

Original Article

## Evaluation of skin irritation and hypoallergenic potential of Venusia Cleanser using the human repeat insult patch test

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### ABSTRACT

**Objectives:** The objective of the study is to evaluate the dermatological safety, tolerability, and hypoallergenic potential of Venusia Cleanser using the human repeat insult patch test (HRIPT).

**Materials and Methods:** A single-center, evaluator-blinded HRIPT was conducted on 220 healthy adults aged 18–65 years, including participants with sensitive skin identified through the lactic acid sting test. The test product (1% diluted Venusia Cleanser) and a negative control (0.9% isotonic saline) were applied occlusively on the participant's back in three phases: Induction (nine 24 h applications), rest (14 days), and challenge (single 24 h application). Skin reactions were assessed using the Draize scale during induction and the International Contact Dermatitis Research Group scale during challenge. Safety and sensitization potential were evaluated qualitatively.

**Results:** Of 220 participants recruited, 218 participants completed the study. 36% ( $n = 78$ ) were confirmed to have sensitive skin. During the induction phase, the mean cumulative irritation score for Venusia Cleanser was 0.08, significantly below the threshold of 2.0, which defines non-irritant status according to Bureau of Indian Standards - Indian Standard 4011: 2018 Methods of Test for Safety Evaluation of Cosmetics (Third Revision) guidelines. Throughout the challenge phase, no strong positive or irritant reactions were observed at any time point. Only a few participants experienced transient, minor, doubtful reactions at 48 h, which resolved quickly without any lasting effects. No adverse events or safety concerns were reported during the entire study period.

**Conclusion:** Venusia Cleanser demonstrated a non-irritant and hypoallergenic profile under HRIPT conditions, supporting its safe repeated use, including in individuals with sensitive skin. Being a single-center study, the findings may limit the generalizability of the results to broader and more diverse populations.

**Keywords:** Human repeat insult patch test, Hypoallergenicity, Sensitive skin, Skin irritation, Skin sensitization

### INTRODUCTION

The skin barrier serves as the body's primary defense against environmental insults, pathogens, and chemical irritants, yet it remains vulnerable to disruption through daily exposures and improper skincare practices. Facial skin faces constant challenge from cosmetics, pollutants, and ultraviolet (UV) radiation, making it especially susceptible to irritation and sensitization reactions. Skin irritation, characterized by inflammation, erythema, and compromised barrier function, affects a substantial portion of the population, with studies indicating that up to 50% of individuals report experiencing sensitive skin at some point in their lives.<sup>[1,2]</sup>

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Among the various products used on facial skin, cleansers play a critical role in dermatological care. Although they are crucial for eliminating dirt, sebum, and potential irritants, they can also compromise the stratum corneum's integrity and trigger inflammatory cascades.<sup>[3,4]</sup> Using harsh or inappropriate cleansers can remove natural lipids, disrupt the skin's natural moisturizing factor, and disturb the delicate pH balance essential for maintaining an effective barrier.<sup>[5]</sup> As a result, cleansers must be formulated with mild, hypoallergenic ingredients to minimize the risk of irritation and adverse reactions. Dermatologists are increasingly advising the use of cleansers and moisturizers that are not only gentle and dermatologically approved but also particularly appropriate for sensitive skin.<sup>[6,7]</sup> Sensitive skin is a clinical condition characterized by the self-reported sensory symptoms that include tightness, stinging, burning, tingling, pain, and pruritus.<sup>[1]</sup> Such heightened cutaneous reactivity necessitates the use of well-tolerated skincare products that minimize irritation. This, in turn, enhances patient adherence and consequently improves patient outcomes, as people will be more inclined to stick to regular skincare routines when the products are well-tolerated and do not cause adverse effects.<sup>[8]</sup>

Given the significant proportion of the population affected by skin sensitivity and the recognized need for gentle yet effective cleansing solutions, rigorous evaluation of new formulations is essential. The present study aims to evaluate the skin irritation and hypoallergenic (skin sensitization) potential of Venusia Cleanser by employing standardized dermatological testing methods.

