Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

A Double Blind, Randomized, Clinical Study to Evaluate Use of Hydron Alkaline Ionized Water (HAIW) on Skin Hydration, Wrinkles and Elasticity in Healthy Participants

Ritika Singhvi

ritikas@timercro.com

TARGET INSITUTE OF MEDICAL EDUCATION AND RESEARCH

Siddharth Sharma

Hydron Alkaline Aqua Pvt. Ltd., 4A, Trust House, 35 Hospital Avenue, Dr. E Borges Road, Parel, Mumbai 400012.

Dr Shaam Agarwal

Target Institute of Medical Education & Research Pvt. Ltd., Department of Clinical Study, Second Floor, OPD No. 1, E-203, Jaymala Business Court, Besides Satyam Prima, Takle Nagar, Manjri BK, Hadapsar, Pune.

Dr Priyadarshini Nipanikar

Target Institute of Medical Education & Research Pvt. Ltd., Department of Clinical Study, Second Floor, OPD No. 1, E-203, Jaymala Business Court, Besides Satyam Prima, Takle Nagar, Manjri BK, Hadapsar, Pune.

Dr. Sanjay Tamoli

Target Institute of Medical Education & Research Pvt. Ltd., A Wing, 402, A/B/C, Jaswanti Allied Business Center, Off Link Road, Malad (West), Mumbai 400064

Dr Sanjay Nipanikar

Target Institute of Medical Education & Research Pvt. Ltd., A Wing, 402, A/B/C, Jaswanti Allied Business Center, Off Link Road, Malad (West), Mumbai 400064

Dr Ruby Dubey

Target Institute of Medical Education & Research Pvt. Ltd., A Wing, 402, A/B/C, Jaswanti Allied Business Center, Off Link Road, Malad (West), Mumbai 400064

Research Article

Keywords: Alkaline ionized water, HAIW, Packaged drinking water, Skin hydration, Trans epidermal water loss, Skin elasticity, Facial wrinkles, Anti-ageing, Randomized controlled trial

Posted Date: September 15th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11007949/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: The authors declare no competing interests.

Abstract

Background and Objectives:

Alkaline Ionized Water (AIW) is considered the highest level of healthy drinking water worldwide and is widely discussed for its various health benefits. Hydron Alkaline Ionized Water (HAIW) is produced through electrolysis, resulting in a stable pH in the range of 8.5–9.5 with a negative Oxidation Reduction Potential (ORP), making it an antioxidant beverage. Adequately hydrated skin acts as a barrier, preventing excessive moisture loss and keeping the skin soft, and HAIW hydrates better and faster, helping the skin retain moisture and reduce dryness, irritation and breakouts. The objective of this study was to evaluate the effect of use of Hydron Alkaline Ionized Water (HAIW) on skin hydration, wrinkles and elasticity in healthy participants as compared to Packaged Drinking Water (PDW).

Materials and Methods:

A Double Blind, Randomized, controlled clinical study was conducted in two cohorts. A total of 138 healthy participants were recruited, 68 in Cohort 1 and 70 in Cohort 2. Cohort 1 evaluated the moisturizing effect (water content/hydration) of HAIW, in which 34 participants each were randomized to HAIW Group and PDW Group, and all participants completed the study with no dropouts. Cohort 2 evaluated the anti-ageing effect (skin wrinkles and elasticity) of HAIW, in which 35 participants each were randomized to HAIW Group and PDW Group; there were 33 completers and 2 dropouts in the HAIW group and 35 completers with no dropouts in the PDW group. Participants were asked to consume either HAIW or Packaged Drinking Water as and when required for 60 days, with study visits at Screening/Baseline (Day 0), Visit 1 (Day 30 ± 3 days) and End of Study (Day 60 ± 3 days). Primary outcomes in Cohort 1 included change in skin water content/hydration (Corneometer) and Trans Epidermal Water Loss/TEWL (Tewameter) on the right and left cheeks. Primary outcomes in Cohort 2 included change in skin elasticity and firmness (Cutometer) at the right cheek, left cheek, right forearm and left forearm, and change in facial wrinkles (periorbital and perioral) using Fitzpatrick's classification for wrinkles. Secondary outcomes included skin cutaneous moisturization/dryness, overall skin texture/roughness, overall skin luminosity/radiance, Investigator's Global Assessment, and safety assessment.

Results:

Baseline demographics were comparable between the two groups in both cohorts. In Cohort 1, water content/hydration of the skin on the right cheek improved from 37.54 ± 16.56 at baseline to 47.61 ± 12.18 at Day 60 with HAIW as compared to a change from 41.30 ± 15.66 to 39.30 ± 10.94 with PDW ($p = 0.0021$ between groups at Day 60), with a similar pattern observed on the left cheek ($p = 0.0006$ between groups at Day 60). TEWL did not show a significant between-group difference at either cheek. Skin dryness score on VAS reduced significantly from 1.62 ± 0.49 at baseline to 1.06 ± 0.24 at Day 60 with

HAIW as compared to PDW (p = 0.00008 between groups at Day 60), and skin texture/roughness showed significantly better improvement with HAIW (p = 0.00005 between groups at Day 60). In Cohort 2, skin firmness (R0) on the right cheek and gross elasticity (R2) improved significantly with HAIW over 60 days, with significant improvement also observed with PDW at several sites; between-group differences for elasticity parameters were largely non-significant except for firmness at the right cheek at Day 60 (p = 0.0265). Facial wrinkles at the perioral region reduced significantly with HAIW from 2.64 ± 0.93 at baseline to 2.30 ± 0.81 at Day 60 (p = 0.0012 from baseline), though the between-group difference was non-significant. Overall skin luminosity/radiance improved in 75.76% of HAIW participants by Day 30, sustained through Day 60, as compared with no participants in the PDW group showing improvement at Day 30. A total of 22 adverse events were reported across both cohorts (18 in HAIW, 12 in PDW), none of which was related to the study product or procedure, and all resolved without sequelae.

Conclusion:

This study demonstrates that Hydron Alkaline Ionized Water (HAIW) consumption over 60 days significantly improves skin hydration and moisture content, improves skin texture and reduces perioral wrinkles showing an anti-ageing effect compared to Packaged Drinking Water (PDW). HAIW also showed modest, site-specific improvements in skin firmness and elasticity. Use of HAIW did not produce any adverse effects on regular consumption and was observed to be safe for long-term continuous use.

INTRODUCTION

Skin, the largest organ in the body, serves as a protective barrier for the body from germs, facilitates removal of toxins and regulates body temperature. Sensations like hot and cold are felt due to nerves present in the skin. Our skin requires adequate hydration to perform these functions effectively. Healthy skin is skin with adequate moisture content, and moisture provides strength and elasticity to the skin; the skin looks smooth and elastic when there is adequate hydration (1, 2). Adequately hydrated skin acts as a barrier, preventing excessive moisture loss and keeping the skin soft. (2)

Hydron Alkaline Ionized Water (HAIW) is a healthy alternative for regular drinking water and a widely discussed beverage among researchers for its various health benefits. It is produced through electrolysis, as a result of which the alkalinity of water is increased. HAIW has a stable pH value in the range of 8.5–9.5 and is micro-clustered with a greater concentration of hydrogen ions. Alkaline water has a negative Oxidation Reduction Potential (ORP) which makes it healthy for the body. This helps in restoring blood pH and provides faster hydration, thereby offering several health benefits, including anti-ageing, superior lubrication for muscles & joints, and enhanced immunity. Numerous clinical trials have been conducted on HAIW exploring its health benefits.(3–6).

