

Original Research Article

A pilot, exploratory study to demonstrate the efficacy of a novel adjuvant herbal liquid supplement for skin rejuvenation and anti-aging

C. S. Janaki^{1*}, Mukta Sachdev², Ritambhara Kr²

¹Department of Anatomy, Bhaarith Medical College, Chennai, Tamil Nadu, India

²MS Clinical Research Pvt Ltd, Bangalore, Karnataka, India

Received: 19 July 2021

Revised: 23 August 2021

Accepted: 27 August 2021

*Correspondence:

Dr. C S Janaki,

E-mail: csjacedemics@gmail.com

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ABSTRACT

Background: Aging of skin has both intrinsic and extrinsic causative factors. Impact of oral consumption of a novel multi-component herbal skin water supplement (HSWS) by Diabliss was investigated in a pilot exploratory study.

Methods: A 90-day open label clinical study among 40 healthy male and female subjects aged 35-50 years, presenting with signs of aging including wrinkles and pigmentation related skin concerns. The study assessments included dermatological, instrumental and imaging evaluation to investigate the impact of the Diabliss HSWS in providing improvements in various signs of aging.

Results: The Diabliss HSWS was able to show significant improvement in skin elasticity parameters by Cutometer® with improvements that ranged between 23-54% for the six elasticity parameters, skin hydration improvements as measured by Corneometer® in forehead (30%) and cheek (34%), wrinkles reduction reductions by Antera® in forehead (25%) and crows feet area (20%), texture improvements in forehead (25%) and crows feet area (20%), reduction L* value on the localized pigmentation/ spot of 6%. The subject assessments also reported improvement in the skin and general health. There were no product related AE/SAE and the product was found to be safe and well tolerated.

Conclusions: In conclusion, this water based oral nutraceutical is a novel delivery system for a skin rejuvenation adjuvant therapy which is cosmetically and systemically acceptable and tolerable for patients.

Keywords: Diabliss herbal skin water supplement, Anti-aging, Wrinkles, Hydration, Pigmentation

INTRODUCTION

Every day the cells of the outer layer of skin die, shed, and regenerate. In young individuals, skin cells turn over quickly, but that turnover rate begins to slow down with age. As a result, the skin loses its lustre and begins to look dull. Aging of skin has both intrinsic and extrinsic causative factors. Ultraviolet radiation in particular results in premature skin ageing. This is also referred to as photoaging. It is one of the most prevalent causative factors associated with the ageing process.^{1,2}

While the causative reasons for ageing may be different, both intrinsic and extrinsic ageing have mechanistic similarities in terms of underlying processes that accelerate skin aging. This mainly involves formation of reactive oxygen species (ROS) which is the root cause of both intrinsic and extrinsic ageing processes. Rate of ageing tends to be more concentrated in the dermis and epidermis areas for UV-induced extrinsic ageing process.³ Loss of Collagen, a fibrous protein which provides elasticity to the skin in photoaged skin cells is a characteristic finding in aged skin.⁴

A recent focus area in skin care pertains to botanical treatments to reduce skin aging and wrinkling. Among botanicals, cosmeceuticals with claims of improvements in elasticity and texture are showing significant promise.¹² Extracts of soy, tea (green and black teas), chamomile, caffeine, coffeeberry have been studied due to diverse compounds including isoflavones, saponins, essential amino acids, phytosterols, polyphenols, flavonoids, although evidence-based science is still emerging.^{12,13} Oral nutraceuticals are newer adjunctive anti-aging products gaining increasing popularity globally.

The HSWS is a proprietary oral nutraceutical combination of various herbal extracts, where the potency of many of its ingredients have been documented in literature. The pilot clinical study to investigate the effectiveness of the formula in its present combination and dosage form in comparison to the baseline. The product has improved organoleptic properties, making it easy to consume daily and provide required micronutrients for skin benefits.

Aim and objective

This pilot exploratory study was conducted to investigate the impact of oral consumption of a novel multi-component herbal skin water supplement (HSWS) by Diabliss.

METHODS

The study was designed as a pilot, exploratory CRO based investigator driven single group clinical trial involving daily oral intake of a multi-component water based herbal cosmeceutical. Prior clearance from an independent ethics committee followed by written informed consent was taken from each study participants before initiating study. The study was conducted in MS Clinical Research, Bangalore between February 2020 to August 2020. A total of 40 male and female subjects (11:29 ratio) who satisfied the inclusion and exclusion criteria were enrolled in the study with 39 subjects completing the study. Based on the skin assessment criteria and overall treatment duration a sample size of more than 30 subjects was sufficient to achieve statistical significance with 80% power and 5% level of significance. The sample size was calculated considering the primary endpoint data generated in previously conducted 3-month anti-aging study. The trial was registered on the clinical trial registry of India (CTRI) (CTRI number).

