the grapes also interfere with melanogenesis by targeting its rate-limiting enzyme called tyrosinase.[40-42] Given their versatile benefits, grape and green tea polyphenols are now gaining recognition for oral photoprotection, melasma treatment, and lowering inflammation.[18,25,43-45]

Periorbital hyperpigmentation can be caused by a number of genetic and lifestyle factors. The skin in the periorbital region is already relatively thinner than other parts, and it thins further with age and skin damage.[46] This parameter was monitored by clinical grading which showed a significant improvement on Day 60 of consuming NCA. Other than UV protection with antioxidants, the collagen peptides may be increasing dermal collagen thickness, as has been previously demonstrated by animal studies in dermal and skeletal tissue.[47,48] While most improvements in skin parameters occurred on Day 30, periorbital hyperpigmentation was improved significantly only on Day 60, perhaps due to the multifactorial nature

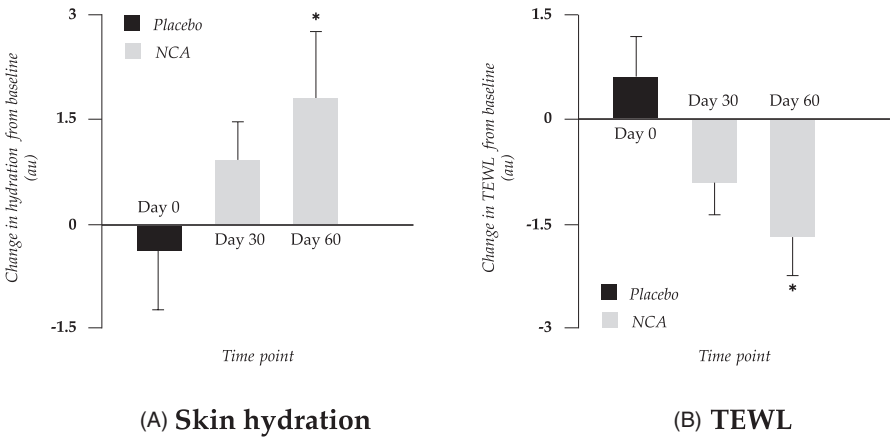


FIGURE 6 (A), Skin hydration was measured by MoistureMeter SC and (B), TEWL was quantified using a Vapometer for the different time points in this study (expressed as mean differences ± SEM). A statistically significant improvement of both these parameters was seen after 60 days of NCA consumption from baseline, as indicated by * (*t* test; $P \leq .05$)

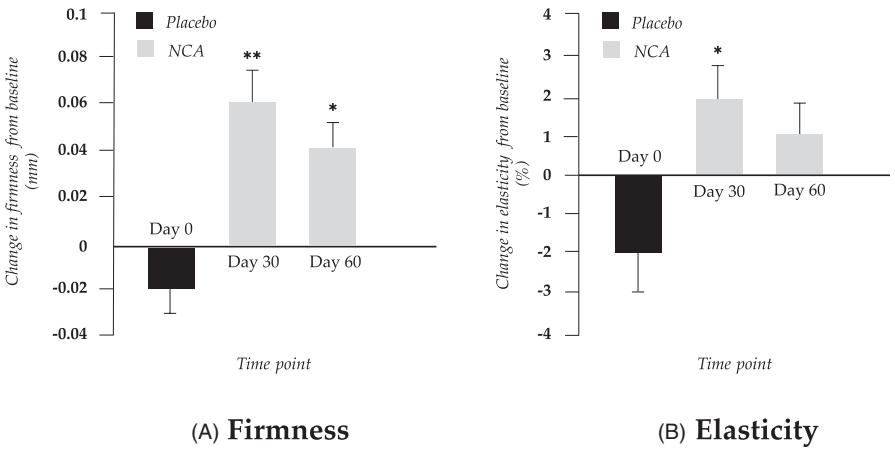


FIGURE 7 Changes in skin (A) firmness and (B) elasticity from baseline after 30 days of placebo consumption (Day 0), and 30 and 60 days of NCA consumption (Day 30 and Day 60, respectively). These were measured using a Cutometer® and presented as means ± SEM. Improvement in firmness is presented in positive numbers as opposed to its scale. Statistical significance (*t* test) is indicated with * ($P \leq .05$) and ** ($P \leq .001$)

of this cosmetic condition. While collagen peptides may have improved the thickness of skin in the periorbital region, carotenoids could have reduced their appearance, due to their ability to deposit in skin and affect its b^* value (yellowness) on the CIE $L^*a^*b^*$ scale.[16] An improvement in blood circulation by virtue of lycopene and grape seed polyphenols[49] could have also reduced the dark appearance in the periorbital region.

