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ORIGINAL ARTICLE

Revitalizing skin, hair, nails, and muscles: Unlocking beauty and wellness with vegan collagen

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Abstract

Background: Collagen, a key protein in the body maintains hair, skin and bone health and its production tends to decrease in synthesis as humans age. The demand for vegan collagen-builder has increased worldwide due to increased adaptability to vegan diet.

Objective: This clinical study was designed aim to evaluate the safety and efficacy of vegan collagen builder (VEGCOL™) at different dosages (2.5, 5, and 10 g) in adult participants.

Methods: Total 66 subjects (22 subjects/dose) aged 30 to 50 years were enrolled, and 63 subjects completed the study. Duration of study was 60 days. Evaluations included change in skin elasticity, hydration, crow's feet area wrinkles, fine lines, skin, Glogau skin age, change in pain scale score, muscle strength and subject perception assessment about test treatment use.

Results: After 60 days of treatment, there was significant improvement in hair growth rate by 45.01%, 38.54% and 50.37% with $p < 0.01$ for doses 2.5, 5, and 10 g respectively. Additionally, 19.64% ($p < 0.0001$) and 20.51% ($p < 0.0001$) increase in hair density and hair thickness respectively was observed with 10 g dose. 2.5 g dose resulted in 33.03% ($p < 0.01$) increase in skin smoothness and 49.94% ($p < 0.0001$) decrease in crow's feet area wrinkles, decreased retraction time by 21.71 milliseconds ($p < 0.05$). 52.54% reduction in pain score ($p < 0.001$). No any adverse events were reported.

Conclusion: Vegan collagen-builder effectively improved multiple age-related concerns such as wrinkles, fine lines, joint pain, muscle strength and hair growth. All respondents perceived the product as beneficial in improving the aesthetics of the skin, hair, and nails. The findings support the use of vegan collagen-builder as safe and efficacious in promoting healthier skin, stronger muscles, and improved hair and nail conditions.

KEYWORDS
fine lines, hair fall, muscle strength, vegan collagen- builder, wrinkles

1 | INTRODUCTION

Collagen is a crucial scaffold protein that gives skin its smoothness and flexibility, but as people become older, their bodies synthesize less of collagen. Due to its proven ability to repair skin damage, collagen-based supplements have emerged as an integral component in the management of the aging process and provide the youthful, healthy appearance that is desired in the quest of beauty. Over the past few decades, additional collagen-based products and tissue engineering biomaterials have been developed owing to increased knowledge of collagen sources, extraction methods, structure, and characteristics.[1] Products containing collagen have been shown to have a significant positive impact on human health, particularly in older individuals due to its low immunogenicity and great biocompatibility.[1,2]

The loss or defect of collagen can cause skin aging and other skin conditions which result in the occurrence of fine lines and wrinkles. The collagen treatments have demonstrated effective improvements in skin hydration, skin elasticity, and medical scaffold treatment.[3] Collagen acts as a supportive layer beneath the skin by enhancing hair growth and reducing the hair fall by strengthening the scalp to withhold the hair.[4]

As the popularity of vegan diets continues to rise, accompanied by a notable decline in the consumption of animal products, there has been a growing interest in vegan supplements that increase collagen levels in the body. Despite its increasing adaptability, there is currently limited research available on the specific benefits and potential risks associated with vegan collagen.[5]

A clinical study has shown that taking supplements containing bioactive collagen peptides can improve the condition and aesthetics of the skin, nails, and hair.[6] These oligopeptides are derived through the enzymatic hydrolysis of natural collagen. Following ingestion, these compounds undergo metabolism within the gastrointestinal tract, where they get converted into bioactive di- and tri-peptides. Subsequently, these peptides are released into the bloodstream and accumulate in the skin, contributing to the formation of the collagen biomatrix.[6]

The test treatment used in the present study contains ingredients including papain enzyme, carrot and broccoli. Papain is a proteolytic enzyme which is rich in vitamin A and C that improves collagen content in body.[7,8] Carrots and broccoli are another excellent sources of vitamin A and C and have a vital use in cosmetic industries.[9,10] Various amino acids including proline, glycine, glutamic acid, hydroxyproline, lysine, threonine and many more, which are the building blocks of collagen are easily obtained by the consumption of vegetables like broccoli and carrot.[11,12]

The test treatment was used in three different doses: 2.5, 5, and 10 g. This clinical study was designed with the objectives to assess comparative clinical safety, and efficacy of test treatment with three different doses by its continuous use for 60 days in order to determine improvement in skin radiance, hydration, elasticity, wrinkles and fine lines, and skin texture, improvement in sleep by providing deep, calm, and restful sleep, reduction in joints pain, stiffness, swelling, improvement in muscles strengths, improvement in hair growth rate, hair thickness and density, improvement in hair and nails health in adult human subjects.

2 | MATERIALS AND METHODS

2.1 | Ethical considerations

This study was conducted in accordance with the Declaration of Helsinki (Brazil, October 2013), ethical standards for medical research involving human subjects, the Indian Council of Medical Research's ethical guidelines for biomedical research, new drugs and clinical trials rules 2019, and International Conference of Harmonization Guideline for Good Clinical Practice E6 (R2) for Good Clinical Practice {ICH-GCP E6 (R2)}, including Indian GCP. Prior to commencing the trial, an Independent Ethics Committee (IEC) thoroughly reviewed and approved the study protocol. The trial was registered in the Clinical Trial Registry India (CTRI) under the registration number CTRI/2022/11/047149 on November 9, 2022. Additionally, the trial was also registered with ClinicalTrials.gov and was assigned the trial identifier number NCT05613660. Written informed consent was obtained from all participants before commencing any study-related procedures. The participants made a voluntary decision to participate based on their understanding of the provided information regarding the study.

2.2 | Study design and selection criteria

This was proof of science/concept clinical study with randomization, comparative, single-blinded design to evaluate safety and efficacy of collagen peptide obtained from vegetables in adult human subjects. This clinical study was conducted at NovoBliss Research Private Limited, Ahmedabad, India. In this study, a total of 66 adults aged 30 to 60 were recruited with the goal of having 22 subjects in each dose group i.e. 2.5, 5, and 10 g. Out of these participants, 63 (95.45%) successfully completed the study, while 3 (4.54%) were lost to follow-up. Ultimately, data analysis was done on 63 subjects who completed the study, with an equal distribution of 21 subjects in each dose group. This study had a total duration of 60 days (+2 days) comprising a total of 6 Visits. Visit 01 (Day −4) was scheduled 4 days before Day 1, followed by Visit 2 on Day 1, Visit 3 on Day 10 (+2 day), Visit 4 on Day 30 (+2 days), Visit 5 on Day 57 (+2 days) and Visit 6 held on Day 60 (+2 days).

2.2.1 | Inclusion criteria

The study enrolled healthy adult males and non-pregnant/non-lactating females aged between 30 and 50 years. The participants were in good overall health and presented with mild to moderate crows' feet wrinkles, as well as mild to moderate joint pain,

swelling, stiffness, and decreased range of motion. They also had a Physician's Global Assessment (PGA) score indicating at least mild skin aging and a Glogau skin age of II or III. Additionally, they reported hair fall, decreased hair growth, and self-declared non-pathological thin, dry, and brittle hair and nails and refrained from undergoing any cosmetic procedures for a period of 3 months prior to and throughout the study. They avoided using any new skincare products during the study duration. Changes in baseline medications, nutritional supplements, and the use of any other collagen peptide powder were not allowed during the study period for the participants. Participants used stable doses of contraceptive or replacement hormonal therapy for at least 6 weeks prior to and throughout the study. Those of childbearing potential agreed to use a reliable method of birth control during the study. Participants were requested not to introduce any new soaps, cleansers, lotions, creams, or other face products for the duration of the study. They provided written informed consent before initiation of the study procedures, and refrained from using medicated/prescription shampoo, hair products containing minoxidil/anti-thinning agents, or any other hair growth treatments or products aside from the test treatment. Individuals with osteoarthritis who experienced joint pain and stiffness based on their current prescription and medical history were also included in the study.

2.2.2 | Exclusion criteria

Excluded were the subjects who had a history of allergy or sensitivity to the ingredients of the test treatment, specifically fish, bovine, chicken, broccoli, and carrots, were excluded from the study. Participants with pre-existing or dormant dermatologic conditions such as psoriasis rashes, eczema, seborrheic dermatitis, acne, or any other condition that could interfere with the study outcomes, as determined by the investigator, were also excluded. Those who had received systemic therapy with chronic antibiotic therapy, retinoids, and/or oral steroids within the 4 weeks prior to the study start or anticipated needing to use them at any point during the study were not eligible for participation. Additionally, participants who were not willing to avoid unprotected sun exposure or other ultraviolet radiation during the study period were also excluded. Individuals with a history of prior hair growth treatment within the last 3 months, such as hair transplants or laser procedures, those with a history of alcohol or drug addiction, plans to shave scalp hair during the study, participation in or planning to start a weight loss program that could significantly impact body weight, a history of skin radiance or wrinkle laser treatment within the last 3 months, or any other condition that, in the dermatologist's/investigator's discretion, could warrant exclusion from the study, were also excluded. Pregnant or breastfeeding individuals, those with a history of chronic illnesses that may influence the condition of the skin, and participants currently participating in other similar cosmetic, device, or therapeutic trials or using skin care products within the last 4 weeks were not eligible for participation in the study.