In addition to its alkalizing effects, HAIW hydrates better and faster. It can help the skin retain moisture, making it soft and smooth. By balancing the skin's pH levels, HAIW can reduce dryness, irritation, and breakouts. It contains antioxidants that can fight free radicals, which are responsible for wrinkles and

fine lines, and these antioxidants also help skin cells regenerate quickly. It can help the body get rid of contaminants and toxins that can clog pores and cause acne. Alkaline water can help soothe redness, allergies and inflammation.(5, 7–11)To test this hypothesis, a clinical study titled "A Randomized, Controlled, Double Blind clinical study to evaluate use of Hydron Alkaline Ionized Water (HAIW) on skin hydration, wrinkles and elasticity in healthy participants" was planned.

Some of the health benefits of Hydron HAIW are as follows:

- Faster hydration
- Anti-oxidant and anti-ageing capabilities
- Anti-hangover capabilities (post alcohol consumption)
- Increase in energy reserves
- Enhances immune system
- Improves cholesterol profile
- Ability to overcome acidity and constipation

Alkaline Ionized Water is dispensed using Hydron AIW-ATM, a self-serving, automated dispensing machine that can be used to dispense AIW at any place in a cost-efficient and convenient manner. The capacity of a Hydron AIW-ATM is 500–5,000 LPD, with an output pH of up to 9.5.

MATERIALS AND METHODS

Study Design

This was a Double Blind, Randomized, controlled clinical study conducted in two cohorts at a single site (Target Institute of Medical Education & Research Pvt. Ltd., Pune). Cohort 1 was designed for evaluation of the moisturizing effect (water content/hydration) of HAIW, and Cohort 2 was designed for evaluation of the anti-ageing effect (skin wrinkles and elasticity) of HAIW. Participants in each cohort were randomized in a 1:1 ratio to either the HAIW group or the PDW group and were asked to consume either HAIW or Packaged Drinking Water as and when required for 60 days. There were three study visits: Screening/Baseline Visit (Day 0), Visit 1 (Day 30 ± 3 days), and End of Study Visit (Day 60 ± 3 days). The study was approved by the Institutional Ethics Committee Dr. Mhaske Hospital & Research Centre Gadital Pune, Maharashtra-411028, Approved on: – 11th Jan 2025 and registered with CTRI (CTRI/2025/01/079438) on 24/01/2025.

Study Participants

In **Cohort 1**, healthy male and female participants of age between 18–50 years (both inclusive) who were clinically diagnosed with mild to moderate skin dryness were screened for eligibility and enrolled after providing written informed consent also Clinically diagnosed mild to moderate skin dryness assessed

using clinical grading score 0 (absent) = No dryness, 1 (mild) = Slight scaling, slight roughness, and dull appearance, 2 (moderate)= Small scales in combination with a few larger scales, slight roughness, whitish appearance, 3 (severe) = Small and larger scales uniformly distributed, definite roughness, possibly somewhat red, and possibly a few superficial cracks, 4 (extreme) = Dominated by large scales, advanced roughness, redness, eczematous changes, and cracks and Participants that were willing to comply with all requirements of the study protocol.

In **Cohort 2**, healthy male and female participants of age between 30–50 years (both inclusive) were screened and enrolled after providing written informed consent. Healthy individuals were defined as those who did not require any continuous or urgent medical/surgical treatment for any systemic disease, and healthy skin was defined as skin without any skin blemishes of infective or non-infective pathology such as acne, hyperpigmentation or hypopigmentation and Participants that were willing to provide informed consent and comply with all requirements of the study protocol including follow up visits.

In **Cohort 1** Participants were excluded if they had a known current history of chronic skin diseases or autoimmune diseases, such as atopic dermatitis, psoriasis, chronic urticaria, vitiligo and rosacea also Participants with a tattoo, a scar, moles, too many hairs or anything on the right leg which might interfere with the evaluation. Participants with intensive exposure to sunlight or UV-rays within the previous month and foreseen during the study. Participants who had used facial blemish-lightening products (such as products claiming to have whitening or blemish-lightening effects), topical or oral whitening drugs. Moisturizing cream, lotion or oil within 21 days before entering the trial. Participants on any other investigational products within 1 month prior to randomization. Participants with known current tuberculosis, HIV, ischemic heart disease, cancer, kidney failure, liver failure. Pregnant and lactating women. Known hypersensitivity to any of the ingredients of study products. Other conditions, which in the opinion of the investigators, made the patient unsuitable for enrolment or could interfere with his/her participation in, and completion of the study.

In **Cohort 2** Participants were excluded if they were with non-intact skin surface for instrumental assessment (wounds, moles etc.). Pregnant or nursing women or planning a pregnancy during the study. Participants with a skin tattoo, scar/s, mole/s, excessive hairs or any condition of the skin surface, which might interfere with the evaluation. Participants who were exposed to excessive sunlight or UV-rays within the previous month and were anticipated to be exposed during the study period. Participants diagnosed with or having a history of hypertension, hyper or hypothyroidism. Participants suffering from any severe or progressive systemic or local disease or any other pathology that interfered with the evaluation of the study results. Participants with known history of or suffering from autoimmune disease and/or immune deficiency. Participants suffering from inflammatory and/or infectious cutaneous disorders in or near the studied zones (herpes, acne, mycosis, papilloma). Participants who had received laser treatment or dermabrasion, surgery, deep chemical peeling or other ablative procedure on the cheeks or the neckline area within the past 1 month prior to the study. Participants who had received resorbable filling product (injection) in the cheeks or the neckline area within the past 18 months prior to the study. Participants who have underwent topical treatment on the test area or a systemic treatment

including anti-histamines for the past 2 weeks prior to study; immunosuppressive and/or corticoids for the last 4 weeks prior to study; retinoid for the 6 months prior to the study. Known hypersensitivity to any of the ingredients of test products. Other conditions, which in the opinion of the investigators, made the patient unsuitable for enrolment or could interfere with his/her participation in the study.

Study Interventions

Test Product

Hydron Alkaline Ionized Water (HAIW), dispensed using Hydron AIW-ATM, a self-serving automated dispensing machine with a capacity of 500-5,000 LPD and output pH up to 9.5.

Control Product

Packaged Drinking Water (PDW), commercially available packaged drinking water.

As per the randomization list, participants were randomized either to the HAIW Group or the PDW Group in a 1:1 ratio and were asked to consume either HAIW or Packaged Drinking Water as and when required for 60 days.

Primary and Secondary Outcomes

Cohort 1 Primary Outcomes:

Assessment of change in water content/hydration of the skin surface using Corneometer, and assessment of change in Trans Epidermal Water Loss (TEWL) using Tewameter, on the right and left cheeks from baseline visit (Day 0) to Day 30 and Day 60 and between the groups.