## MATERIALS AND METHODS

### Study design

This single-center, evaluator-blinded study was designed to evaluate the skin irritation and skin sensitization (hypoallergenic) potential of a test product, Venusia Cleanser (Batch No: TR2572A; Dr. Reddy's Laboratories Ltd., Hyderabad, India), using the human repeat insult patch test (HRIPT) technique. The study aimed to compare skin reactions to the test product against baseline and a negative control (0.9% isotonic saline).

Participants were screened before product application using the lactic acid sting test (following a modified version of the method described by Kim *et al.*<sup>[9]</sup>) to identify skin sensitivity status. 10% aqueous lactic acid was randomly applied using cotton on the nasolabial fold and cheek area on the one side of face and water was randomly applied to the contralateral side on the other half of face. 10% aqueous lactic acid and water were blended such that neither the investigator nor the participants knew which was lactic acid and which was water. Readings for stinging were taken after 2.5 min, 5 min, and 8 min (if required), where the participant rated any irritation

on a 4-point scale. Any volunteer getting a cumulative score of (readings at 2/3 time points)  $\geq 3$  was considered positive on the lactic acid sting test (i.e., having sensitive skin) and included in the study. This procedure was used to classify participants into sensitive-skin and normal-skin subgroups; both subgroups were then included in the study.

### Study population

Participants were included if they met the following conditions:

- Healthy male and female participants aged 18–65 years
- Participants are willing to undergo a lactic acid sting test to classify skin as sensitive or normal
- Participants with skin phototypes III (tans after initial burn), IV (burns minimally, tans easily), or V (rarely burns, tans darkly easily), as these phototypes are predominant in the Indian population<sup>[10]</sup>
- Presence of apparently healthy skin at the test site (back).

Participants were excluded if any of the following applied:

- Pregnancy (confirmed by urinary pregnancy test at screening) or lactation
- Presence of scars, tattoos, or excessive terminal hair at the test site
- Dermatological infections or pathologies at or near the test area
- History or current evidence of hypersensitivity or allergy to cosmetics, hair dye, or raw materials relevant to the test product
- Presence of any clinically significant systemic or cutaneous disease that could interfere with study outcomes
- Use of systemic or topical medications within the past month that could affect skin reactivity
- Participant in an exclusion period or participating in another food, cosmetic, or therapeutic trial.

### Study intervention and follow-up

- Test Product: Filter papers soaked with about 0.04 mL of the 1% diluted test product (Venusia Cleanser) were placed in patch chambers and applied occlusively to the participants' backs.

The test product contained the following key ingredients: sodium cocoyl isethionate, allantoin, niacinamide, Hydrosella, provitamin B5 (panthenol), oatmeal extract, and a triple ceramide complex with hyaluronic acid (HA)

- Negative Control: Filter papers soaked with approximately 0.04 mL of 0.9% isotonic saline (which serves as an aqueous-matched negative control) were placed in patch chambers and applied occlusively to the participants' backs.

### Determination of non-irritant concentration

A preliminary assessment to determine the highest non-irritant concentration was conducted on 10 participants. A series of dilutions of the product was applied to the participant's back under occlusive patches for 24 h.<sup>[11]</sup> Reactions were graded at 48 h post-removal using a clinical grading system. The highest concentration producing no irritation in this small cohort was determined as 1% w/w, which was then used for the main HRIPT study.