2.3 | Evaluation parameters

The primary efficacy parameters include various aspects of skin related changes like reduction in fine lines of crow's feet area, facial wrinkles, and improvements in skin texture using Visioscan® VC 20 Plus (Courage + Khazaka electronic GmbH, Cologne, Germany); skin elasticity, retraction time and young's modulus using DermaLab® Combo (Cortex Technology, Denmark); deep skin hydration using MoistureMeterEPiD (Delfin Technology Ltd., Finland). Parameters related to hair health included hair growth, thickness and density using CASLite-Nova (Catseye Systems & Solutions Pvt Ltd, India); anagen to telogen (A:T) ratio and total hair count using hair pluck test; number of hair breakage and hair with bulb using 60 s hair count technique.[13] Anthropometric measurements such as height, weight, and BMI were recorded. Study also evaluated change in muscle strength; joint pain effect using 10-point Visual Analog Scale (VAS) scale.[14]

Secondary evaluation included improvement in Glogau skin age[15] and dermatologist scoring with the Griffiths scale to assess treatment effectiveness based on severity of facial skin. The scale comprises of 9 points, categorized as follows: 0 (no sign), 1–3 (mild), (4–6) moderate, and (7–9) severe, measured at Days −4, 1, 10, 30, and 60. Hair strength was measured using hair pull test. Sleep quality was evaluated using Leeds Sleep Evaluation Questionnaire (LSEQ) consisting of 10 self-rating 100-mm (10 cm)-line analogue questions concerned with aspects of sleep and early morning behaviour.[16] Evaluation of nail appearance was done using PGA; general appearance of hair was evaluated by dermatologist and a hedonic questionnaire was used to gather feedback from the subjects regarding skin elasticity, suppleness, sleep quality, digestion and gut health, joint health, and nail and hair health. By utilizing these various instruments and assessment methods, a comprehensive evaluation of the efficacy parameters was achieved, providing objective and subjective data to assess the effects of the test treatment on multiple aspects of skin, hair, nails, joint health, and overall well-being.

2.4 | Assessments of safety and tolerability

Safety assessments were conducted by the dermatologist and study physician to evaluate possible treatment-emergent adverse effects such as intolerable irritability, excessive bloating, nausea, diarrhea, and skin-related issues such as redness, tingling, dryness, and burning. By assessing these treatment-emergent adverse effects, the safety profile of the test treatment was determined.

2.5 | Internal training and validation

An internal validation study was conducted to ensure consistency in readings and maintain standardized evaluation techniques for clinical safety and efficacy studies involving hair care products on healthy adult human subjects. The study focused on various evaluations and

scoring activities such as the 60 s combing test, pull test, pluck test, subjective questionnaires, and scoring for hair quality appearance and scalp condition. To maintain consistency in evaluating skin-related parameters, the dermatologist conducted internal training sessions. These sessions aimed to establish documentary evidence and ensure adherence to procedural steps, processes, and scoring activities. By implementing these internal validation measures, the study aimed to ensure the reliability and consistency of their evaluation techniques and maintain the quality and accuracy of their clinical studies for hair care product evaluation.[17]

The internal training conducted by the dermatologist involved explaining the anatomy of the skin and hair through a PowerPoint presentation (Microsoft, Washington, United States). This was followed by detailed explanations and discussions of each evaluation using photographic examples, providing a comprehensive understanding for each evaluator. Standard images were utilized for photographic evaluations. Prior to conducting the study evaluations in a blinded manner, test evaluations were conducted between dermatologists and trainee/trained evaluators. The dermatologist selected 50 high-resolution pictures for each parameter throughout the training. These pictures were examined and scored by the dermatologist and all evaluators on a computer system using the same light-emitting diode (LED) screen. The scoring system for the images followed predetermined standards and parameters, with high definition graphics details, a resolution of 1920 × 1080, and a frame rate of 60 Hz. Only single, same LED screen with a screen light level of 90 were used for the photographic evaluation, and display scaling was kept constant. To ensure inter-evaluator dependability and maintain consistency between dermatologists, and trained evaluators, the scoring of these groups was analyzed. These measures were implemented to establish reliable and consistent evaluation techniques across the study.[13]

Physiotherapists conducted training sessions to demonstrate the procedural steps, processes, and scoring activities related to parameters such as VAS scoring and muscle strength tests. Through a PowerPoint presentation, the physiotherapists explained the anatomy of the knee and elbow joints, range of motion, and measurements of muscle strength. They also performed push-ups and planks to demonstrate correct posture and provided training on the correct techniques, steps involved, and things to consider while performing the activities.

2.6 | Test treatment and doses (2.5, 5, and 10 g)

The test treatment is a patent-pending formulation researched and manufactured by Collagen Lifesciences Private Limited, an entity of Krishna Enzytech, India. It is a unique blend of special vegetables including broccoli and carrot, proteolytic enzyme papain and water. Papain enzyme is treated and added to facilitate the liberation of the specific amino acids from the extract. It contains 26 g per 100 g of collagen tripeptide (CTP) and 3.6 g per 100 g of glycine-proline-hydroxyproline (GPH) with a strong amino acid profile with higher concentration of proline, glutamic acid, hydroxyproline, glycine, lysine, threonine and various other essential and non-essential amino acids. Moreover, its amino acid composition and molecular structure closely resembles Type 1 and Type 3 collagen, having molecular weight around 300 kDa. It is designed to provide equal benefits as animal-derived collagen and is suitable for consumption all over the world, particularly in vegetarian-populated countries like India and many other countries globally. The scientific rationale for implementing a multi-dose study was to methodically evaluate the treatment across various pharmaceutical and nutraceutical applications and varied treatment durations. This approach was undertaken to establish a standardized dosage regimen tailored to the specific use of the treatment. The test treatment was used in three different doses: 2.5, 5, and 10 g as the study designed with the objectives to assess comparative clinical safety, and efficacy of test treatment with three different doses by its continuous use for 60 days in order to determine improvement in skin radiance, hydration, elasticity, wrinkles and fine lines, and skin texture, improvement in sleep by providing deep, calm, and restful sleep, reduction in joints pain, stiffness, swelling, improvement in muscles strengths, improvement in hair growth rate, hair thickness and density, improvement in hair and nails health in adult human subjects. Subjects consumed 1 sachet (scoop) of test treatment daily, accompanied by a glassful of water (approximately 250 mL) for a total of 60 days.

2.7 | Statistical methods

Descriptive statistics were used to summarize the continuous variables, such as the number of observations (*N*), mean, standard deviation (SD), median, minimum, and maximum values. For categorical variables, frequencies and percentages were presented, and when necessary, graphical representations were utilized. Paired *t*-tests were conducted to compare continuous variables between the baseline and post-treatment measurements, allowing for the assessment of any significant changes. The statistical analysis was performed using R software (Version 4.0) and GraphPad Prism [Version 9.5.1 (733)] with a significance level set at 5%. GraphPad Prism was used to analyze data of Investigator Global Assessment and VAS score. Subjects who withdrew from the study were not included in the statistical analysis to ensure the integrity of the data analysis.

3 | RESULTS

3.1 | Demographics and baseline characteristics

A total of 66 subjects were enrolled in the study, with an equal distribution of 22 subjects in each dosage group. The inclusion and exclusion criteria were pre-defined and used to select eligible subjects. Among the enrolled subjects, there were 27 females (40.90%) and 39 males (59.10%). The average age of the subjects at the study visit was 38.65 years and the average height was 164.01 cm. Further

detailed demographics and baseline characteristics are summarized in Table 1.

3.2 | Endpoint assessments

3.2.1 | Crow's feet area wrinkles and fine lines, skin texture–roughness, dryness, wrinkles, smoothness

Statistical analysis revealed that on Day 10, subjects in the 2.5, 5, and 10 g dosage groups exhibited a statistically significant decrease in crow's feet area wrinkles from their baseline values. Specifically, the values decreased from 103.22 ± 52.04 to 69.55 ± 22.02, showing a reduction of 32.62% ($p < 0.001$) in the 2.5 g group; from 91.83 ± 37.20 to 69.16 ± 20.80, representing a reduction of 12.83% ($p < 0.001$) in the 5 g group; and from 100.98 ± 48.00 to 73.11 ± 18.47, indicating a reduction of 27.17% ($p < 0.005$) in the 10 g dosage group. On Day 30, the test treatment reduced mean values from 69.55 ± 22.02 to 53.79 ± 12.77 with 48.30% reduction ($p < 0.0001$); from 69.16 ± 20.80 to 53.16 ± 15.32 with 42.12% reduction ($p < 0.0001$) and 73.11 ± 18.47 to 52.65 ± 10.20 with 47.85% reduction ($p < 0.0001$) in dosage group 2.5, 5, and 10 g respectively. On Day 60, 10 g dosage group demonstrated the highest attenuation with mean value of 47.30 ± 9.86 with 53.16% reduction from baseline ($p < 0.0001$). 2.5 g dosage group had mean value 44.23 ± 7.35 with 49.94% reduction ($p < 0.0001$) while 5 g dosage group showed mean value of 45.03 ± 14.40 with 46.81% reduction from baseline ($p < 0.0001$) (Table 2; Figures 1 and 2).