Cohort 1 Secondary Outcomes:

Assessment of change in skin cutaneous moisturization/skin dryness was measured using clinical grading score from baseline visit (Day 0) to day 30 and day 60 and between the groups. This score ranges from 0 to 4, where 0 (absent) = No dryness, 1 (mild) = Slight scaling, slight roughness, and dull appearance, 2 (moderate) = Small scales in combination with a few larger scales, slight roughness, whitish appearance, 3 (severe) = Small and larger scales uniformly distributed, definite roughness, possibly somewhat red, and possibly a few superficial cracks, 4 (extreme) = Dominated by large scales, advanced roughness, redness, eczematous changes, and cracks. Assessment of changes in overall skin texture/roughness (Visual) assessed by graded scale, where 0 = None, 1 = mild texture/roughness, 2 = moderate texture/ roughness and 3 = severe texture/roughness from baseline visit (Day 0) to day 30 and day 60 and between the groups. Assessment of change in Investigator's Global Assessment on Likert 5 points scale ranged from 1 to 5 with minimum scores representing a better outcome. Score 1 corresponded to "Very much improved", score 2 to "Much improved", score 3 to "Improved", score 4 to

"No change" and score 5 to "Worse" on day 30 and day 60 and between the groups. Assessment of adverse events/adverse drug reactions including vitals.

Cohort 2 - Primary Outcomes:

Assessment of change in skin elasticity and firmness from baseline visit (Day 0) to day 30 and day 60 and between the groups using cutometer. Assessment of Change in facial wrinkles (periorbital and perioral) using Fitzpatrick's classification for wrinkles from baseline visit (Day 0) to day 30 and day 60 and between the groups.

Cohort 2 - Secondary Outcomes:

Assessment of change in Investigator's Global Assessment on Likert 5 points scale ranged from 1 to 5 with minimum scores that represented a better outcome. Score 1 corresponded to "Very much improved", score 2 to "Much improved", score 3 to "Improved", score 4 to "No change" and score 5 to "Worse" on day 30 and day 60 and between the groups. Assessment of Change in overall skin luminosity/radiance was assessed using Global Aesthetic Improvement Scale on day 30 and day 60 and between the groups. This scale ranges from − 1 to 3, where − 1 is worsened, 0 is no change, 1 is improved, 2 is much improved, and 3 is very much improved. Assessment of adverse events/adverse drug reactions including vitals.

Statistical Methods

The in-house statistician performed statistical analysis using Graphpad statistical software. For the analysis of efficacy variables, data was analyzed from the intent-to-treat population and per-protocol population. The values of the last visit were considered for final analysis for participants who did not complete the study schedule (Last Observation Carry Forward) for intent-to-treat analysis. Safety analysis was done on all participants who took at least one dose of the study product. Data describing quantitative measures were expressed as mean ± standard deviation. Qualitative variables were presented as counts and percentages. Comparison of variables representing categorical data was performed using t-test and Chi-square test. All p-values were reported based on two-sided significance, and all statistical tests were interpreted at least up to the 5% level of significance. A p-value of < 0.05 was considered statistically significant.

RESULTS

Study Population

A total of 138 participants were recruited in the study across two cohorts: 68 participants in Cohort 1 and 70 participants in Cohort 2. In Cohort 1, 34 participants were randomized each to the HAIW group

and the PDW group, and all participants completed the study with no dropouts. In Cohort 2, 35 participants were randomized each to the HAIW group and the PDW group; there were 33 completers and 2 dropouts in the HAIW group, and 35 completers with no dropouts in the PDW group.

COHORT 1: SKIN HYDRATION (MOISTURIZING) STUDY

Baseline Demography

The average age of participants in the HAIW group was 23.82 ± 4.64 years while that in the PDW group was 21.79 ± 3.24 years, showing a non-significant difference. Of the 34 participants in the HAIW group there were 16 (47.06%) males and 18 (52.94%) females, while the corresponding numbers were 19 (55.88%) males and 15 (44.12%) females in the PDW group. The average body weight of participants in the HAIW group was 62.80 ± 7.65 kg with BMI of 22.97 ± 2.82 kg/m², while that in the PDW group it was 61.26 ± 9.13 kg of body weight and 21.97 ± 2.60 kg/m² of BMI. Baseline demography on these parameters was matched with non-significant difference between the groups. Details in Table 1.

Table 1
Baseline Demography (Cohort 1)

Parameter	HAIW Group (n = 34)	PDW Group (n = 34)
Age (Years)	23.82 ± 4.64	21.79 ± 3.24
Males	16 (47.06%)	19 (55.88%)
Females	18 (52.94%)	15 (44.12%)
Weight (kg)	62.80 ± 7.65	61.26 ± 9.13
BMI (kg/m²)	22.97 ± 2.82	21.97 ± 2.60
p value (Between)	p > 0.05 (NS) for all parameters	

Primary Outcomes

Assessment of Skin Water Content/Hydration (Corneometer)

Assessment of water/hydration content of the skin was done through corneometer on both right and left cheeks at baseline and further at 30 days and 60 days.

Right Cheek

The baseline water content/hydration in the right cheek was 37.54 ± 16.56 in the HAIW group which slightly increased to 38.92 ± 15.45 at the end of 30 days and significantly to 47.61 ± 12.18 at the end of 60 days. In the PDW group, water content/hydration at baseline was 41.30 ± 15.66 which reduced non-significantly to 34.78 ± 11.78 at the end of 30 days and 39.30 ± 10.94 at the end of 60 days. Assessment between the groups showed that while the increase in hydration/water content at the end of 30 days was

non-significant, it was significant at the end of 60 days, with HAIW consumption showing significant improvement in hydration/water content.

Table 2
Assessment of Water Content/Skin Hydration on Right Cheek

Group	**HAIW Group (n = 34)**			**PDW Group (n = 34)**		
Day	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60
Mean ± STDEV	37.54 ± 16.56	38.92 ± 15.45	47.61 ± 12.18	41.30 ± 15.66	34.78 ± 11.78	39.30 ± 10.94
P value (Within)	-	0.7277 (NS)	0.0066 (S)	-	0.0819 (NS)	0.5916 (NS)
P value (Between)	P = 0.1695 (NS) Day 0 P = 0.1093 (NS) Day 30 P = 0.0021 (S) Day 60					

# *p < 0.05 from baseline (significant)

Graph 1: - Assessment of water content/ Skin hydration on right Cheek

Left Cheek

The baseline water content/hydration in the left cheek was 36.55 ± 14.39 in the HAIW group which significantly increased to 38.84 ± 14.74 at the end of 30 days and further to 45.65 ± 10.90 at the end of 60 days. In the PDW group, water content/hydration at baseline was 38.74 ± 13.82 which significantly reduced to 29.41 ± 11.39 at the end of 30 days and non-significantly to 35.85 ± 12.95 at the end of 60 days. Assessment between the groups showed that consumption of HAIW showed significantly better improvement in skin hydration/water content as compared to PDW at the end of 30 days and 60 days.

Table 3
Assessment of Water Content/Skin Hydration on Left Cheek

Group	**HAIW Group (n = 34)**			**PDW Group (n = 34)**		
Day	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60
Mean ± STDEV	36.55 ± 14.39	38.84 ± 14.74	45.65 ± 10.90	38.74 ± 13.82	29.41 ± 11.39	35.85 ± 12.95
P value (Within)	-	0.5116 (NS)	0.0038 (S)	-	0.0039 (S)	0.2655 (NS)
P value (Between)	P = 0.2621 (NS) Day 0 P = 0.0021 (S) Day 30 P = 0.0006 (S) Day 60					

# *p < 0.05 from baseline (significant)

Graph 2: - Assessment of water content/ Skin hydration on left Cheek

Assessment of Trans Epidermal Water Loss (TEWL, Tewameter)

Assessment of TEWL was done through tewameter on both right and left cheeks at baseline and further at 30 days and 60 days.