The study was conducted in three phases, modified from the research institute for fragrance materials standard HRIPT protocol:<sup>[12]</sup>

1. Induction Phase: Nine 24 h occlusive patches of the test product and control were applied on alternating days over a 3-week period. Each patch was removed after 24 h, and the application site was evaluated 48 h later for skin reactions using the Draize scoring scale for erythema/dryness/wrinkles (A) and edema (O) [Table 1].<sup>[13-15]</sup> This phase was designed to assess cumulative irritation and the potential for sensitization.
2. Rest Phase: A 14-day patch-free period followed the induction phase to allow for the resolution of any skin reactions.
3. Challenge (Elicitation) Phase: A single 24-h patch application of the test product and control was applied to new adjacent sites on the participants' backs. Patches were removed after 24 h, and the test sites were evaluated at 48, 72, and 96 h post-removal according to the International Contact Dermatitis Research Group (ICDRG) scoring scale<sup>[16]</sup> to assess allergic sensitization reactions [Table 2].

Participants were advised to avoid water contact, intense UV exposure, and activities causing sweating to maintain patch integrity and avoid confounding variables during the study.

An identical HRIPT protocol was applied across skin types.

Detailed data on the measurements and their respective time points related to the induction and challenge phases are presented in Tables 3 and 4.

Score for erythema/dryness/wrinkles (A)	Score for edema (O)
0=No reaction	0=No reaction
1=Very slight erythema/dryness with shiny appearance	1=Very slight edema
2=Slight erythema/dryness/wrinkles	2=Slight edema
3=Moderate erythema/dryness/wrinkles	3=Moderate edema
4=Severe erythema/wrinkles/scales	4=Severe edema

### Blinding and randomization

The test product and control solutions were coded and labeled by an independent third party (C.L.A.I.M.S Pvt. Ltd., India) to ensure evaluator blinding throughout the study. Participants and investigators were blinded to the product identities.

### Primary outcomes

The primary outcome was to determine the skin irritation and sensitization (hypoallergenicity) potential of the test cleanser in adult men and women using the HRIPT method. The participants' skin was clinically examined, and reactions were scored at each scheduled visit using the Draize and ICDRG scale.

### Statistical analysis

A total of 220 subjects were enrolled to account for potential dropouts. Data were summarized using descriptive statistics. Continuous variables, such as age, were expressed as mean and standard deviation (SD). Categorical variables, including sex distribution and skin sensitivity status, were presented as frequencies. For the induction phase, the mean cumulative irritation scores on the Draize scale were calculated for both the test product (Venusia Cleanser) and the control (0.9% isotonic saline solution). Scores were interpreted according to the relevant Bureau of Indian Standards - Indian Standard (BIS - IS) 4011: 2018 Methods of Test for Safety Evaluation of Cosmetics (Third Revision) guidelines criteria to assess irritation potential. In the challenge phase, frequencies of each reaction grade were tabulated according to the ICDRG scoring system. No inferential statistical analyses were conducted, as the primary objective was to assess safety

**Table 2:** International Contact Dermatitis Research Group scale for scoring the challenge phase reactions.

Symbol	Morphology	Interpretation
-	No reaction	Negative reaction
?	Erythema only, no infiltration	Doubtful reaction
+	Erythema, infiltration, possibly discrete papules	Weak positive
++	Erythema, infiltration, papules, vesicles	Strong positive
+++	Erythema, infiltration, confluent vesicles	Extreme positive
Ir	Different types of reactions (soap effect, vesicles, blister, necrosis)	Irritant
- : Negative reaction, ?: Doubtful reaction, +: Weak positive reaction, ++: Strong positive reaction, +++: Extreme positive reaction, Ir: Irritant reaction		

**Table 3:** Measurement timepoints for the induction phase.

Procedure/ Assessment	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19
	D1	D2	D3	D4	D5	D6	D8	D9	D10	D11	D12	D13	D15	D16	D17	D18	D19	D20	D21
Explaining trial procedures to participants	X																		
Written informed consent	X																		
Inclusion/ Exclusion criteria	X																		
Demographic information	X																		
Medical history/ Prior treatments	X																		
Clinical examination	X																		
Patch application	X		X		X		X		X		X		X		X		X		
Patch removal		X		X		X		X		X		X		X		X		X	
Concomitant treatment recording	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Reading skin reaction			X		X		X		X		X		X		X		X		X
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

D: Day, V: Visit

**Table 4:** Measurement timepoints for the challenge phase.

Procedure/Assessment	V20 (0 h)	V21 (24 h)	V22 (48 h)	V23 (72 h)	V24 (96 h)
Patch application	X				
Patch removal		X			
Concomitant treatment recording	X	X	X	X	X
Reading skin reaction			X	X	X
Adverse events	X	X	X	X	X

V: Visit

and sensitization qualitatively based on observed reactions. Adverse events were monitored and reported descriptively throughout the study.