Skin roughness is a crucial parameter for assessing cosmetic skin properties. The test treatment demonstrated its effectiveness in reducing skin roughness across different dosage groups. At 10 days, compared to baseline measurements, a significant reduction in skin roughness was observed in the 2.5 g dosage group by 36.77% ($p = 0.20$) with mean value 0.61 ± 0.50; in 5 g dosage group by 43.08% ($p = 0.05$) with mean value 0.65 ± 0.39 and in the 10 g dosage group by 28.54% ($p = 0.33$). By Day 30, there was a substantial reduction in skin roughness in all dosage groups 2.5 g dosage group showed a significant reduction of 140.13% ($p < 0.05$) with mean 1.06 ± 0.93; the 5 g dosage group exhibited a reduction of 147.03% ($p < 0.01$) with mean 1.12 ± 0.82, and the 10 g dosage group demonstrated a reduction of 158.79% ($p < 0.01$) with mean 1.21 ± 0.97, compared to baseline measurements. On Day 60, the 2.5 g dosage group displayed a significant reduction of 120% ($p < 0.01$) with mean 0.99 ± 0.68; the 5 g dosage group showed a reduction of 185.28% ($p < 0.01$) with mean 1.30 ± 0.71, and the 10 g dosage group demonstrated a reduction of 145.92% ($p < 0.01$) with mean 1.15 ± 0.61, compared to baseline measurements. These findings indicate a perceptible effect of the test treatment in achieving smoother and suppler skin, as evidenced by the significant reductions in skin roughness across the study duration and dosage groups.

The administration of test treatment demonstrated significant improvements in reducing skin scaliness. On the 10th day, compared to the first day, the 5 g dose group experienced a significant reduction in skin wrinkles by 28.83% ($p < 0.05$) with mean 3.95 ± 2.14, while the 10 g dose group exhibited a reduction of 32.72% ($p < 0.001$) with mean 3.74 ± 1.86. Furthermore, on the 30th day, compared to the first day, the 2.5 g dose group demonstrated a significant reduction in skin scaliness by 50.31% ($p < 0.001$) with mean 2.46 ± 1.40; the 5 g dose group showed a reduction of 49.74% ($p < 0.0001$) with mean 2.78 ± 1.76, and the 10 g dose group exhibited a reduction of 55.58% ($p < 0.001$) with mean 2.60 ± 1.19. By the 60th day the 2.5 g dose group displayed a significant reduction in skin scaliness by 51.99% ($p < 0.0001$) with mean 2.26 ± 1.27, the 5 g dose group showed a reduction of 62.01% ($p < 0.0001$) with mean 2.11 ± 1.72, and the 10 g

TABLE 1 Demographics and baseline characteristics.

Parameters	Statistical values	Test treatment (2.5 g) ($N = 22$)	Test treatment (5 g) ($N = 22$)	Test treatment (10 g) ($N = 22$)
Age	Mean (SD)	37.00 (6.16)	39.27 (5.37)	39.68 (5.15)
Gender	Female	9 (41.00%)	9 (41.00%)	9 (41.00%)
	Male	13 (59.10%)	13 (59.10%)	13 (59.10%)
Height (cm)	Mean (SD)	166.86 (10.91)	161.68 (7.93)	163.5 (11.65)
Weight (kg)	Mean (SD)	65.93 (13.07)	62.17 (14.29)	63.08 (9.37)
BMI	Mean (SD)	23.82 (4.34)	23.61 (4.30)	23.82 (4.42)
Waist circumference	Mean (SD)	35.23 (5.00)	35.50 (4.30)	35.20 (3.30)
Hip circumference	Mean (SD)	39.00 (5.512)	39.14 (3.80)	38.82 (3.35)
Current medical/Concomitant medicine history (yes/no)	Number (%)	22 (100.0%)	22 (100.0%)	22 (100.0%)
Type of skin (normal, sensitive, mixed, dry, oily)	Dry	3 (13.60%)	3 (13.60%)	3 (13.60%)
	Mixed	7 (31.90%)	4 (18.20%)	6 (27.30%)
	Normal	12 (54.50%)	13 (59.10%)	11 (50.0%)
	Oily	0 (0.0%)	2 (9.10%)	2 (9.10%)

Abbreviations: BMI, body mass index; *N*, number of non-missing observation; SD, standard deviation.

TABLE 2 Enhancing hair and skin metrics with the administration of test treatment.

Percentage change from baseline				
Variables	**Visit days**	**Test treatment (2.5 g)**	**Test treatment (5 g)**	**Test treatment (10 g)**
Crow's feet area wrinkles	Day 10	32.62% ↓	12.83% ↓	22.70% ↓
	Day 30	48.30% ↓	42.12% ↓	47.85% ↓
	Day 60	49.94% ↓	50.97% ↓	53.16% ↓
Skin roughness	Day 10	36.77%↓	43.07%↓	28.54% ↓
	Day 30	140.13%↓	147.03% ↓	158.79%↓
	Day 60	121.74%↓	185.27%↓	145.90% ↓
Skin scaliness	Day 10	14.44%↓	28.83%↑	32.72%↓
	Day 30	50.31%↓	49.74% ↓	62.01% ↓
	Day 60	51.99%↓	62.01%↓	61.22%↓
Skin viscoelasticity	Day 10	5.13% ↑	0.91% ↓	14.86 ↑
	Day 30	17.65% ↓	2.14% ↓	1.53% ↑
	Day 60	33.03% ↓	36.28%↓	32.53% ↓
Skin smoothness	Day 10	13.74%↑	11.66%↑	17.13%↑
	Day 30	15.54%↑	18.91%↑	25.08%↑
	Day 60	33.03%↑	36.28%↑	32.53%↑
Skin retraction time	Day 10	11.78% ↓	13.59% ↓	19.04% ↓
	Day 30	11.84% ↓	10.52% ↓	19.87% ↓
	Day 60	10.97% ↓	13.71% ↓	21.37% ↓
Skin hydration	Day 10	13.29%↑	14.26%↑	25.82%↑
	Day 30	14.02%↑	21.37%↑	27.81%↑
	Day 60	3.63%↑	14.37%↑	22.39%↑
Hair density	Day 10	11.42%↑	4.46%↑	5.10%↑
	Day 30	11.73%↑	8.71%↑	8.70%↑
	Day 60	17.39%↑	16.57%↑	19.64%↑
Hair thickness	Day 10	10.75%↑	9.68%↑	10.56%↑
	Day 30	18.86%↑	14.58%↑	20.51%↑
	Day 60	22.44%↑	26.35% ↑	20.51%↑
Hair fall	Day 10	12.54%↑	17.26%↑	19.46%↑
	Day 30	29.01%↑	27.07%↑	34.34%↑
	Day 60	42.13%↑	40.27%↑	46.84%↑

dose group exhibited a reduction of 61.22% ($p < 0.0001$) with mean 2.26 ± 1.44.

The test treatment demonstrated significant effectiveness in improving skin smoothness. On the 10th day, compared to baseline, the 10 g dose group showed highest significant improvement in skin smoothness by 17.13% ($p < 0.01$) with mean 297.01 ± 67.32. Further improvements in skin smoothness were observed on the 30th day. Compared to baseline, 2.5 g showed 15.54% improvement but the change was not significant. 5 g dose group exhibited a significant improvement of 18.91% ($p < 0.05$) with mean 288.19 ± 110.25, while the 10 g dose group showed a significant improvement of 25.08% ($p < 0.01$) with mean 270.05 ± 92.28. By the 60th day the 2.5 g dose group demonstrated a significant improvement of 33.03% ($p < 0.01$) with mean 218.77 ± 42.92; 5 g dose group showed a significant improvement of 36.28% ($p < 0.01$) with mean 226.46 ± 88.98, while the 10 g dose group exhibited a significant improvement of 32.53% ($p < 0.01$) with mean 243.18 ± 72.95. These results indicate that the test treatment effectively enhances skin smoothness, with significant improvements observed across multiple dose groups and over various time points during the study period (Table 2).

3.2.2 | Skin viscoelasticity, young's modulus, retraction time

The usage of test treatment resulted in significant improvements in skin viscoelasticity. After 10 days, compared to baseline, the 2.5 g dose group showed non-significant improvement of 5.12%,

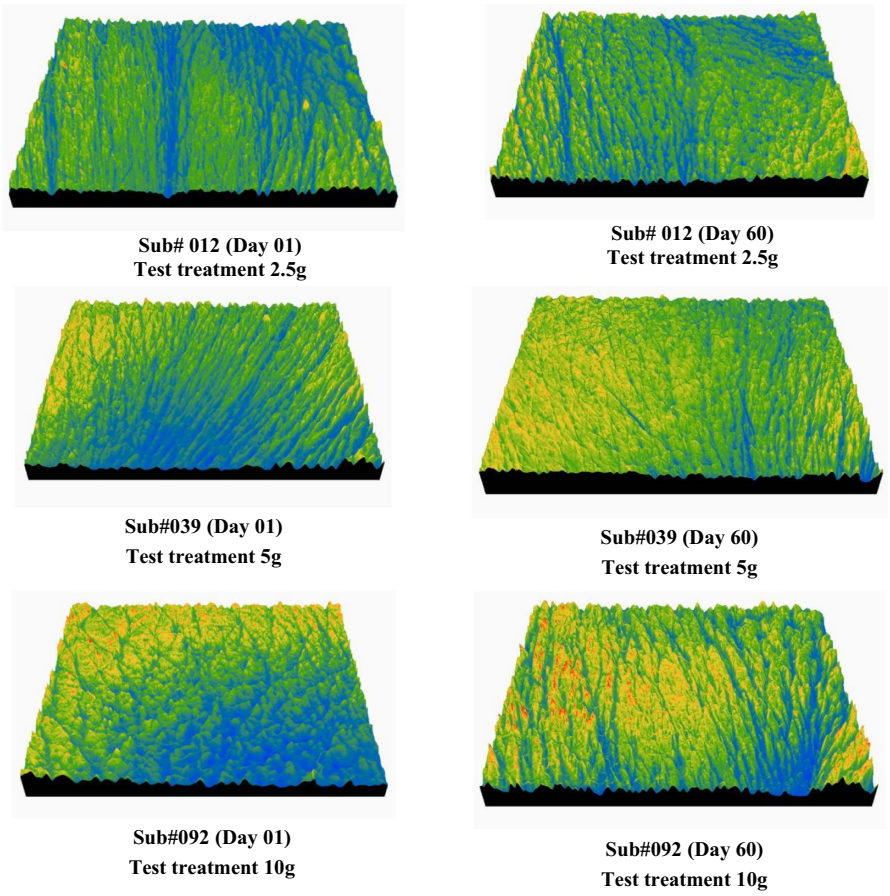


FIGURE 1 3D images of improvement in crow's feet area wrinkles.