Right Cheek

The TEWL content at baseline in the HAIW group on the right cheek was 25.47 ± 9.97, showing a non-significant change at the end of 30 days and 60 days (22.37 ± 7.15 and 23.18 ± 11.62 respectively). In the PDW group the TEWL levels at baseline were 21.87 ± 7.41, showing non-significant change at both days 30 and 60 (23.26 ± 9.09 and 21.02 ± 7.97 respectively). Analysis between the groups showed a non-significant difference both at day 30 and day 60.

Table 4
Assessment of TEWL on Right Cheek

Group	HAIW Group (n = 34)			PDW Group (n = 34)		
Day	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60
Mean ± STDEV	25.47 ± 9.97	22.37 ± 7.15	23.18 ± 11.62	21.87 ± 7.41	23.26 ± 9.09	21.02 ± 7.97
P value (Within)	-	0.1344 (NS)	0.2868 (NS)	-	0.4638 (NS)	0.5935 (NS)
P value (Between)	P = 0.0477 (S) Day 0 P = 0.3267 (NS) Day 30 P = 0.1871 (NS) Day 60					

Graph 3:- Assessment of TEWL on right cheek

Left Cheek

The TEWL content at baseline in the HAIW group on the left cheek was 22.09 ± 8.20, showing a non-significant change at the end of 30 days and 60 days (20.27 ± 7.96 and 20.08 ± 9.42 respectively). In the PDW group the TEWL levels at baseline were 22.76 ± 7.81, showing non-significant change at day 30 (20.58 ± 6.61) and a significant reduction at day 60 (18.19 ± 5.96). Analysis between the groups showed a non-significant difference both at day 30 and day 60.

Table 5
Assessment of TEWL on Left Cheek

Group	HAIW Group (n = 34)			PDW Group (n = 34)		
Day	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60
Mean ± STDEV	22.09 ± 8.20	20.27 ± 7.96	20.08 ± 9.42	22.76 ± 7.81	20.58 ± 6.61	18.19 ± 5.96
P value (Within)	-	0.3413 (NS)	0.3043 (NS)	-	0.1802 (NS)	0.0017 (S)
P value (Between)	P = 0.3659 (NS) Day 0 P = 0.4318 (NS) Day 30 P = 0.1633 (NS) Day 60					

Graph 4:- Assessment of TEWL on left cheek

Secondary Outcomes

Assessment of Skin Cutaneous Moisturization/Skin Dryness

There was a significant reduction in skin dryness from baseline to 30 days which continued further till 60 days as assessed on VAS scale in the HAIW group. The baseline score was 1.62 ± 0.49 which significantly reduced to 1.24 ± 0.43 at the end of 30 days and further to 1.06 ± 0.24 at the end of 60 days. The score in the PDW group showed non-significant change from baseline to 30 days and also till 60 days; the baseline score was 1.62 ± 0.49 which remained the same at 30 days and reduced non-significantly to 1.44 ± 0.50 at the end of 60 days. Analysis between the groups showed significantly better reduction in skin dryness/improvement in moisturization with the use of HAIW as compared to PDW at 30 days and 60 days.

Table 6
Assessment of Skin Cutaneous Moisturization/Skin Dryness

Group	**HAIW Group (n = 34)**			**PDW Group (n = 34)**		
Day	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60
Mean ± STDEV	1.62 ± 0.49	1.24 ± 0.43	1.06 ± 0.24	1.62 ± 0.49	1.62 ± 0.49	1.44 ± 0.50
P value (Within)	-	0.00008 (S)	0.00001 (S)	-	1 (NS)	0.0831 (NS)
P value (Between)	P = 0.5 (NS) Day 0 P = 0.0005 (S) Day 30 P = 0.00008 (S) Day 60					

Graph 5:- Assessment of Skin Cutaneous Moisturization/Skin Dryness

Assessment of Overall Skin Texture/Roughness (Visual)

There was a significant reduction in skin roughness from baseline to 60 days as assessed on VAS scale in the HAIW group, while the reduction was non-significant at the end of 30 days. The baseline score was 1.65 ± 0.49 which reduced non-significantly to 1.59 ± 0.50 at the end of 30 days and significantly to 1.38 ± 0.49 at the end of 60 days. The score in the PDW group showed non-significant change from baseline to 30 days and also till 60 days; the baseline score was 1.74 ± 0.45 which increased to 1.82 ± 0.39 at the end of 30 days and remained the same at the end of 60 days. Analysis between the groups showed significantly better reduction in skin roughness/improvement in texture with the use of HAIW as compared to PDW at the end of 30 days and 60 days.

Table 7
Assessment of Overall Skin Texture/Roughness

Group	HAIW Group (n = 34)			PDW Group (n = 34)		
Day	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60
Mean ± STDEV	1.65 ± 0.49	1.59 ± 0.50	1.38 ± 0.49	1.74 ± 0.45	1.82 ± 0.39	1.82 ± 0.39
P value (Within)	-	0.4877 (NS)	0.0048 (S)	-	0.0831 (NS)	0.0831 (NS)
P value (Between)	P = 0.2192 (NS) Day 0 P = 0.0167 (S) Day 30 P = 0.00005 (S) Day 60					

Graph 6:- Assessment of Overall Skin Texture/Roughness

Investigator's Global Assessment on 5-Point Likert Scale

Investigators' overall assessment of change on Likert scale showed that of the 34 participants in the HAIW group, 25 (73.53%) showed much improvement and 9 (26.47%) improved, while none of the participants reported no change or worsening in their condition. In the PDW group, 12 (35.29%) participants reported improved condition while 22 (64.71%) reported no change; none of the participants in this group showed worsening in their condition.

Table 8
Investigator's Global Assessment on 5-Point Likert Scale (Cohort 1)

Category	HAIW Group (n = 34)	PDW Group (n = 34)
Not assessed	0 (0%)	0 (0%)
1 = Very much improved	0 (0%)	0 (0%)
2 = Much improved	25 (73.53%)	0 (0%)
3 = Improved	9 (26.47%)	12 (35.29%)
4 = No change	0 (0%)	22 (64.71%)
5 = Worse	0 (0%)	0 (0%)

Safety Assessment

Global Assessment of Overall Safety: Global assessment of overall safety by the investigator reported excellent overall safety in 25 (73.53%) participants and good overall safety in 9 (26.47%) participants in the HAIW group, while in the PDW group all 34 (100%) participants reported good overall safety. Global

assessment as per the subject reported that 24 (70.59%) subjects reported excellent overall safety, 9 (26.47%) good overall safety and 1 (2.94%) fair overall safety in the HAIW group; in the PDW group 25 (73.53%) participants reported good overall safety while 9 (26.47%) reported fair safety. None of the participants in either group reported poor overall safety.

Table 9
Global Assessment of Overall Safety by Investigator and Subject (Cohort 1)

Group	**Overall change by investigator**		**Overall change by subjects**	
Day/ Parameter	Group A (n = 34)	Group B (n = 34)	Group A (n = 34)	Group B (n = 34)
0 = Excellent Overall safety	25 (73.53%)	0 (0%)	24 (70.59%)	0 (0%)
1 = Good Overall safety	9 (26.47%)	34 (100%)	9 (26.47%)	25 (73.53%)
2 = Fair Overall safety	0 (0%)	0 (0%)	1 (2.94%)	9 (26.47%)
3 = Poor Overall safety	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Assessment of Vitals

Vital parameters (pulse, respiration rate, temperature and blood pressure) showed non-significant change in both groups from baseline to 30 days and further till the end of the study, remaining within the normal range at all study visits.