Baseline assessment on day 1 involved dermatological assessment, self-assessment, non-invasive instrument assessment and imaging. The selected subjects were provided with the HSW cosmeceutical supplied by Diabliss. Subjects were advised not to change their lifestyle and follow the same routine activities (diet, work, exercise, sleep etc.) during the study. Their general

health parameters such as appetite, bowel movements, digestion, fatigue, stress level were monitored during the study. Subjects were given face wash and lotion to limit the differences in skin care products.

Subjects visited study sites every 30 days for follow up. On every follow-up visit the product compliance was checked and the subjects were asked to report any discomfort or adverse events. Subjects were assessed for overall change in health and fitness levels.

After ensuring compliance and routine health monitoring, subjects underwent dermatological assessment, self-assessment, skin measurements with non-invasive instruments and imaging to evaluate the efficacy of the treatment product.

Assessment details

Clinical evaluations were carried with various established instruments along with clinical dermatological assessments for skin parameters associated with ageing as summarized below:

Dermatological visual clinical grading: Evaluation of skin attributes in relation to the signs of aging including facial fine lines, wrinkles, skin texture, firmness, elasticity, crow's feet, skin evenness and skin using internal standards. Cutometer® for skin elasticity measurements. Corneometer® for skin hydration measurements. Antera® for measurements of wrinkles, skin texture and changes in skin pigmentation. Product safety by dermatological skin assessment for intolerances or AE/SAE. Images capture with VISIA® CR.

Test product details

The HSWS was prepared by Diabliss Consumer Products from a combination of water-based extracts of the following ingredients with known skin care benefits:

Carrot Seed (*Daucus Carota*), Purple Amaranth (Known as *Amaranthus Tricolor L.*), False Daisy (*Eclipta Prostrata*), Indian Gooseberry (*Phyllanthus emblica*), Turmeric (*Curcuma Longa*).¹⁵⁻²¹ Long Pepper (*Piper Longum*), Guava Leaves (*Psidium Guajava*), Basil (*Ocimum Basilicum*), Chia Seed (*Salvia Hispanica*), Walnut (*Juglans Regia*), Flaxseed (*Linum Usitatissimum*), Almond (*Prunus Dulcis*), Black Pepper (*Piper Nigrum*), Sunflower (*Helianthus Annuus*), Curry Leaves (*Murraya Koenigii*), Licorice (*Glycyrrhiza Glabra*), Cinnamon (*Cinnamomum Zeylanicum Blume*), Cumin (*Cuminum Cyminum*), Beetroot (*Beta Vulgaris*), Pomegranate (*Punica Granatum*).²²⁻⁴³

Daily intake

15 ml of Diabliss HSWS was mixed in 500 ml of drinking water. 150 ml each was consumed at breakfast, lunch and dinner and balance 50 ml at bed time.

Statistical methodology

Statistical software R version 3.1.2 was used to analyse the data. Shapiro-Wilk Test was performed to check the normality of data. Paired t-test/Wilcoxon signed rank test for paired samples was performed for mean value comparison. P<0.05 was considered to be significant.

Study demographics

40 subjects enrolled in the study and 39 completed the study. The age distribution was in the ratio of 1:1 for 35-40 years and 40-50 years. Subjects of Fitzpatrick skin type III, IV and V of Indian ethnicity participated in the study as per the distribution mentioned in the table 2 below. The table also enlists the distribution of subjects by their skin type.

RESULTS

Under dermatological clinical grading, a significant improvement in overall changes in skin appearance such as skin texture, skin firmness, evenness of skin tone, skin

clarity and spot intensity were observed as early as one month (Day 30) and improved progressively till the end of the study in comparison to the baseline. The improvement in wrinkles (Crows Feet) was noted from Month 2 but significance was attained at month 3 (Day 90) in comparison to baseline (Table 1).

As per Antera® evaluations, the study population showed significant improvement in texture, facial fine lines (Crow’s feet); wrinkles and hyperpigmentation were observed as early as one month (Day 30) and improved progressively till the end of the study in comparison to baseline.

Significant improvement in skin hydration was observed as early as one month (Day 30) and was noted to be progressive till the end of the study in comparison to baseline when assessed by Corneometer®.