while the 10 g dose group demonstrated a more substantial improvement of 14.86% ($p = 0.92$) with mean reading 4.28 ± 1.52. By Day 30, a significant improvement of 17.65% in skin viscoelasticity was observed in the 2.5 g dose group while non-significant improvements of 2.14% ($p = 0.84$) and 1.53% ($p = 0.84$) were observed in the 5 g and 10 g dose groups, respectively. By the 60th day, improvements in skin viscoelasticity was increased in 2.5 g dose group by 33.03%. 5 g dose group exhibited a 36.28% improvement while 10 g dose group demonstrated a 32.53% improvement. These findings indicate that test treatment enhances skin viscoelasticity, which is a positive indicator of healthy and elastic skin. It demonstrates the skin's ability to maintain its shape, resist deformation, and recover from stress, contributing to overall skin health and elasticity. No significant change was observed in the Young's modulus of the skin on 60th day compared to baseline (Table 2).

In terms of skin retraction, in comparison with baseline, on Day 10, there was a statistically significant decrease by 11.78% ($p < 0.01$) with mean time 135.19 ± 20.50 ms, and 13.59% ($p < 0.05$) with mean time 136.81 ± 22.16 ms, in dosage groups 2.5 and 5 g respectively. 19.40% ($p = 0.09$) attenuation of skin retraction time was seen in dosage group 10 g with mean time 137.31 ± 22.30 ms. In comparison with baseline, on Day 30, there was attenuation noted in the skin retraction time by 19.87% ($p = 0.07$) in dosage group of 10 g. In dosage group 2.5 g and 5 g there was significant attenuation in skin retraction time of 11.84% ($p < 0.05$) with mean time 137.05 ± 23.29 ms and 10.52% ($p < 0.01$) with mean time 141.66 ± 27.72 ms respectively. On Day 60, there was improvement of 10.97% ($p < 0.01$) with mean time 136.42 ± 21.01 ms; 13.71% ($p < 0.05$) with mean time 136.61 ± 14.92 ms and 21.37% ($p = 0.05$) with mean time 132.42 ± 15.03 in dosage group 2.5, 5, and 10 g respectively. The decrease in retraction time indicated that the skin was more elastic and increased elasticity is more often associated with younger appearance and better overall skin health (Table 2).

3.2.3 | Skin hydration

Keeping skin hydrated protects skin integrity and avoids tissue damage, which can cause many disabling problems. There was significant increase noted in moisture content of skin by 13.29% ($p < 0.01$) with mean content 41.18 ± 7.54%, and 14.02% ($p < 0.001$) with mean content 42.51 ± 6.48% in dosage group of 2.5 g and 5 g respectively on Day 10. Improvement in skin moisture content was also observed by 3.63% ($p = 0.33$) with mean content 42.86% in dosage group 10 g. In comparison with baseline, on Day 30, significant increase perceived in moisture content of skin by 14.28% ($p < 0.01$) with mean 42.22 ± 8.21%, 21.37% ($p < 0.0001$) with mean content 45.26 ± 5.12% and 14.38% ($p < 0.01$) with mean content 47.23 ± 4.83% in dosage groups 2.5, 5, and 10 g respectively. On Day 60, significant increase perceived in moisture content of

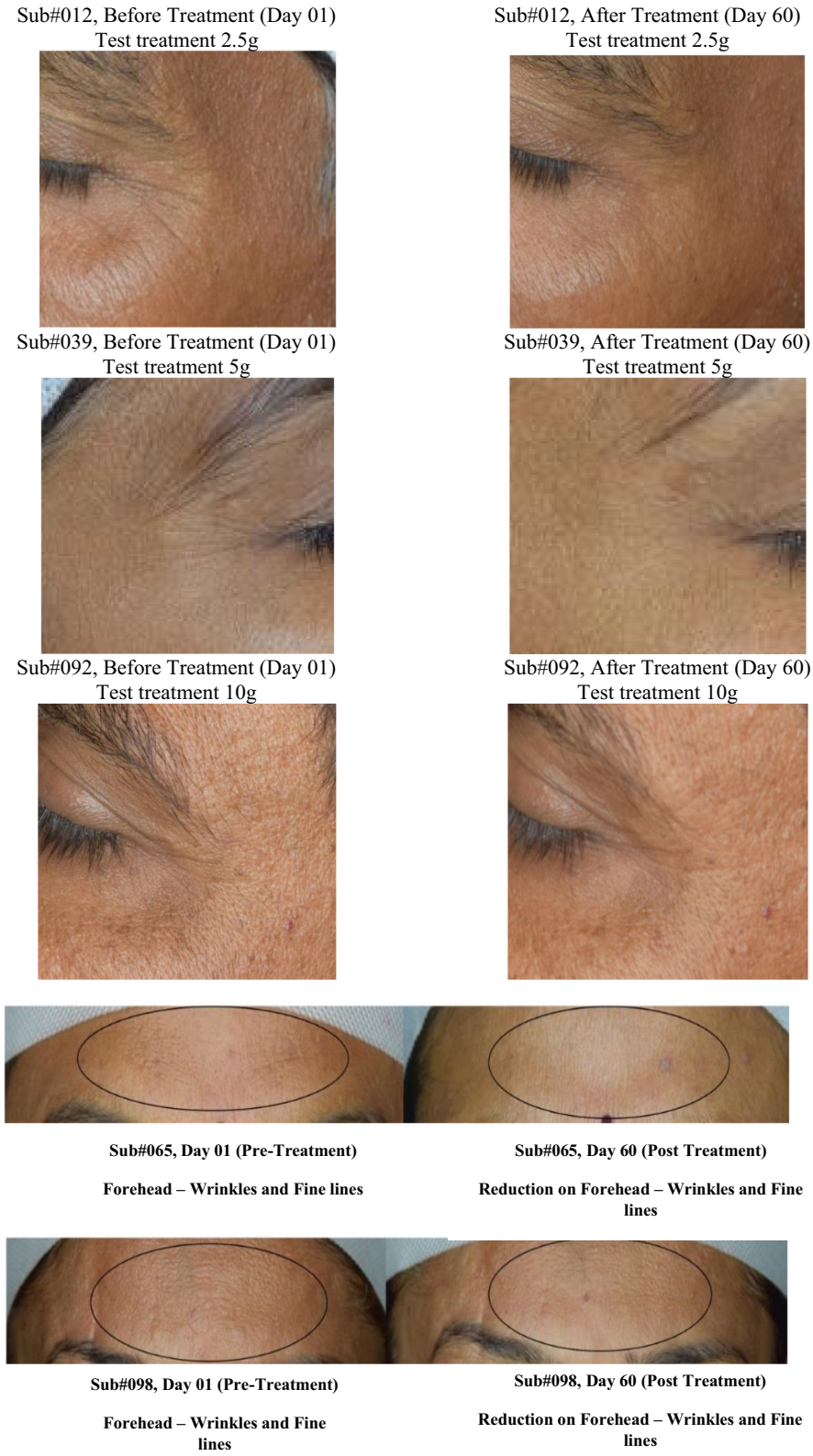


FIGURE 2 Photographs of improvement in crow's feet area wrinkle and fine lines.

skin by 25.84% ($p < 0.0001$) with mean 45.73 ± 8.16%, 27.81% ($p < 0.0001$) with mean 47.66 ± 6.38% and 22.40% ($p < 0.0001$) with mean 50.54 ± 5.52% in dosage group of 2.5, 5, and 10 g respectively. The increased skin hydration helps to maintain healthy skin as increased hydration helps to plump skin cells, sustain their structure, and regulates their functions (Table 2).

3.2.4 | Hair fall, hair thickness, hair density and hair growth

It was observed that after receiving treatment by test treatment, there was a significant reduction in total hair fall seen on Day 10 by 12.55% ($p < 0.01$) with mean count 31.19 ± 27.71 hairs, 17.28%

($p<0.01$) with count 25.33±27.54 hairs and 19.45% ($p<0.05$) with count 27.57±23.03 hairs in dosage group of 2.5, 5, and 10 g respectively, as compared with Day 1 (baseline reading). Significant reduction in hair fall was also seen on Day 30 by 23.01% ($p<0.001$) with mean 24.80±24.83 hairs, 27.07% ($p<0.001$) with mean 22.33±26.71 hairs and 34.34% ($p<0.01$) with count 20.38±16.42 hairs in dosage groups 2.5, 5, and 10 g respectively in comparison with Day 1. Significant reduction was also seen on Day 60 by 42.13% ($p<0.001$) with count 20.61±22.46 hairs, 40.27% ($p<0.001$) with count 18.28±24.06 hairs and 46.84% ($p<0.01$) with count 16.52±13.62 hairs in dosage groups of 2.5, 5, and 10 g respectively in comparison with Day 1.