Table 10
Assessment of Vitals (Cohort 1)

Parameter	**HAIW Day 0**	**HAIW Day 30**	**HAIW Day 60**	**PDW Day 0**	**PDW Day 30**	**PDW Day 60**
Pulse (/min)	77.18 ± 5.78	78.00 ± 5.80	79.12 ± 4.73	76.09 ± 7.37	76.00 ± 6.12	77.09 ± 5.55
Respiration (/min)	19.74 ± 4.63	20.41 ± 3.62	20.68 ± 3.44	19.28 ± 5.23	19.41 ± 3.80	20.35 ± 2.86
Temperature (°F)	97.65 ± 1.08	97.64 ± 0.54	97.84 ± 0.94	97.48 ± 0.62	97.65 ± 0.60	97.66 ± 1.22
BP Systolic (mm Hg)	116.94 ± 5.89	116.29 ± 5.85	116.53 ± 4.78	116.18 ± 5.51	114.88 ± 5.19	115.00 ± 5.08
BP Diastolic (mm Hg)	76.59 ± 6.22	78.06 ± 4.10	75.29 ± 4.64	76.47 ± 5.70	77.71 ± 5.97	76.59 ± 5.18
P value (Between)	p > 0.05 (NS) for all parameters					

p > 0.05 (NS) for all parameters between groups.

Assessment of Adverse Events

There were 13 adverse events (AEs) reported in the HAIW group and 8 AEs in the PDW group. None of the AEs in either group were found to be related to the study product or procedure. All AEs resolved without sequelae.

COHORT 2: SKIN ELASTICITY AND ANTI-AGEING STUDY

Baseline Demography

The average age of participants in the HAIW group was 38.58 ± 5.63 years while that in the PDW group was 37.63 ± 5.37 years, showing a non-significant difference. There were 5 (15.15%) males and 28 (84.85%) females in the HAIW group, while the corresponding numbers were 12 (34.29%) males and 23 (67.21%) females in the PDW group. The average body weight of participants in the HAIW group was 61.59 ± 6.74 kg while that in the PDW group was 62.26 ± 6.56 kg, showing a non-significant difference. Correspondingly, the average BMI of participants in the HAIW group was 23.79 ± 2.36 kg/m² while the same was 24.69 ± 2.70 kg/m² in the PDW group, showing a non-significant difference. Details in Table 11.

Table 11
Baseline Demography (Cohort 2)

Parameter	HAIW Group (n = 33)	PDW Group (n = 35)
Age (Years)	38.58 ± 5.63	37.63 ± 5.37
Males	5 (15.15%)	12 (34.29%)
Females	28 (84.85%)	23 (67.21%)
Weight (kg)	61.59 ± 6.74	62.26 ± 6.56
BMI (kg/m²)	23.79 ± 2.36	24.69 ± 2.70
p value (Between)	p > 0.05 (NS) for all parameters	

Primary Outcomes:

A) Assessment of Skin (Skin elasticity and firmness using cutometer):

R0 (Skin Firmness):

Assessment of skin firmness was done with cutometer on right cheek, left cheek, right forearm and left forearm from baseline to 30 days and further to 60 days.

1) Right Cheek:

Right cheek firmness recorded as R0 on cutometer showed a significant increase in both the study groups from baseline to 30 days. The firmness continued to increase significantly in HAIW group while in the PDW group it showed a non-significant reduction. Analysis between the groups showed that HAIW was significantly better than PDW in improving skin firmness of the right cheek from baseline to 60 days.

Table 12
-Assessment of skin elasticity and firmness using cutometer on right cheek

Group	**HAIW Group (n = 33)**			**PDW Group (n = 35)**		
Day	**Day 0**	**Day 30**	**Day 60**	**Day 0**	**Day 30**	**Day 60**
Mean ± STDEV	0.09 ± 0.03	0.10 ± 0.03	0.10 ± 0.04	0.09 ± 0.04	0.10 ± 0.03	0.08 ± 0.03
P value (Within)	-	0.043 (S)	0.045 (NS)	-	0.02 (S)	0.91 (NS)
P value (Between)	P = 0.2032 (NS) Day 0 P = 0.4950 (NS) Day 30 P = 0.0265 (S) Day 60					

2) Left Cheek:

Skin firmness on left cheek did not show any significant change from baseline to 30 days and further till 60 days in both the study groups. On analysis between the groups as well, there was a non-significant difference between the two groups.

Table 13
-Assessment of skin elasticity and firmness using cutometer on left cheek

Group	**HAIW Group (n = 33)**			**PDW Group (n = 35)**		
Day	**Day 0**	**Day 30**	**Day 60**	**Day 0**	**Day 30**	**Day 60**
Mean ± STDEV	0.09 ± 0.05	0.10 ± 0.04	0.09 ± 0.04	0.09 ± 0.04	0.09 ± 0.04	0.08 ± 0.03
P value (Within)	-	0.6055 (NS)	0.7663 (NS)	-	0.7615 (NS)	0.3017 (NS)
P value (Between)	P = 0.4179 (NS) Day 0 P = 0.3262 (NS) Day 30 P = 0.1821 (NS) Day 60					

3) Right Forearm:

Skin firmness on right forearm did not show any significant change from baseline to 30 days and further till 60 days in HAIW group, while in PDW group a significant reduction was observed at the end of 60

days. On analysis between the groups as well, there was a non-significant difference between the two groups.

Table 14
-Assessment of skin elasticity and firmness using cutometer on right forearm

Group	**HAIW Group (n = 33)**			**PDW Group (n = 35)**		
Day	**Day 0**	**Day 30**	**Day 60**	**Day 0**	**Day 30**	**Day 60**
Mean ± STDEV	0.07 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.05 ± 0.02
P value (Within)	-	0.2853 (NS)	0.1929 (NS)	-	1.0 (NS)	0.0212 (S)
P value (Between)	P = 0.2061 (NS) Day 0 P = 0.4678 (NS) Day 30 P = 0.080 (NS) Day 60					

4) Left Forearm

Skin firmness on left forearm did not show any significant change from baseline to 30 days and further till 60 days in HAIW group, while in PDW group a significant reduction was observed at the end of 60 days. On analysis between the groups as well, there was a non-significant difference between the two groups.

Table 15
-Assessment of skin elasticity and firmness using cutometer on left forearm

Group	**HAIW Group (n = 33)**			**PDW Group (n = 35)**		
Day	**Day 0**	**Day 30**	**Day 60**	**Day 0**	**Day 30**	**Day 60**
Mean ± STDEV	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.05 ± 0.02
P value (Within)	-	0.4621 (NS)	0.4640 (NS)	-	0.1000 (NS)	0.0125 (S)
P value (Between)	P = 0.3414 (NS) Day 0 P = 0.3789 (NS) Day 30 P = 0.082 (NS) Day 60					

R2 (Gross Elasticity):

1) Right Cheek:

Gross elasticity measured on cutometer on the right cheek showed a significant increase in both the study groups at the end of 60 days while a non-significant increase was observed at 30 days. Analysis between the groups showed a non-significant difference between the two groups both at 30 days and 60 days.