**Ethical considerations**

The protocol followed all relevant ethical and regulatory guidelines, including the BIS (IS 4011: 2018 Methods of Test for Safety Evaluation of Cosmetics (Third Revision) guidelines), ICMR (2017) guidelines, ICH E6(R3) Good Clinical Practice (2016), Good Clinical Laboratory Practices, the Declaration of Helsinki (2013), and other applicable regulations. Ethical clearance was obtained from an Independent Ethics Committee (Re-Registration number:

ECR/245/Indt/MH/2015/RR-22). The trial was registered on the Clinical Trial Registry of India (CTRI/2025/06/088112). All participants provided written informed consent after full disclosure of study objectives, procedures, benefits, and risks.

**RESULTS**

A total of 220 participants were recruited, of whom 218 participants completed the study. Two participants were lost to follow-up.

**Demographic and baseline data**

Among the 218 participants, the average age of the participants was 38.84 years (SD: 10.32). There were 12 males

and 206 females in the study. Based on the lactic acid sting test, 78 participants (36%) had sensitive skin, while 140 participants (64%) had normal skin.

### Induction phase reaction

The mean cumulative score for Venusia Cleanser was well below 2, indicating the product is non-irritant during repeated use [Table 5].

### Challenge phase

Challenge phase reactions were scored using the ICDRG scale [Table 6]. No strong positive reactions (++) or higher were observed at any time point for Venusia Cleanser. A few ( $n = 38$ , 17%) participants showed doubtful and weak-positive reactions at 48 h, which were resolved by 72 and 96 h, indicating transient and mild skin responses. The challenge phase thus demonstrated no meaningful sensitization.

Representative HRIPT images from participants with normal skin [Figure 1] and sensitive skin [Figure 2] show no visible reactions for Venusia Cleanser versus negative control during induction (patch application at visit 1 and scoring after

9<sup>th</sup> application) and challenge (application and 96-h scoring) phases. Images were captured only for reactions with Grade ++ or higher.

### Adverse events

No adverse events were reported during the entire duration of the study.

## DISCUSSION

This study demonstrates that Venusia Cleanser is non-irritant even after nine induction applications and a challenge exposure and is hypoallergenic, with no strong positive skin reactions or adverse events observed. This suggests that there is no significant sensitization potential in a diverse population, including individuals with sensitive skin. The mean cumulative irritation score of 0.08 recorded for Venusia Cleanser during the induction phase was significantly below the non-irritant threshold of 2.0, as specified by the BIS - IS 4011: 2018 Methods of Test for Safety Evaluation of Cosmetics (Third Revision) guidelines. This suggests that repeated occlusive application of the cleanser did not result in clinically significant irritation.

The non-irritant profile of Venusia Cleanser is particularly important for patients with sensitive skin, who constituted 36% of the study cohort. Sensitive skin poses distinct challenges due to complex interactions between climate, lifestyle, and traditional skincare practices.<sup>[6]</sup> Dermatologists and cosmetic scientists define “sensitive skin” as skin that is more prone to contact irritants or allergic reactions compared to the general population.<sup>[17]</sup> In an Indian survey involving 3012 participants, 27.9% of men and 36.7% of women reported having “sensitive” or “very sensitive” skin.<sup>[18]</sup> The underlying causes of sensitive skin are not well understood and may involve neurosensory dysfunction or a compromised skin barrier.<sup>[19]</sup>

The lactic acid sting test in this study identifies sensitive skin through a rigorous methodological approach to selecting at-risk individuals.<sup>[9]</sup> Lactic acid sting test identifies those with compromised skin barriers rather than relying on self-reporting alone. By ensuring the study included participants who were identified as having sensitive skin through this validated test, the design provided the framework to confirm that the test product does not compromise barrier function in individuals with this pre-existing vulnerability.