In terms of hair breakage on Day 10, in comparison with baseline it was noted that test treatment at dosage of 2.5 and 10 g attenuated the hair breakage by 6.70% ($p=0.64$), and 3.35% ($p=0.63$) respectively, whereas same treatment at dosage of 5 g attenuates hair breakage significantly by 25.33% ($p<0.05$), with mean broken hair count 7.86±9.08 hairs. On Day 30, in comparison with baseline, it was observed that test treatment at dosage of 2.5 g attenuated hair breakage by 35.71% ($p=0.13$) and same treatment at dosage of 5 and 10 g significantly attenuated hair breakage by 33.45% ($p<0.01$) with count 6.43±9.05 hairs and 57.06% ($p<0.05$) with count 5.71±6.56 hairs respectively. On Day 60 it was noted that treatment by 5 g test treatment attenuated hair breakage significantly by 44.39% ($p<0.001$) with mean 6.81±8.59 hairs. Treatment at dosage of 2.5 and 10 g also attenuated the hair breakage by 26.30% ($p=0.06$) and 32.19% ($p=0.20$) respectively.

On Day 10, in comparison with baseline it was noted that dosage of 2.5 g attenuated the lost hair with bulbs significantly by 14.60% ($p<0.05$) with mean count 22.57±20.53 hairs. Further decrease was observed by 12.60% ($p=0.05$) and 22.48% ($p=0.08$) in dosage group 5 and 10 g respectively. On Day 30, in comparison with baseline, it was noted that dosage of 2.5 and 5 g attenuated hair count with bulbs significantly by 24.37% ($p<0.05$) with count 19.10±22.34 hairs and 20.50% ($p<0.01$) with count 15.90±2.60 hairs respectively. In dosage group 10 g there was a non-significant reduction by 25.40% ($p=0.07$). On Day 60 the count decreased significantly in dosage group 2.5, 5, and 10 g by 44.43% ($p<0.0001$) with mean count 14.67±17.24 hairs, 38.10% ($p<0.001$) with mean count 12.38±20.54 hairs and 51.31% ($p<0.01$) with mean count 10.81±11.57 hairs respectively in comparison with baseline.

As per the obtained results by hair pluck test, it was observed after the treatment, percentage of hairs in anagen phase were hiked significantly by 36.96% ($p<0.0001$), 50.02% ($p<0.0001$) and 51.55% ($p<0.0001$) in dosage group 2.5, 5, and 10 g respectively on Day 60 in comparison with Day 1. The percentage of hairs in telogen phase were decreased significantly by 50.02% ($p<0.0001$), 53.83% ($p<0.0001$) and 58.40% ($p<0.0001$) in dosage group 2.5, 5, and 10 g respectively. On Day 60 for dose 2.5 g, hair density showed improvement by 17.39% ($p<0.001$) with mean change from 255.71±51.41 to 300.19±50.37 hairs/cm^2. For dose 5 g, improvement was seen from 242.24±29.08 to 282.38±36.26 hairs/cm^2 ($p<0.0001$). Similarly, 10 g dose showed improvement from 253.91±42.67 to 303.76±50.63 hairs/cm^2 ($p<0.0001$). Hair growth rate improved significantly on Day 60 from 286.52±66.16 to 415.47±173.78 μm ($p<0.05$); from 280.38±120.77 to 388.43±129.29 μm ($p<0.05$) and from 305.95±95.48 to 460.05±166.41 μm ($p<0.05$) for dose group 2.5, 5, and 10 g respectively, compared to baseline reading. Hair thickness significantly improved on Day 60 from 15.91±2.07 to 19.48±2.27 μm ($p<0.0001$); from 15.71±2.51 to 19.85±2.95 μm ($p<0.0001$) and from 16.48±3.34 to 19.85±2.65 μm ($p=0.0001$) for dosage groups 2.5, 5, and 10 g respectively, compared to baseline. This shows the efficacy of treatment in all dosages in increasing the hair growth rate, hair density, hair thickness (Table 2). Photographs of A:T and hair growth rate has been shown in Figures 3 and 4 respectively.

3.2.5 | Joint pain and stiffness evaluation (VAS)

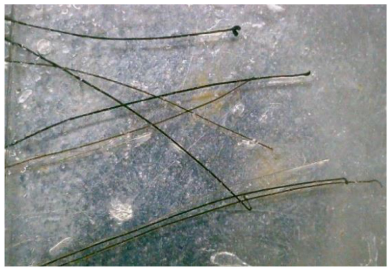
After test treatment consumption there was significant reduction noted in pain and stiffness by 10.04% ($p<0.01$) with mean score 4.95±0.69; by10.30% ($p<0.05$) with mean score 4.52±0.98 and 14.31% ($p<0.01$) with score 4.68±0.82 in dosage group 2.5, 5, and 10 g respectively, on Day 10 as compared with baseline. There was significant reduction observed by 21.90% ($p<0.001$) with mean score 4.30±0.86; by 22.57% ($p<0.01$) with score 3.90±1.00 and by 28.08% ($p<0.001$) with mean score 4.05±1.05 in dosage group 2.5, 5, and 10 g respectively, as compared to baseline on Day 30. On Day 60 there was significant reduction observed by 47.45% ($p<0.001$) with score 3.00±0.85, by 41.58% ($p<0.001$) with score 2.95±0.97 and by 52.54% ($p<0.001$) with mean score 2.71±0.94 in dosage groups 2.5, 5, and 10 g respectively as compared to baseline.

3.2.6 | Muscles strength–upper body, lower body, handgrip, elbow flexion and extension and knee flexion and extension strength

After treatment it was observed that the upper body muscle strength as measured on the basis of push-ups increased significantly on Day 10 in dosage groups 5 g and 10 g by 18.86% ($p<0.001$) with mean push-ups 5.67±3.18 and by 17.98% with mean 6.00±3.86 ($p<0.001$) respectively. In dosage group 2.5 g, there was also non-significant increase observed by 9.17% ($p=0.47$) and 23.12% ($p=0.12$) in comparison with baseline on the Days 10 and 30. Upper body muscle strength measured on the basis of push-ups increased significantly in dosage groups 5 and 10 g by 33.95% ($p<0.001$) with mean 6.76±3.91 and by 33.29% with mean 7.81±4.40 ($p<0.001$) respectively on Day 30. It also significantly increased at Day 60 in dosage group 2.5, 5, and 10 g by 65.14% ($p<0.01$) with mean 8.57±3.11, by 66.98% ($p<0.001$) with mean 8.43±4.08 and by 60.17% ($p<0.001$) with mean value 9.38±4.78 respectively as compared to baseline.

After treatment it was observed that the muscle strength measured on the basis of hand grip strength increased significantly on

Sub# 012 Baseline (Before Treatment) – Day 01
Anagen Hair: 05 (55.5%),
Telogen Hair: 04 (44.4%)
Test treatment 2.5g

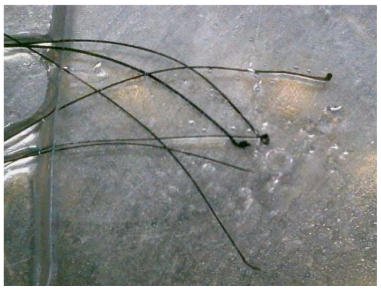


Sub#012 After Treatment – Day 60
Anagen Hair: 04 (57.1%)
Telogen Hair: 03 (42.8%)
Test treatment 2.5g

Sub#039 Baseline (Before Treatment) – Day 01
Anagen Hair: 05 (62.5%)
Telogen Hair: 03 (37.5%)
Test treatment 5g

Sub# 039 After Treatment – Day 60
Anagen Hair: 05 (71.4%)
Telogen Hair: 02 (28.6%)
Test treatment 5g

Sub#092 Baseline (Before Treatment) – Day 01
Anagen Hair: 02 (33.3%)
Telogen Hair: 04 (66.7)
Test treatment 10g



Sub#092 After Treatment – Day 60
Anagen hair: 03 (50%)
Telogen Hair: 03 (50%)
Test treatment 10g

FIGURE 3 Change in anagen to telogen ratio (A:T ratio).

Day 10 in dosage group 5 and 10 g by 11.37% ($p < 0.05$) with mean 17.23 ± 6.04 and by 4.36% ($p < 0.01$) with mean 16.23 ± 5.90, respectively. In dosage group 2.5 g there was also non-significant increase observed in muscle strength by 4.73% ($p < 0.46$) in comparison with baseline on the Day 10. On Day 30, it increased significantly in dosage group 2.5, 5, and 10 g by 18.54% ($p < 0.0001$) with mean 21.49 ± 7.20, by 21.28% ($p < 0.01$) with mean 18.77 ± 6.63 and by 18.98% ($p < 0.05$) with mean 19.25 ± 7.25 respectively, in comparison to baseline. On Day 60 significant increase was observed in dosage groups 2.5, 5, and 10 g by 27.27% ($p < 0.0001$) with mean 22.65 ± 7.49, by 47.82% ($p < 0.0001$) with mean 22.88 ± 6.83 and by 46.84% ($p < 0.0001$) with mean 23.76 ± 6.79 respectively, in comparison to baseline.