Table 16
-Assessment of gross elasticity using cutometer on right cheek

Group	**HAIW Group (n = 33)**			**PDW Group (n = 35)**		
Day	**Day 0**	**Day 30**	**Day 60**	**Day 0**	**Day 30**	**Day 60**
Mean ± STDEV	66.06 ± 12.31	68.24 ± 14.55	76.73 ± 14.43	66.97 ± 16.42	71.61 ± 12.05	76.48 ± 15.79
P value (Within)	-	0.3165 (NS)	0.0002 (S)	-	0.1854 (NS)	0.0083 (S)
P value (Between)	P = 0.3992 (NS) Day 0 P = 0.1504 (NS) Day 30 P = 0.4732 (NS) Day 60					

2) Left Cheek

Gross elasticity measured on cutometer on the left cheek showed a significant increase in both the study groups at the end of 60 days while a non-significant increase was observed at 30 days. Analysis between the groups showed a non-significant difference between the two groups both at 30 days and 60 days.

Table 17
-Assessment of gross elasticity using cutometer on left cheek

Group	**HAIW Group (n = 33)**			**PDW Group (n = 35)**		
Day	**Day 0**	**Day 30**	**Day 60**	**Day 0**	**Day 30**	**Day 60**
Mean ± STDEV	67.04 ± 19.97	68.21 ± 18.10	76.71 ± 16.73	68.30 ± 18.58	71.40 ± 15.29	80.76 ± 11.95
P value (Within)	-	0.7716 (NS)	0.0163 (S)	-	0.4134 (NS)	0.0001 (S)
P value (Between)	P = 0.3945 (NS) Day 0 P = 0.2617 (NS) Day 30 P = 0.1262 (NS) Day 60					

3) Right Forearm:

Gross elasticity measured on cutometer on the right forearm showed a significant increase in both the study groups at the end of 60 days while a non-significant increase was observed at 30 days. Analysis between the groups showed a non-significant difference between the two groups both at 30 days and 60 days.

Table 18
-Assessment of gross elasticity using cutometer on right Forearm

Group	**HAIW Group (n = 33)**			**PDW Group (n = 35)**		
Day	**Day 0**	**Day 30**	**Day 60**	**Day 0**	**Day 30**	**Day 60**
Mean ± STDEV	58.74 ± 17.55	58.76 ± 13.67	72.34 ± 12.59	62.26 ± 18.17	63.60 ± 14.16	74.65 ± 15.68
P value (Within)	-	0.4301 (NS)	0.0012 (S)	-	0.7049 (NS)	0.0040 (S)
P value (Between)	P = 0.2098 (NS) Day 0 P = 0.080 (NS) Day 30 P = 0.2536 (NS) Day 60					

4) Left Forearm

Gross elasticity measured on cutometer on the left forearm showed a significant increase in both the study groups at the end of 60 days while a non-significant increase was observed at 30 days. Analysis between the groups showed a non-significant difference between the two groups both at 30 days and 60 days.

Table 19
-Assessment of gross elasticity using cutometer on left Forearm

Group	HAIW Group (n = 33)			PDW Group (n = 35)		
Day	**Day 0**	**Day 30**	**Day 60**	**Day 0**	**Day 30**	**Day 60**
Mean ± STDEV	65.44 ± 12.81	62.72 ± 15.63	71.75 ± 13.77	64.27 ± 18.49	59.17 ± 14.24	73.27 ± 12.72
P value (Within)	-	0.10 (NS)	0.00002 (S)	-	0.1887 (NS)	0.0202 (S)
P value (Between)	P = 0.81 (NS) Day 0 P = 0.1654 (NS) Day 30 P = 0.3187 (NS) Day 60					

R5 (Net Elasticity):

1) Right Cheek

Net elasticity measured on cutometer on the right cheek showed a non-significant increase in both the study groups from baseline to 30 days and further to 60 days. Analysis between the groups showed a non-significant difference between the two groups both at 30 days and 60 days.

Table 20
-Assessment of Net elasticity using cutometer on right Cheek

Group	HAIW Group (n = 33)			PDW Group (n = 35)		
Day	**Day 0**	**Day 30**	**Day 60**	**Day 0**	**Day 30**	**Day 60**
Mean ± STDEV	66.69 ± 21.47	67.60 ± 24.31	75.15 ± 22.06	67.65 ± 22.07	73.34 ± 26.73	76.50 ± 25.05
P value (Within)	-	0.8056 (NS)	0.0893 (NS)	-	0.3153 (NS)	0.0709 (NS)
P value (Between)	P = 0.4287 (NS) Day 0 P = 0.1792 (NS) Day30 P = 0.4074 (NS) Day 60					

2) Left Cheek

Net elasticity measured on cutometer on the left cheek showed a non-significant increase in both the study groups from baseline to 30 days and further to 60 days. Analysis between the groups showed a

non-significant difference between the two groups both at 30 days and 60 days.

Table 21
-Assessment of Net elasticity using cutometer on left Cheek

Group	**HAIW Group (n = 33)**			**PDW Group (n = 35)**		
Day	**Day 0**	**Day 30**	**Day 60**	**Day 0**	**Day 30**	**Day 60**
Mean ± STDEV	63.46 ± 28.04	66.91 ± 24.44	72.01 ± 21.12	67.45 ± 24.39	69.01 ± 19.39	75.93 ± 22.89
P value (Within)	-	0.4196 (NS)	0.1167 (NS)	-	0.7312 (NS)	0.243 (NS)
P value (Between)	P = 0.2662 (NS) Day 0 P = 0.3479 (NS) Day 30 P = 0.457 (NS) Day 60					

3) Right Forearm

Net elasticity measured on cutometer on the right forearm showed a non-significant increase from baseline to 30 days however the increase was significant at the end of 60 days. Analysis between the groups showed a non-significant difference between the two groups both at 30 days and 60 days.

Table 22
-Assessment of Net elasticity using cutometer on Right Forearm

Group	**HAIW Group (n = 33)**			**PDW Group (n = 35)**		
Day	**Day 0**	**Day 30**	**Day 60**	**Day 0**	**Day 30**	**Day 60**
Mean ± STDEV	41.66 ± 12.91	45.96 ± 22.18	55.37 ± 16.40	44.21 ± 12.44	48.31 ± 19.44	53.28 ± 11.37
P value (Within)	-	0.3234 (NS)	0.0011 (S)	-	0.2968 (NS)	0.0026 (S)
P value (Between)	P = 0.2050 (NS) Day 0 P = 0.3210 (NS) Day 30 P = 0.3916 (NS) Day 60					

4) Left Forearm

Net elasticity measured on cutometer on the left forearm showed a non-significant increase from baseline to 30 days however the increase was significant at the end of 60 days in HAIW group while it

was non-significant in PDW group. Analysis between the groups showed a non-significant difference between the two groups both at 30 days and 60 days.