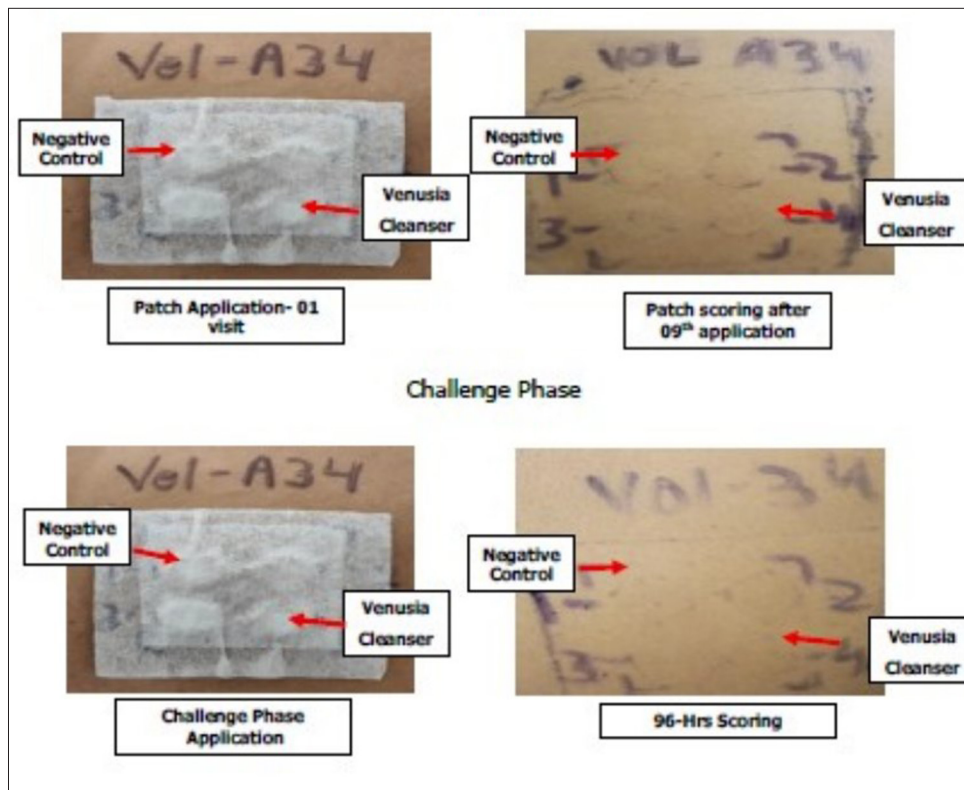
Ensuring that facial cleansers are hypoallergenic is an essential safety feature, as cosmetics and personal care items are increasingly identified as significant triggers of allergic contact dermatitis. Common cosmetic allergens include fragrances, preservatives, and certain surfactants, which can cause delayed-type (type IV) hypersensitivity reactions, particularly in people with sensitive or compromised skin.

**Table 5:** Scores for the induction phase for the test product and saline.

Product	Mean cumulative score (n=218)	Median	Range
Venusia Cleanser	0.08	0.00	0.00–6.00
0.9% Isotonic saline solution	0.00	0.00	0.00–0.00

**Table 6:** Scores for the challenge phase for the test product and saline.

Reaction category	48 h (n=218 participants)	72 h (n=218 participants)	96 h (n=218 participants)
0.9% Isotonic saline solution	218 had negative reactions	218 had negative reactions	218 had negative reactions
Venusia Cleanser			
Negative reaction (-)	180	217	218
Doubtful reaction (?)	37	1	0
Weak positive (+)	1	0	0
Strong positive (++)	0	0	0
Extreme positive (+++)	0	0	0
Irritant (Ir)	0	0	0