Significant improvement in skin elasticity (in terms of R2, R3, R5, R6 and R7 values) was observed as early as one month (Day 30) and improved progressively till the end of the study in comparison to baseline as assessed by Cutometer®.

Table 1: Dermatological clinical evaluation.

Skin Parameter	Scale		Day-1 (baseline)	Day-30	Day-60	Day-90
Skin Texture	Min=0, Max=9, Lower value is a sign of improvement	Mean	5.59	4.82	4.59	4.00
		Std Dev	1.07	1.11	1.06	0.82
		CFB Mean	-	-0.77**	-1**	-1.59**
Skin firmness / elasticity	Min=0, Max=9, Lower value is a sign of improvement	Mean	5.44	5.04	4.69	4.26
		Std Dev	0.88	0.91	0.95	0.85
		CFB Mean	-	-0.4**	-0.74**	-1.18**
Crow’s feet grading scale in unanimated face using photo-numeric scale	Min=0, Max=9, Lower value is a sign of improvement	Mean	3.46	3.46	3.46	3.29
		Std Dev	1.02	1.02	1.02	0.92
		CFB Mean	-	0	0	-0.17**
Spot intensity (spot colour)	Min=0, Max=9, Lower value is a sign of improvement	Mean	6.26	5.5	5.33	4.22
		Std Dev	0.79	1.01	0.92	1.54
		CFB Mean	-	-0.76**	-0.92**	-2.04**
Skin clarity-visibility of spots	Min=0, Max=9, Lower value is a sign of improvement	Mean	5.69	5.31	5.14	4.47
		Std Dev	1.34	1.22	1.19	1.11
		CFB Mean	-	-0.38**	-0.55**	-1.22**
Evenness of skin tone across the face	Min=0, Max=9, Lower value is a sign of improvement	Mean	5.67	5.21	5.08	4.42
		Std Dev	1.15	1.09	0.99	1.07
		CFB Mean	-	-0.46**	-0.59**	-1.24**

1. CFB= Change from Baseline, **= significant value.
2. Improvement Scale: Min=0, Max=9, Lower value is a sign of improvement.

Table 2: Subjects distribution by Fitzpatrick and skin type.

Fitzpatrick Skin Type	Count of subjects	Skin Type	Count of subjects
III	13	Oily	8
IV	22	Combination	3
V	5	Normal	23
		Dry	6

Table 3: Cutometer®, Corneometer® and Antera® measurements summarizing effectiveness (% of study subjects showing improvement) and efficacy (average % improvement).

Instrument	Measurement	Measurement Details	% of subjects showing improvement (Day 90 versus Day 1)	Average improvement, % (Day 90 versus Day 1)
Cutometer®	R0	Passive behaviour of skin	90%	44%
	R2	Gross Elasticity	100%	43%
	R3	Tiring Effects	90%	39%
	R5	Net Elasticity	83%	23%
	R6	Viscoelasticity	90%	-49%
	R7	Portion of the elasticity compared to the complete curve	100%	54%
Corneometer®	Dielectric Strength	Skin Hydration - Forehead	100%	30%
	Dielectric Strength	Skin Hydration - Cheek Area	100%	34%
Antera®	Wrinkles	Forehead Area	100%	-25%
	Wrinkles	Crows Feet Area	100%	-20%
	Texture	Forehead Texture	98%	-25%
	Texture	Crows Feet Area	100%	-23%
	Pigmentation	L* value on the localized pigmentation area/ spot	100%	6%

Table 4: Subjective feedback.

	Time point	Day-1 N (%)	Day-30 N (%)	Day-60 N (%)	Day-90 N (%)
Q.1 Does your skin look firmer (tight)?	Disagree	39 (100)**	31 (79.49)**	4 (10.26)	0 (0)
	Agree	0 (0)	8 (20.51)	35 (89.74)**	39 (100)**
Q2. Does your skin look younger?	Disagree	39 (100)**	30 (76.92)**	2 (5.13)	0 (0)
	Agree	0 (0)	9 (23.08)	37 (94.87)**	39 (100)**
Q3. Does your skin look free of fine lines/wrinkles on your face (do not see small lines or folds observed near eye area, forehead etc.?)	Disagree	39 (100)**	25 (64.1)	0 (0)	0 (0)
	Agree	0 (0)	14 (35.9)	39 (100)**	39 (100)**
Q4. Do you see reduction on age spots (pigments, dark spots) on your face?	Disagree	39 (100)**	25 (64.1)	8 (20.51)	0 (0)
	Agree	0 (0)	14 (35.9)	31 (79.49)**	39 (100)**
Q5. Is your skin moisturized?	Disagree	39 (100)**	15 (38.46)	0 (0)	0 (0)
	Agree	0 (0)	24 (61.54)	39 (100)**	39 (100)**
Q6. Does your skin look bright?	Disagree	39 (100)**	15 (38.46)	0 (0)	0 (0)
	Agree	0 (0)	24 (61.54)	39 (100)**	39 (100)**
Q7. Does your skin look even tone?	Disagree	39 (100)**	26 (66.67)*	15 (38.46)	0 (0)
	Agree	0 (0)	13 (33.33)	24 (61.54)	39 (100)**