Muscle strength (measured on the basis of time of low plank hold) increased significantly in dosage group 2.5, 5, and 10 g by 32.67% ($p < 0.01$) with mean time 42.38 ± 34.83 s, by 25.89% ($p < 0.001$) with mean time 30.33 ± 30.14 s and 17.83% ($p < 0.01$) with mean time 32.84 ± 20.00 s respectively on Day 10, in comparison to baseline. On Day 30 muscle strength increased significantly in dosage group 2.5, 5, and 10 g by 25.49% ($p < 0.05$) with mean 40.05 ± 25.37 s, by 34.19% ($p < 0.001$) with mean 32.33 ± 29.68 s and by 23.77% ($p < 0.01$) with mean time 34.71 ± 17.55 s respectively, in comparison to baseline. On Day 60 it increased significantly in dosage groups 2.5, 5, and 10 g by 77.88% ($p < 0.0001$) with mean 56.48 ± 39.14, by 63.83% ($p < 0.0001$) with mean 39.48 ± 34.14 and by 42.27% ($p < 0.0001$) with mean 39.91 ± 21.35 respectively, in comparison to baseline. Maximum improvement in muscle movement was observed on the Day 60. Test treatment improved the muscle strength as assessed by improvement in hand grip, strength during knee flexion, knee extension, hip abduction, hip adduction, internal hip rotation, and external hip rotation movements.

During the baseline assessment, 47.62%, 19.05%, and 38.10% of the subjects had Glogau skin type II in treatment group with dosage 2.5, 5, and 10 g respectively. After usage of the test treatment for 60 days, it was observed that 95.24%, 80.95%, and 80.95% of the subjects had Glogau skin type II in dosage groups 2.5, 5, and 10 g respectively. This states that the test treatment was found effective in improving the Glogau skin type (Table 3).

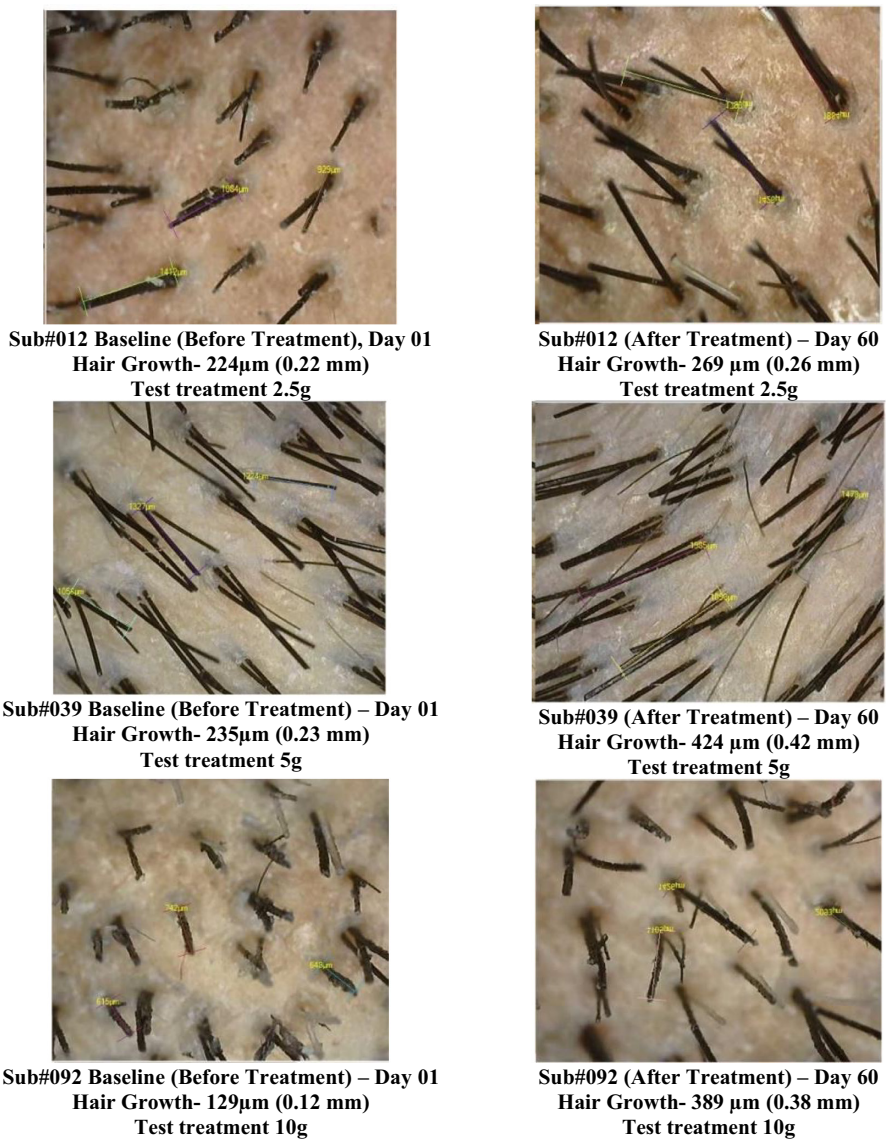


FIGURE 4 Phototrichogram images of improvement in hair growth rate.

The Physician Global Assessment (PGA) score as assessed by dermatologist for skin fine wrinkles, mottled hyperpigmentation, lentigines, tactile roughness, telangiectasia, pore size, elastosis and laxity showed reduction at Days 30 and 60 across all dosage groups. The PGA score of nail appearance as assessed by dermatologist for lamellar onychoschizia, ridging, longitudinal splitting, fragility/breakage, thickness of nail, surface roughness, raggedness, and peeling were reduced at Days 30 and 60 in all dosage groups. It can be said that all dosage of test treatment had effectively improved the overall appearance of the skin and nail. According to the hair pull test, test treatment across all doses was found to improve hair strength with 100% subjects rating as good.

3.2.7 | Investigator global assessment, sleep quality and subject perception questionnaire

100% of the subjects had clear skin as evaluated by dermatologist's trained evaluator using total Investigator Global Assessment scoring for acne at Day 60 from baseline across all the dosage groups. Change in sleep quality was assessed for deep and sound sleep using Leeds Sleep Evaluation Questionnaire. In comparison with baseline, it was observed that there was an improvement in overall sleep quality and the patients woke up with a fresh feeling. The response and improvement on sleep quality increased with the increasing dose at Days 30 and 60, with an overall increase in mean score of response to questions asked (Figure 5). The subjective perception questionnaire was prepared and process of interrogation was carried out in terms of reducing wrinkles, fine lines, improvement in skin elasticity, suppleness and smoothness, improvement in skin glow and radiance, improvement in hair growth and thickness and nail health, improvement in skin moisturization, improvement in food digestion capacity and gut health, improvement in quality and texture of skin, effect in reducing hair fall, improvement in hair growth and thickness, improvement in overall nails health, effect in terms of reducing joint pain and stiffness, improvement in terms of food digestion, bowel movement and gut health, improvement in overall sleep, likeness of test treatment and overall satisfaction with test treatment. As per

TABLE 3 Improvement in Glogau skin age after consumption of test treatment.

Parameter	Visit	Test treatment (2.5 g)	Test treatment (5 g)	Test treatment (10 g)
Glogau skin age type	Visit 1	*N* = 21	*N* = 21	*N* = 21
	Type II	10 (47.62%)	4 (19.05%)	8 (38.10%)
	Type III	11 (52.38%)	17 (80.95%)	13 (61.90%)
	Visit 3	*N* = 21	*N* = 21	*N* = 19
	Type II	10 (47.62%)	7 (33.33%)	7 (36.84%)
	Type III	11 (52.38%)	14 (66.67%)	12 (63.16%)
	Visit 4	*N* = 20	*N* = 21	*N* = 21
	Type II	10 (50.00%)	15 (71.43%)	14 (66.67%)
	Type III	10 (50.00%)	6 (28.57%)	7 (33.33%)
	Visit 6	*N* = 21	*N* = 21	*N* = 21
	Type I	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Type II	20 (95.24%)	17 (80.95%)	17 (80.95%)
	Type III	1 (4.76%)	4 (19.05%)	4 (19.05%)

Abbreviation: *N*, number of non-missing observation.

the responses, was found that 100% of subjects had improvement across all the parameters compared to baseline and had a satisfaction by the use of test treatment (Table 4).

3.2.8 | Age related enhancement in muscle strength, joint, skin, hair and nail health

A sub-group analysis on age-group revelated that improvement in joint pain and muscle strength was seen at a higher dose of 10 g. The improvement was much higher in age group ≥40 years when compared age group <40 years on Day 60 of treatment compared to baseline (Figure 6). On contrary, 2.5 g dose was sufficient to show significant improvements at Day 60 in age-related hair density, hair thickness, crow's feet area wrinkles, skin roughness and scaliness, lamellar onychoschizia, and nail surface roughness. It is noteworthy that skin, hair and nail parameters showed greater improvements in age group <40 years compared to age group ≥40 years (Figure 7).

3.3 | Safety assessments

The dermatologist and study physician assessed the safety of the test treatment by monitoring treatment-emergent adverse symptoms, including severe irritability, excessive bloating, nausea, diarrhea, and skin redness, tingling, dryness, and burning. However, no adverse events were observed during the study period, indicating the safety of the test treatment.