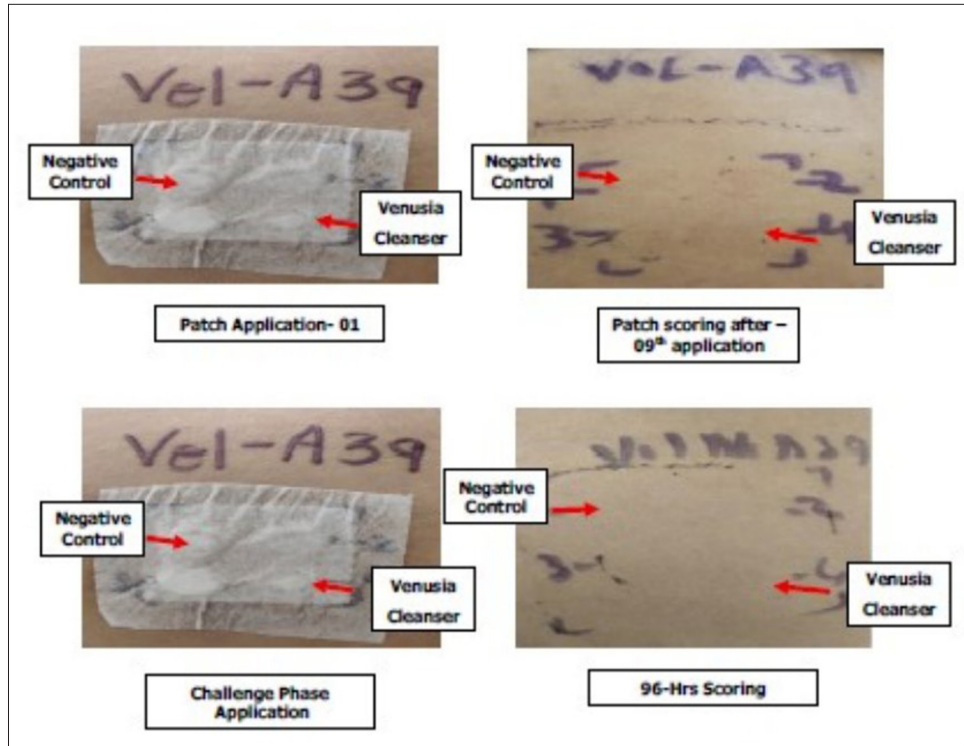
**Figure 1:** Representative human repeat insult patch test images on normal skin showing no irritation or sensitization from Venusia Cleanser versus negative control during induction (9<sup>th</sup> application) and challenge (96 h score) phases.

Therefore, cosmetic products designed for dry sensitive skin should be subjected to thorough testing to confirm their hypoallergenic properties.<sup>[6]</sup>

The assessment of potential sensitization through the HRIPT is an important component of this study's safety evaluation. Unlike irritant contact dermatitis, which occurs on first exposure and is dose-dependent, allergic contact dermatitis develops only in individuals who have been previously sensitized.<sup>[20]</sup> The HRIPT is considered the gold-standard clinical method for evaluating sensitization potential<sup>[21]</sup> because it encompasses the two critical stages necessary for the development of allergic contact dermatitis: The induction phase (where the immune system becomes sensitized to potential allergens through repeated exposure) and the challenge phase (where sensitized individuals exhibit visible allergic reactions upon re-exposure). In this study, the absence of strong positive reactions to Venusia Cleanser at any assessment timepoint, combined with the rapid resolution of minor doubtful and weak-positive reactions observed in 38 participants at 48 h, strongly indicates that the cleanser does not contain significant sensitizing agents.