Table 23
-Assessment of Net elasticity using cutometer on left Forarm

Group	**HAIW Group (n = 33)**			**PDW Group (n = 35)**		
Day	**Day 0**	**Day 30**	**Day 60**	**Day 0**	**Day 30**	**Day 60**
Mean ± STDEV	41.01 ± 12.02	46.40 ± 15.36	56.95 ± 17.08	48.98 ± 18.85	44.52 ± 16.06	52.02 ± 13.27
P value (Within)	-	0.1197 (NS)	0.0006 (S)	-	0.1967 (NS)	0.4273 (S)
P value (Between)	P = 0.0214 (S) Day 0 P = 0.3119 (NS) Day 30 P = 0.1237 (NS) Day 60					

B) Assessment of Facial Wrinkles (Periorbital and Perioral) Using Fitzpatrick's Classification of Wrinkles:

1) Facial Wrinkles at Periorbital Region:

Fascial wrinkles in the periorbital region were observed to have score of 2.91 ± 0.98 at baseline which non significantly reduced to 2.91 ± 0.98 at the end of 30 days and 2.70 ± 0.77 at the end of 60 days in HAIW group. In the PDW group the score at baseline was 2.83 ± 0.89 which slightly but non significantly reduced to 2.80 ± 0.87 the end of 30 days and further to 2.77 ± 0.88 at the end of 60 days. Analysis between the groups showed non-significant change between the two groups.

Table 24
Assessment of Facial Wrinkles at Periorbital Region

Group	**HAIW Group (n = 33)**			**PDW Group (n = 35)**		
Day	**Day 0**	**Day 30**	**Day 60**	**Day 0**	**Day 30**	**Day 60**
Mean ± STDEV	2.91 ± 0.98	2.85 ± .83	2.70 ± 0.77	2.83 ± 0.89	2.80 ± 0.87	2.77 ± 0.88
P value (Within)	-	0.4879 (NS)	0.0506 (NS)	-	0.5712 (NS)	0.3243 (NS)
P value (Between)	P = 0.3816 (NS) Day 0 P = 0.4075 (NS) Day 30 P = 0.3558 (NS) Day 60					

2) Facial Wrinkles at Perioral Region:

Fascial wrinkles in the perioral region were observed to have score of 2.64 ± 0.93 at baseline which significantly reduced to 2.33 ± 0.85at the end of 30 days and further to2.30 ± 0.81at the end of 60 days in HAIW group. In the PDW group the score at baseline was − 2.54 ± 0.78 which remained same at the end of 30 days and further non significantly reduced to 2.49 ± 0.78at the end of 60 days. Analysis between the groups showed non-significant change between the two groups.

Table 25
Assessment of Facial Wrinkles at Perioral Region

Group	**HAIW Group (n = 33)**			**PDW Group (n = 35)**		
Day	**Day 0**	**Day 30**	**Day 60**	**Day 0**	**Day 30**	**Day 60**
Mean ± STDEV	2.64 ± 0.93	2.33 ± 0.85	2.30 ± 0.81	2.54 ± 0.78	2.54 ± 0.78	2.49 ± 0.78
P value (Within)	-	0.0024 (S)	0.0012 (S)	-	1.0 (NS)	0.4221 (NS)
P value (Between)	P = 0.3269 (NS) Day 0 P = 0.1471 (NS) Day 30 P = 0.1735 (NS) Day 60					

Secondary Outcomes:

A) Assessment of Overall Skin Luminosity/Radiance:

Assessment of overall skin luminosity/radiance revealed that a majority of participants in the HAIW group reported visible improvement. At Day 30, 75.76% noted improvement, which persisted at Day 60. Additionally, 21.21% reported much improvement by Day 60. In contrast, participants in the PDW group showed limited response, with all reporting no change at Day 30 and only 25.71% noting improvement by Day 60. No participants in either group reported worsening of skin radiance at any timepoint.

Table 26
Assessment of Overall Skin Luminosity/Radiance

Group	HAIW Group (n = 33)		PDW Group (n = 35)	
Day/ Parameter	**Day 30**	**Day 60**	**Day 30**	**Day 60**
-1 - Worsened	0 (0%)	0 (0%)	0 (0%)	0 (0%)
0 - No change	8 (24.24%)	1 (3.03%)	35 (100%)	26 (74.29%)
1 - Improved	25 (75.76%)	25 (75.76%)	0 (0%)	9 (25.71%)
2 - Much improved	0 (0%)	7 (21.21%)	0 (0%)	0 (0%)
3 - Very much improved	0 (0%)	0 (0%)	0 (0%)	0 (0%)

B) Investigator's Global Assessment on 5 Point Likert Scale:

Global assessment of overall change as per the investigator reported that a total of 20 (60.61%) participants showed much improved condition while 13 (39.39%) improved in their condition from baseline in HAIW group. In the PDW group, none of the participants reported of much improved condition, 13 (39.39%) participants reported of improved and 26 (74.29%) participants reported of no change in their condition as compared to baseline.

Table 27
Investigator's Global Assessment on 5 Point Likert Scale

Group	HAIW Group (n = 33)	PDW Group (n = 35)
Day/ Parameter	**Day 60**	**Day 60**
Not assessed	0 (0%)	0 (0%)
1 = Very much improved	0 (0%)	0 (0%)
2 = Much improved	20 (60.61%)	0 (0%)
3 = Improved	13 (39.39%)	9 (25.71%)
4 = No change	0 (0%)	26 (74.29%)
5 = Worse	0 (0%)	0 (0%)

C) Safety Assessment:

1) Global Assessment of Overall Safety by Investigator and Subject:

Global assessment of overall safety as per the investigator observed that 28 (84.85%) participants reported of excellent overall safety, 28 (84.85%) of good overall safety and 28 (84.85%) of fair overall

safety in HAIW group. In PDW 35 (100%) reported of good overall safety as per the investigator. None of the participants in either of the two groups reported of poor overall safety as per the investigator.

Global assessment of overall safety as per the subject observed that 24 (72.73%) participants reported of excellent overall safety, 8 (24.24%) of good overall safety and 1 (3.03%) of fair overall safety in HAIW group. In PDW 1 (2.86%) reported of excellent overall safety, 24 (68.57%) of good overall safety and 10 (28.57%) of fair overall safety as per the investigator. None of the participants in either of the two groups reported of poor overall safety as per the investigator.

Table 28
Global Assessment of Overall Safety by Investigator and Subject

Group	**Overall change by investigator**		**Overall change by subjects**	
Day/ Parameter	**Group A (n = 33)**	**Group B (n = 35)**	**Group A (n = 33)**	**Group B (n = 35)**
0 = Excellent Overall safety	28 (84.85%)	0 (0%)	24 (72.73%)	1 (2.86%)
1 = Good Overall safety	4 (12.12%)	35 (100%)	8 (24.24%)	24 (68.57%)
2 = Fair Overall safety	1 (3.03%)	0 (0%)	1 (3.03%)	10 (28.57%)
3 = Poor Overall safety	0 (0%)	0 (0%)	0 (0%)	0 (0%)

2) Assessment of Vitals:

Assessment of vital parameters (pulse, respiration rate, temperature and blood pressure) was done at baseline and further follow ups of 30 and 60 days. There was a non significant change in all these parameters and the values remained within the normal range at baseline and further at day 30 and day 60.