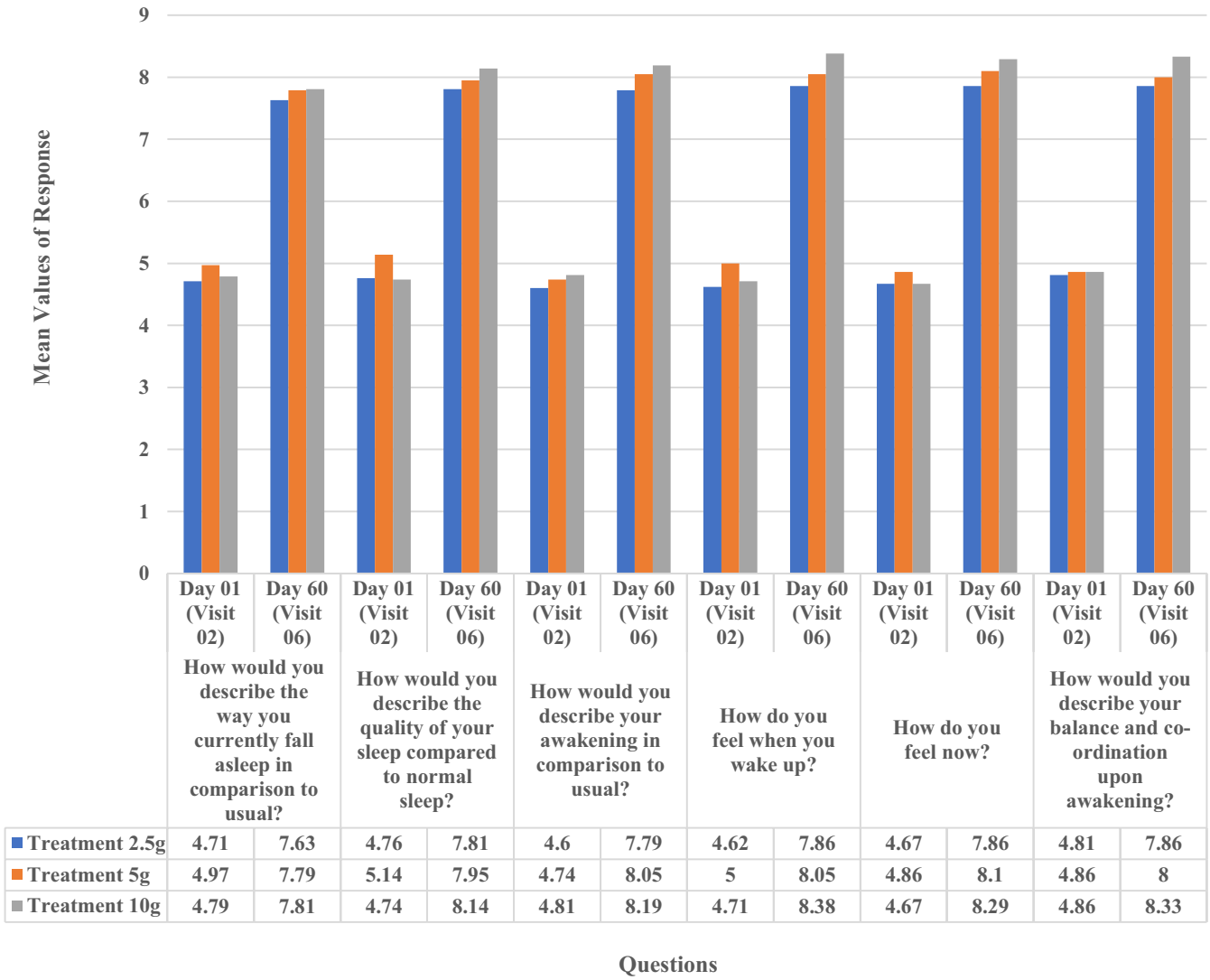
4 | DISCUSSION

The study significantly achieved its pre-determined objectives with improvement across various hair and skin parameters. The test treatment across all the doses, showed improvement in skin smoothness, hydration, viscoelasticity, retraction time, hair growth rate, strength, thickness, density and reduced skin dryness, crow's feet area wrinkles, skin scaliness, roughness and hair fall. However, dosage difference did not make significant change in skin health and showed significant improvement with small to medium dose (2.5–5 g). However, higher dose had positive impact on joints pain and muscle strength. The test treatment also improved nail health, gut health and overall sleep quality.

Currently, there are very limited studies done and literature available on the use of vegan supplements to build collagen and its overall impact on skin, hair and nail health.

The test treatment contains papain and vegetables like broccoli and carrot which contain essential and non-essential amino acids, vitamin C and A in abundance, which are required for collagen synthesis. Papain, which is a proteolytic enzyme and a phytochemical obtained from latex of unripe papaya (*Carica papaya*) has various applications across pharmaceutical, food, cosmetic, and textile industries.[18] Papain possesses excellent antibacterial, antiviral, antifungal properties and exfoliant properties. It also provides smoothing effect to the skin, increase skin hydration, rejuvenates skin and decreases skin spots, thereby, providing a soothing effect to the skin and making the skin look healthier.[7,8] The similar effects have been observed in the present study with improved skin smoothness, hydration and suppleness after administration of test treatment.

Carrots contain beta-carotene which is one of the most important carotenoids in human body and a precursor of vitamin A which exerts its anti-aging properties by penetrating the dermis layer of skin and increasing collagen by stimulation of the fibroblasts.[19] Retinoids have been shown to improve the clinical features of aged appearance by a reduction in fine wrinkling, increased smoothness and decreased hyperpigmentation.[20] The results of the present study stating effect of collagen on skin are in line with a randomized, placebo-controlled, blinded study which included 72 healthy



	Day 01 (Visit 02)	Day 60 (Visit 06)	Day 01 (Visit 02)	Day 60 (Visit 06)	Day 01 (Visit 02)	Day 60 (Visit 06)	Day 01 (Visit 02)	Day 60 (Visit 06)	Day 01 (Visit 02)	Day 60 (Visit 06)	Day 01 (Visit 02)	Day 60 (Visit 06)
	How would you describe the way you currently fall asleep in comparison to usual?		How would you describe the quality of your sleep compared to normal sleep?		How would you describe your awakening in comparison to usual?		How do you feel when you wake up?		How do you feel now?		How would you describe your balance and co-ordination upon awakening?	
Treatment 2.5g	4.71	7.63	4.76	7.81	4.6	7.79	4.62	7.86	4.67	7.86	4.81	7.86
Treatment 5g	4.97	7.79	5.14	7.95	4.74	8.05	5	8.05	4.86	8.1	4.86	8
Treatment 10g	4.79	7.81	4.74	8.14	4.81	8.19	4.71	8.38	4.67	8.29	4.86	8.33

FIGURE 5 Improvement in leads sleep evaluation questionnaire after consumption of test treatment.

subjects and was performed to study the effect nutraceutical collagen preparation. In the study half of the subjects ($n=36$) received this collagen supplement which contained 2.5 g of collagen peptide, biotin, vitamin C and E, acerola extract and zinc while the other half received placebo. After 12 weeks, the test product collagen supplement resulted in improved skin hydration, elasticity, roughness and density when compared to the control group who received placebo.[6] Proline and its precursors like glutamate and pyrroline-5-carboxylate, increase synthesis of collagen in fibroblast cells. Various in vitro studies involving fibroblast cells show the dependence of collagen hydroxylase enzymes on vitamin C has been substantiated through numerous. In the absence of vitamin C, these investigations consistently reveal a dual impact: A reduction in total synthesis and a decrease in crosslinking, underscoring the pivotal role of vitamin C in supporting the enzymatic processes crucial for collagen formation[21] Broccoli is rich in vitamin C which has a proven effect in reducing hair loss and enhancing hair growth rate. A study by Zdzieblik et al. concluded that amino acids rich collagen peptides supplementation can increase overall muscle mass and improve muscle strength.[22]

The outcomes of the present study are in line with the discussed studies and clinical effects of various ingredients of test treatment. The test treatment showed high significant improvements across various tested parameters on Day 60, when compared to baseline readings.

In certain study outcomes, compared to 5 g dosage group, the 10 g dosage group did not exhibit greater effectiveness and showed slightly lower or almost equal percentage improvement. This outcome can be attributed to the phenomenon of dose–response saturation. At certain instances, at higher doses, there comes a point where no further therapeutic response is observed. This occurs because the targeted receptors reach maximum occupancy, thereby limiting additional therapeutic benefits due to the saturation of the dose–response relationship of the test product.[23] Similar responses have been observed in a randomized, placebo-controlled trial conducted by Asserin et al.[24] on collagen supplementation at three varied doses. They observed that upon collagen supplementation at doses of 0.01 and 0.1 mg/mL, the levels of glycosaminoglycan increased significantly by 5 to 18 folds, respectively. However, upon further increasing the dose to 1 mg/mL,

TABLE 4 Subjective perception questionnaire assessment.