** , significant but unfavourable skin condition, *= suggestively significant, **, significant and favourable skin condition.

Table 3 summarizes the percent of the subjects showing improvements in various instrument-based assessments – Cutometer®, Corneometer® and Antera® and shows very high percentage of subjects showing improvements in the various skin parameters, with 100% of the subjects showing improvements in many parameters. Figures 1 to 3 are representative values of the individual subject readings of elasticity measurements by Cutometer®, skin hydration measurements by Corneometer® and Skin wrinkles measurements using Antera®.

Subject self-assessment

Self-Assessment questionnaire on Day 1, Day 30, Day 60 and Day 90 showed improvements in all the seven areas of skin improvement as summarized in Table 3.

Photographic documentation

Photographs were taken for all clinical trial participants to record visual differences in the skin attributes with

product use. Figures 4 and 5 are representative image sets of two study participants showing improvement in skin parameters like fine lines, texture, spots and clarity, tanning and photodamage, respectively.

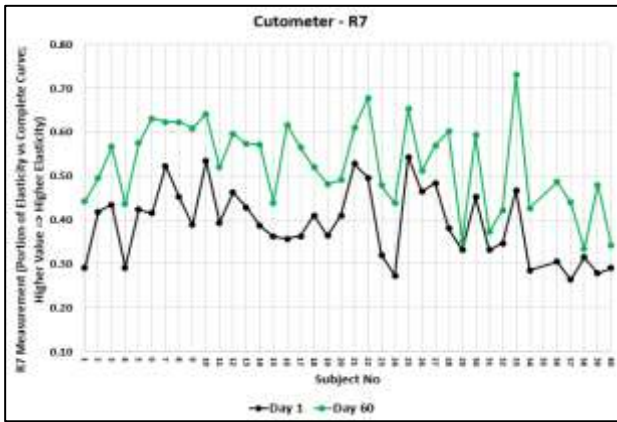


Figure 1: Cutometer R7 measurement which measures portion of elasticity versus complete curve.

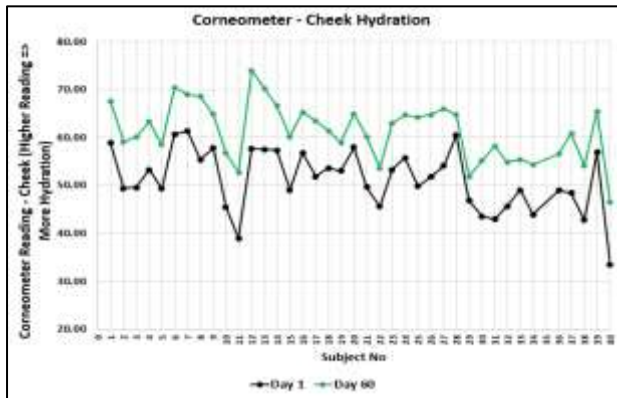


Figure 2: Skin hydration measurements by Corneometer®.

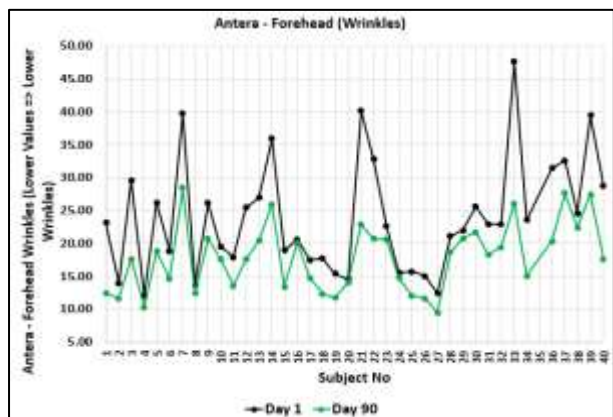


Figure 3: Forehead wrinkles measurements by Antera®.