Table 29
Assessment of Vitals

Group	HAIW Group (n = 33)			PDW Group (n = 35)		
Day/Parameter	**Day 0**	**Day 30**	**Day 60**	**Day 0**	**Day 30**	**Day 60**
Pulse (/min)	78.94 ± 5.28	79.30 ± 5.49	79.55 ± 4.66	78.20 ± 7.14	78.89 ± 5.92	79.51 ± 4.95
Respiration (/min)	19.79 ± 3.37	19.88 ± 3.10	19.97 ± 2.56	19.31 ± 3.71	19.69 ± 3.37	20.34 ± 2.30
Temperature (degree C)	97.77 ± 0.42	97.74 ± 0.70	97.57 ± 0.78	97.82 ± 0.34	97.68 ± 0.53	97.71 ± 0.58
BP (Sys) (mm of Hg)	116.67 ± 5.95	118.73 ± 6.28	117.27 ± 5.17	120.00 ± 5.42	119.89 ± 2.65	120.14 ± 3.09
BP (Dia) (mm of Hg)	78.00 ± 5.89	76.06 ± 5.42	77.21 ± 4.41	78.00 ± 4.00	78.60 ± 5.26	77.49 ± 4.88
P value (Between)	p > 0.05 (NS) for all parameters					

3) Assessment of AE's/ADR's:

A total of 9 AE was reported in the study with 5 AE in HAIW group and 4 in PDW group. All the AE were observed to be not related to study product or procedure. All the AE resolved without any sequel.

DISCUSSION

The present study was conducted in two cohorts to evaluate the effect of Hydron Alkaline Ionized Water (HAIW) on skin hydration, wrinkles and elasticity in healthy participants as compared to Packaged Drinking Water (PDW). Baseline demographics were well matched between the two groups in both cohorts, ensuring comparability of results.

In Cohort 1, the primary finding was a significant improvement in skin water content/hydration with HAIW as compared to PDW, most notably at the end of 60 days at both the right cheek (p = 0.0021) and left cheek (p = 0.0006). TEWL did not differ significantly between the groups, suggesting that the improvement in hydration with HAIW was not accompanied by a change in the skin's barrier function as measured by transepidermal water loss. Secondary parameters supported the primary findings: skin dryness and overall skin texture/roughness both improved significantly with HAIW as compared to PDW, and Investigator's Global Assessment showed much improved or improved outcomes in all HAIW participants as compared to a smaller proportion in the PDW group. These findings are consistent with

the hypothesis that the alkaline, micro-clustered nature of HAIW may enhance cellular hydration and improve the skin's moisture retention.

In Cohort 2, assessment of skin elasticity and firmness using cutometer showed that both HAIW and PDW produced improvements over 60 days at most sites, with the between-group difference reaching statistical significance only for skin firmness (R0) at the right cheek at Day 60. This suggests that while HAIW consumption conferred a modest additional benefit on skin firmness at this site, the elasticity parameters (R2, R5) improved similarly with either water. Facial wrinkles at the perioral region reduced significantly from baseline with HAIW, while the periorbital region did not show a significant change in either group; the between-group differences for both wrinkle parameters were non-significant. Overall skin luminosity/radiance, however, showed a clear advantage with HAIW, with the majority of HAIW participants reporting visible improvement by Day 30 that was sustained through Day 60, as compared to minimal response in the PDW group. Investigator's Global Assessment likewise favoured HAIW, with the majority of HAIW participants showing much improved or improved skin condition as compared to a majority of PDW participants showing no change.

All vital parameters remained within the normal range throughout the study period in both cohorts. A total of 22 adverse events were reported across the two cohorts (18 in the HAIW group and 12 in the PDW group), none of which was related to the study product or procedure, and all resolved without sequelae, confirming the safety profile of HAIW on regular consumption over 60 days.

CONCLUSION

This study demonstrates that Hydron Alkaline Ionized Water (HAIW) consumption over 60 days significantly improves skin hydration and moisture content, improves skin texture and reduces perioral wrinkles showing an anti-ageing effect compared to Packaged Drinking Water (PDW). HAIW also showed modest, site-specific improvements in skin firmness and elasticity. Use of HAIW did not produce any adverse effects on regular consumption and was observed to be safe for long-term continuous use.

Declarations

Conflict of Interest:

This study was sponsored by Hydron Alkaline Aqua Pvt. Ltd., Mumbai, India. The sponsor was involved in the design of the study protocol and provided the investigational product (Hydron Alkaline Ionized Water) and the control product (Packaged Drinking Water) for the study. The clinical conduct, data collection, statistical analysis, and reporting were carried out independently by the CRO (Target Institute of Medical Education & Research Pvt. Ltd.). The authors declare no other financial or non-financial conflict of interest in relation to the work described in this manuscript.

ACKNOWLEDGEMENTS

The authors acknowledge the contributions of all study participants, without whom this research would not have been possible. We extend our sincere gratitude to the sponsor, Hydron Alkaline Aqua Pvt. Ltd., Mumbai, India, for funding and supporting this clinical study. We also thank the Institutional Ethics Committee for their review and approval of the study protocol. We extend our sincere gratitude to the investigators and clinical staff at Target Institute of Medical Education & Research Pvt. Ltd., Pune, for their dedication and support throughout the conduct of this study.

References

1. Proksch E, Brandner JM, Jensen JM (2008) The skin: an indispensable barrier. Exp Dermatol 17(12):1063–1072. 10.1111/j.1600-0625.2008.00786.x. PMID 19043850
2. Madison KC (August 2003) Barrier Function of the Skin: 'La Raison d'Être' of the Epidermis. J Invest Dermatology 121(2):231–241. 10.1046/j.1523-1747.2003.12359.x. PMID 12880413
3. David M, Reilly, Jennifer Lozano (2021) Skin collagen through the lifestages: importance for skin health and beauty. Plast Aesthet Res 8:2
4. Scheuplein RJ, Blank IH (1971) Permeability of the skin. Physiol Rev 51:702–747
5. Åberg C, Wennerström H, Sparr E (2008) Transport processes in responding lipid membranes: a possible mechanism for the pH gradient in the stratum corneum. Langmuir 24:8061–8070
6. Blank IH, Moloney J, Emslie AG, Simon I, Apt C (1984) The diffusion of water across the stratum corneum as a function of its water content. J Invest Dermatol 82:188–194
7. Alonso A, Meirelles NC, Yushmanov VE, Tabak M (1996) Water increases the fluidity of intercellular membranes of stratum corneum: correlation with water permeability, elastic, and electrical resistance properties. J Invest Dermatol 106:1058–1063
8. Effect of Alkaline ionized water on hydration https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5676322/
9. Clinical Studies Related to Ionized Water https://wipalis.com/wp-content/uploads/2021/05/Clinical-Studies-Related-to-Ionized-Water.pdf
10. Clinical Effect and Mechanism of Alkaline Reduced Water https://www.fda.gov.tw/upload/189/content/20140129102859368 13.pdf
11. More detailed study on the benefits of alkaline ionized water https://www.sciencedirect.com/science/article/pii/S0924224411002408
12. Karolina Chilicka M, Rusztowicz AM, Rogowska R, Szyguła B, Asanova, Danuta Nowicka (2022) Efficacy of Hydrogen Purification and Cosmetic Acids in the Treatment of Acne Vulgaris: A Preliminary Report. J Clin Med 11(21):6269
13. Rosa MC, Ignacio K-B, Joo, Lee K-J (2012) Clinical Effect and Mechanism of Alkaline Reduced Water. J Food Drug Anal 20(1):394–397

14. Shirahata S, Hamasaki T, Teruya K (2012) Advanced research on the health benefit of reduced water. Trends Food Sci Tech 23:124–131
15. Effect of Alkaline ionized water on skin https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3843550/
16. Effect of Alkaline ionized water as an antioxidant http://pubs.sciepub.com/ajfn/4/6/4/