Subjective perception questionnaire (percentage of participants in consensus)						
Question for subject perception post usage of test treatment	Test treatment (2.5 g)		Test treatment (5 g)		Test treatment (10 g)	
	Day 30 (*N* = 20)	Day 60 (*N* = 21)	Day 30 (*N* = 21)	Day 60 (*N* = 21)	Day 30 (*N* = 21)	Day 60 (*N* = 21)
Improvement terms of reducing fine lines and wrinkles	95.00%	100.00%	95.24%	100.00%	76.19%	100.00%
Improvement in terms of smoothness and suppleness of skin	90.00%	100.00%	95.24%	100.00%	80.95%	100.00%
Effective in reducing crow's feet area fine lines	95.00%	100.00%	90.48%	100.00%	90.48%	100.00%
Effective in terms of radiance/glow of skin	90.00%	100.00%	95.24%	100.00%	90.48%	100.00%
Improvement in skin moisturization	95.00%	100.00%	95.24%	100.00%	90.48%	100.00%
Improvement in food digestion capacity and gut health	90.00%	100.00%	71.43%	100.00%	76.19%	100.00%
Improvement in quality and texture of skin	95.00%	100.00%	80.95%	100.00%	90.48%	100.00%
Effective in reducing hair fall	95.00%	100.00%	90.48%	100.00%	85.71%	100.00%
Improvement in hair growth and thickness	100.00%	100.00%	76.19%	100.00%	71.43%	100.00%
Improvement in overall nails health	95.00%	100.00%	95.24%	100.00%	85.71%	100.00%
Effective in terms of reducing joint pain and stiffness	95.00%	100.00%	80.95%	100.00%	80.95%	100.00%
Improvement in terms of food digestion, bowel movement and gut health	95.00%	100.00%	95.24%	100.00%	85.71%	100.00%
Improvement in overall sleep	90.00%	100.00%	85.71%	100.00%	80.95%	100.00%
Likeness of test treatment	95.00%	100.00%	80.95%	100.00%	85.71%	100.00%
Overall satisfaction with test treatment	95.00%	100.00%	90.48%	100.00%	90.48%	100.00%

saturation of effect was reached, with only a 17-fold significant increase from baseline. In the same study, 0.01 mg/mL supplementation increased collagen content of papillary dermis by 3% and dose 0.1 mg/mL increased it by 9%. Further increase in dose to 1 mg/mL showed only 5.4% increase from baseline due to reach to the saturation of the effect. Another literature on collagen supplements also showed similar saturation effect at higher doses across hair and skin outcomes similar to the present study.[25] For hair density parameter, 5 g dose underperformed compared to 2.5 g dosage group. This can be attributed to the reduced baseline mean hair density in 5 g dosage population (242.24 ± 29.08) compared to baseline means of 2.5 g (255.71 ± 51.41) and 10 g (253.91 ± 42.67) dosage groups. Due to this variation in baseline mean, the resultant percentage change from baseline is slight lower in five dosage groups. This outcome suggests that the response of the 5 g dose may improve with longer duration of use. Similarly, for Crow's feet area wrinkles, the 2.5 g dose was slightly more effective than the 5 g dose. However, this effect persisted for only 30 days of usage. After the completion of 60 days of product usage, percentage improvement for this parameter followed a linear trend with increased doses. Similar effects were also observed in another collagen supplementation study.[25] Study with longer study duration may provide accurate outcomes. This is one of the limitations of the present study. Additionally, although patient adherence was checked at every visit by weighing of powder (test products container) more reliable methods like blood concentration monitoring of CTP can provide more accurate data on treatment adherence and subsequent outcomes.

Other limitations include, relatively small study sample size with 21 subjects per dose, which may not be sufficient to study in-depth safety and efficacy of the treatment. Additionally, missing biomarkers and lab investigations may have impacted the accuracy of study results. The study also lacked comparison with placebo or animal derived traditional collagen to assess the true

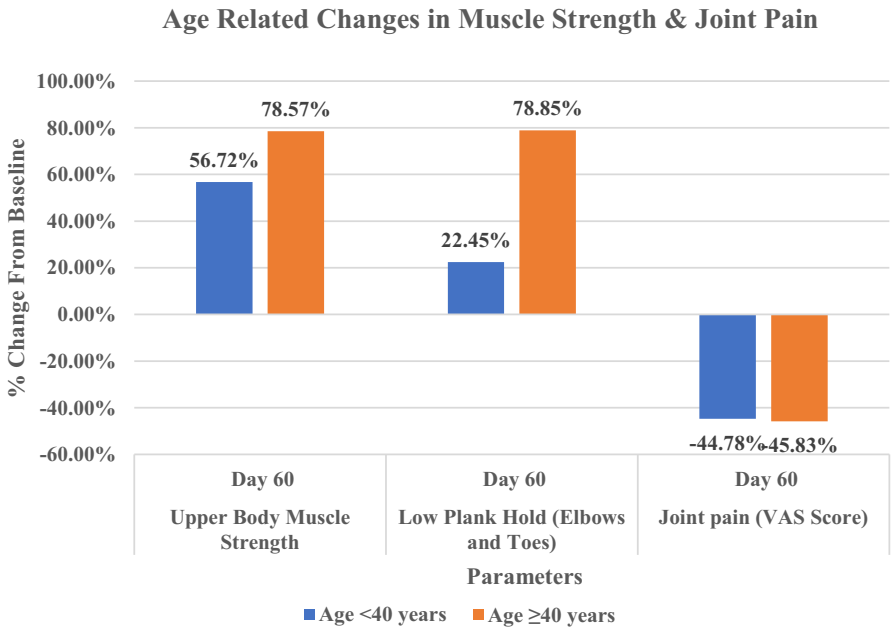


FIGURE 6 Age related improvements in muscle strength and joint pain.

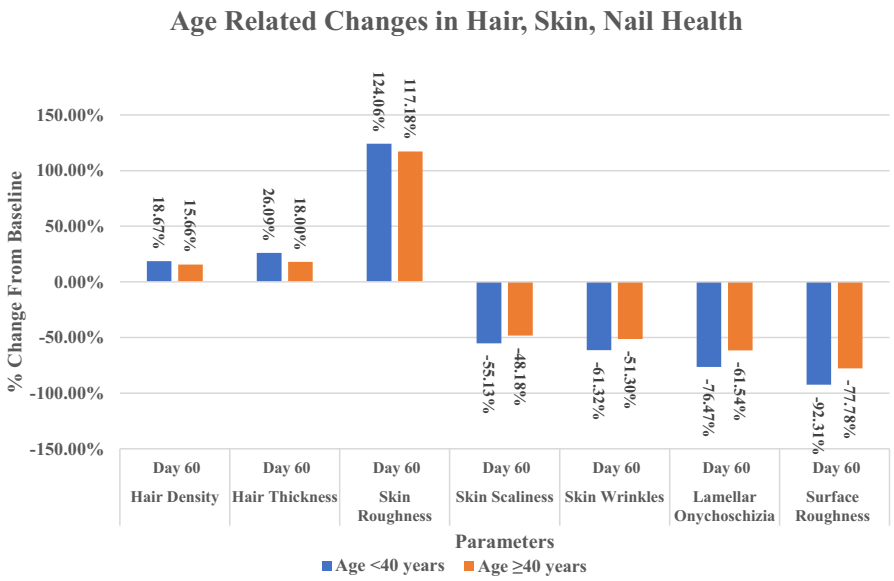


FIGURE 7 Age related improvements in hair, skin, nail health.

efficacy and equivalence of the treatment. Greater variations in results across different doses may be attributed to the study's design and shorter treatment duration which may have potentially influenced the observed effects, as short-term studies may capture immediate responses that differ from longer-term outcomes. Clinical study with larger sample size, longer duration, comparator arm with diverse population and ethnicity may provide more precise safety, efficacy and dose-gradient effects of treatment in such population.

5 | CONCLUSION

As the popularity of veganism continues to rise worldwide, test treatment, made from a blend of nutritional ingredients including broccoli, carrot, and papain, effectively addresses the needs of individuals seeking natural collagen alternatives. This clinical study demonstrated that skin and hair related issues could be addressed using a vegan collagen-builder across doses 2.5, 5, and 10 g. The treatment was found to be effective in reducing the appearance of wrinkles, fine lines, roughness and scaliness of the skin, improving skin elasticity and hydration and provide smooth and supple skin. Treatment demonstrated its worth in hair care by promoting hair growth, hair density, thickness and its general appearance. The study indicated that a dose of 2.5 g was sufficient for improving skin, nail and hair health, while a dose of 10 g showed improvement in joint pain and muscle strength. The treatment satisfied the patients' needs along with improvements in sleep quality, food digestion, bowel movement, gut health, nail health, and a reduction in joint pain and stiffness. VEGCOL™, being a natural collagen

boosting source without any proven adverse events, provides a rich amino acid profile, making it an ideal choice for enhancing hair, skin, nail, gut and joint health.

AUTHOR CONTRIBUTIONS

Conceptualization: Nayan Patel, Samrat Warma, Himanshi Warma and Maheshvari Patel. *Data curation*: Samrat Warma and Himanshi Warma. *Funding acquisition*: Samrat Warma, Himanshi Warma Nayan Patel and Maheshvari Patel. *Investigation*: Apeksha Merja, Nayan Patel and Maheshvari Patel. *Methodology*: Apeksha Merja, Nayan Patel and Maheshvari Patel. *Project administration*: Nayan Patel, Samrat Warma, Himanshi Warma and Maheshvari Patel, Nayan Patel. *Resources*: Nayan Patel and Maheshvari Patel. *Software*: Nayan Patel and Maheshvari Patel. *Supervision*: Samrat Warma, Himanshi Warma and Apeksha Merja, Nayan Patel and Maheshvari Patel. *Validation*: Apeksha Merja and Maheshvari Patel. *Visualization*: Nayan Patel and Maheshvari Patel. *Writing—original draft*: Nayan Patel. *Writing—review and editing*: Apeksha Merja and Maheshvari Patel, Samrat Warma and Himanshi Warma.

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CONFLICT OF INTEREST STATEMENT

Mr. Samrat Warma, Ms. Himanshi Warma are employees of Collagen Lifesciences Private Limited, India. Dr Nayan Patel, Dr. Apeksha Merja and Ms. Maheshvari Patel declare no conflict of interest. All the authors declare that any association have not influenced the work reported in this paper.

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.

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