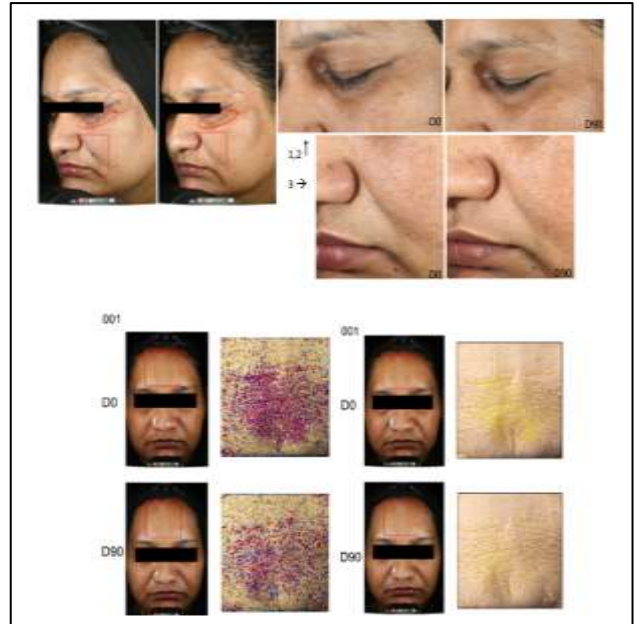


Figure 4: Subject 001, a 47-year-old female showed improvement in fine lines, texture and reduction in spots with 3 months of regular intake of Diabliss HSWS.



Figure 5: Subject 21, a 36-year-old male showed improved skin quality, colour and clarity. The skin showed visible improvement in aging associated skin damages including sun spots, tanning and photodamage.

DISCUSSION

Over the last decade, there has been an increase in scientific interest in reducing the appearance of ageing.⁴⁴ The use of plant extracts and herbs has its origin in ancient times, with the earliest records originating from ancient China and Egypt.⁴ Plants produce a great variety of organic compounds and can be classified into three major groups: terpenoids, alkaloids, and phenolic compounds.⁴⁵

The Diabliss test product contains multiple ingredients (mentioned above) demonstrating individual benefits and efficacy as documented in scientific literature.⁴⁶⁻⁴⁸

The study was conducted to evaluate and explore the skin benefits with regular intake of HSWS on various signs of aging among male and female subjects.

The results obtained from the study show that the regular consumption of the HSWS has positive health benefits and anti-aging efficacy. The efficacy in terms of visual reduction in wrinkles when evaluated over the crow's feet area was noted to be significant in comparison with the baseline values with 3 months of product use. Efficacy on secondary parameters associated with aging, was seen as early as D30, and was noted to progress with time over the study period of 90 days. As per the dermatological clinical evaluation, all assessment parameters showed a statistically significant improvement by the end of the study in comparison to the baseline.

The entire study population saw an improvement in skin parameters in association with skin elasticity, texture, hydration pigmentation as well as fine lines and wrinkles when evaluated with various standard instruments. Regular consumption of Diabliss HSWS water was found to show a statistically significant improvement in all of the study parameters in comparison to baseline values, by the end of the study. Further, there was direct correlation of instrumental analysis, dermatological assessments and subject self-assessment among all parameters assessed.

Since the test product has numerous ingredients, each having individual and synergistic benefits the anticipated positive results and outcome with no demonstrated adverse effects is encouraging.

The limitation of this study was the small sample size. Further larger, placebo controlled clinical studies are recommended to demonstrate and confirm additional and prolonged benefits.

CONCLUSION

In the current scenario, most nutraceutical products are not required by regulatory guidelines to conduct a clinical study, to be available as a commercially marketed product. This product has demonstrated the efficacy and benefits in a scientifically designed clinical study. The study demonstrated Diabliss HSWS based nutraceutical has shown a positive impact on the skin attributes and could possibly be considered as an adjunctive therapy for anti-ageing skin benefits. This water based oral nutraceutical is a novel delivery system for a skin rejuvenation adjuvant therapy which is cosmetically and systemically acceptable and tolerable for patients.

ACKNOWLEDGEMENTS

The authors would like to acknowledge M/s Diabliss Consumer Products Pvt Ltd for providing Herbal Skin Water Supplement for this study.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the institutional ethics committee

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Cite this article as: Janaki CS, Sachdev M, Kr R. A pilot, exploratory study to demonstrate the efficacy of a novel adjuvant herbal liquid supplement for skin rejuvenation and anti-aging. *Int J Res Dermatol* 2021;7:792-9.