Venusia Cleanser contains several complementary ingredients that together promote gentle cleansing, barrier

repair, and anti-inflammatory benefits. Sodium cocoyl isethionate, a syndet surfactant, is notably gentler on the skin barrier compared to traditional soaps. An *in vitro* study has demonstrated that its relatively large micelles have limited penetration into the skin's aqueous pores, leading to less barrier disruption and irritation.<sup>[22]</sup> Allantoin exhibits antioxidant, anti-inflammatory, antimicrobial, and keratolytic properties, aiding the transition of skin from a prolonged inflammatory state to the proliferative phase by promoting extracellular matrix synthesis, granulation tissue formation, and re-epithelialization.<sup>[23]</sup> Niacinamide has been clinically proven to enhance barrier function, reduce transepidermal water loss, increase stratum corneum hydration, and decrease inflammation and hyperpigmentation, making it suitable for sensitive and diseased skin.<sup>[24]</sup> Panthenol (provitamin B5) improves hydration, supports barrier lipid synthesis, and accelerates recovery of irritated skin. It also shows protective effects against experimentally induced irritation; dexpanthenol cream pretreatment significantly reduces stratum corneum barrier damage compared to untreated skin.<sup>[25]</sup> Colloidal oatmeal extract provides antioxidant and anti-inflammatory activity and has demonstrated significant reductions in erythema, itch, and xerosis in patients with eczematous and dry skin.<sup>[26]</sup> Finally, topical formulations



**Figure 2:** Representative human repeat insult patch test images on sensitive skin showing no irritation or sensitization from Venusia Cleanser versus negative control during induction (9<sup>th</sup> application) and challenge (96 h score) phases.

containing ceramides and HA improve water retention and restore barrier function by replenishing intercellular lipids and binding water within the stratum corneum, which is clinically associated with improved hydration and reduced dryness.<sup>[27]</sup>

From a clinical and patient-centered perspective, the daily use of a hypoallergenic, non-irritant facial cleanser is crucial for maintaining skin homeostasis and preventing dermatological complications in real-world settings. Regular cleansing effectively removes excess sebum, environmental pollutants, particulate matter, and residual makeup, thereby reducing the risk of follicular occlusion and subsequent inflammatory conditions such as acne vulgaris.<sup>[17]</sup> This practice is particularly relevant in urban environments where exposure to atmospheric pollutants contributes to oxidative stress and accelerated skin aging.<sup>[28]</sup> The hypoallergenic and non-irritant formulation minimizes the potential for contact sensitization or exacerbation of sensitive skin conditions, thus encouraging long-term adherence to a cleansing regimen without causing the barrier disruption or dryness often associated with harsher surfactants.<sup>[3]</sup>

The present study has several strengths. It used a standardized HRIPT methodology under stringent exposure conditions with evaluator blinding and a negative control. This enhances internal validity and supports robust assessment of the

irritation and sensitization potential of the product. The inclusion of participants with sensitive skin, as determined by the lactic acid sting test, further enhances the clinical applicability of the results for real-world users who may be more susceptible to skin reactions.

### Study limitations

This study also has limitations that should be acknowledged. As a single-center study, the findings may limit the generalizability of the results to broader and more diverse populations. Furthermore, the controlled and relatively short-term exposure period does not fully reflect long-term, real-world usage patterns or the concurrent use with other topical products.

### CONCLUSION

Under HRIPT conditions, Venusia Cleanser exhibited a mean cumulative irritation score well below 2, with no strong positive reactions, no signs of clinically significant sensitization, and no adverse events throughout the study. This evidence supports classifying Venusia Cleanser as a non-irritant, hypoallergenic cleanser suitable for repeated use, even for individuals with sensitive skin.

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**Ethical approval:** The research/study was approved by the Institutional Review Board at the Independent Ethics Committee, number ECR/245/Indt/MH/2015/RR-22, dated April 04, 2025.

**CTRI number:** CTRI/2025/06/088112.

**Declaration of patient consent:** The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given consent for their images and other clinical information to be reported in the journal. The patient understands that the patient's names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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