4.5 | Skin hydration and barrier properties

Skin hydration and TEWL are intrinsically related, and they depend on both, the protective epidermal lipid layer, and the water holding capacity of the underlying ECM. One of the reasons that skin hydration reduces and TEWL increases with age is the reducing amount of collagen in the ECM. It holds water within its structure, which concomitantly declines with reducing collagen. The significant improvement in these factors (at Day 60) is therefore expected, thereby substantiating the effect of collagen peptide consumption on the skin's ECM. Pro-Hyp and Hyp-Gly have shown to increase skin hydration by ameliorating skin barrier dysfunction[50] and perhaps also by reducing inflammation.[51] Playing a key role in maintaining cell volume of epidermal keratinocytes, taurine in NCA may have helped maintain the improvement in skin hydration.[20]

All of the above-mentioned properties of skin are dependent on the skin ECM's structural integrity. Considering the large proportion of collagen in the ECM, increasing skin's rate of collagen synthesis would improve these factors. Therefore, the daily consumption of collagen peptides facilitating collagen anabolism by fibroblasts is a likely mechanism of NCA improving skin parameters. Furthermore, research is revealing phytonutrient benefits far beyond photoprotection and scavenging singlet oxygen radicals. For example, supplementation with a lycopene-rich tomato paste reduced the UV-induction of MMP1, a collagenolytic enzyme elemental to photoaging, and increased deposition of procollagen I.[21] In connection with skin disorders, carotenoids can also scavenge peroxyl radicals at low oxygen tension, thereby preventing lipid peroxidation in skin.[52,53] Antioxidants and collagen peptides, both, may help reduce inflammation, and therefore be useful for inflammatory conditions of skin like atopic dermatitis as well.[51,54,55]

The overall results of this study were positive, supporting the role of collagen peptides and antioxidants, and specifically NCA, in maintaining healthy skin and mitigating common skin concerns of the Indian population. An efficacy questionnaire from participants corroborated these results (Table S5), with 97% reporting that their skin felt firmer and 88% reporting improvements in wrinkles, fine lines, and undereye dark circles. A tolerance and acceptability questionnaire confirmed that there were no adverse reactions reported by the participants. Further studies can explore the specific and long-term benefits of these nutrients, and clarify the mechanisms that underlie the connections made here.

5 | CONCLUSIONS

Skin health parameters did not improve with the placebo, which lacked the ingredients believed to be active in NCA. The presence of collagen peptides, phytonutrients from botanical extracts (tomatoes, grapes, and green tea), vitamins, and taurine in NCA improved metrics of skin health. After 30 days of daily NCA intake, skin roughness and crow's feet wrinkle width were significantly reduced, while skin firmness and elasticity increased. After 60 days of NCA consumption, there was an improvement in metrics of open pores and hyperpigmented spots, and the clinical grade of periorbital hyperpigmentation. Skin hydration also improved at this time point, with a concomitant reduction in TEWL. Skin parameters that improved on Day 30 remained the same or improved further at Day 60, with the exception of skin elasticity that failed to reach a statistically significant improvement from baseline. A longer study duration may help more accurately determine the impact of NCA on skin elasticity. Overall, NCA showed a significant improvement in metrics of skin health and can be used for therapeutic and cosmetic applications.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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APPENDIX A

Blood and urine test panels for participant screening:

1. Blood test parameters

☐Complete blood count
☐Biochemistry profile (complete metabolic panel)
☐Lipid profile

2. Urine test parameters

☐Physical and chemical examination
☐Microscopic examination