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Original Article

Spray-dried Curcumin–Piperine Dry Powder Inhaler Incorporating Suvarnasindoor: Characterization and Acute Pulmonary Tolerability

Journal of Applied Pharmaceutical Science
16(8) 666–677, 2026
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DOI: 10.1177/22313354261460692
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Abstract

The objective of this research was to formulate a respirable dry powder inhaler formulation of Suvarnasindoor, a classical herbomineral formulation, co-formulated with curcumin and piperine. The formulation was optimized using a spray-drying method (F10) and a ball milling method (F6), wherein mannitol and lactose were used as excipients. The formulation was characterized using scanning electron microscopy, X-ray photoelectron spectroscopy, and inductively coupled plasma-atomic emission spectrometry to ascertain that it had a spherical morphology (2–5 µm), was in a stable crystalline phase of α-HgS without any organic mercury complexes. The aerodynamic performance of the formulation was tested using an Andersen Cascade Impactor to evaluate its FPF (31.85% for Suvarnasindoor, 38.56% for Curcumin, 38.86% for Piperine) and mass median aerodynamic diameter (3.971 µm for Suvarnasindoor; 5.593 µm for Curcumin and 5.556 µm for Piperine), which may be suitable for dry powder inhaler delivery to the lungs. The chemical integrity of all phytoconstituents of the formulation was also tested using liquid chromatography–mass spectrometry and differential scanning calorimetry. Acute intratracheal toxicity studies in rats were conducted to further evaluate its safety profile. The results showed no mortality or signs of toxicity in rats at a dose equivalent to humans, thus demonstrating acceptable tolerability under acute exposure conditions. Physicochemical stability was evaluated per ICH Q1A (R2) guidelines over 6 months under real-time and accelerated conditions. These findings collectively suggest that the spray-dried Suvarnasindoor DPI may serve as a feasible formulation approach for pulmonary delivery, warranting further investigation in inflammatory respiratory conditions.

Keywords

Suvarnasindoor, α-HgS, herbo-mineral formulation, dry powder inhaler, spray-drying, pulmonary drug delivery

Received 23 March 2026; **accepted** 14 May 2026

Introduction

Respiratory diseases—including chronic obstructive pulmonary disease (COPD), asthma, pulmonary tuberculosis, and other inflammatory airway conditions—represent a major global health burden, contributing substantially to morbidity and mortality worldwide.[1] Their pathophysiology is characterized by chronic airway inflammation mediated by pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin 1 beta (IL-1β), and interleukin-6 (IL-6), sustained activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and progressive oxidative stress that collectively impair lung function and epithelial integrity.[2] Current pharmacological management relies primarily on bronchodilators and corticosteroids, which provide symptomatic relief but do not adequately address the underlying oxidative stress and immune dysregulation that sustain disease progression.[3] This therapeutic gap has generated considerable interest in multi-target approaches, including those derived from traditional medicine systems with established anti-inflammatory, antioxidant, and immunomodulatory activity.

Ayurveda has employed herbomineral formulations for the management of chronic respiratory and inflammatory

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disorders for centuries, with their preparation and application systematically documented in classical texts including the Charaka Samhita and Rasa Ratna Samuccaya.[4] A unique class of these preparations, known as Bhasmas, is produced through standardized metallurgical-pharmaceutical processes termed Śodhana (purification) and Māraṇa (incineration). These multi-step procedures involve repeated trituration with herbal media and controlled thermal processing, which convert raw metals and minerals into biocompatible therapeutic forms by altering their physicochemical properties, reducing elemental reactivity, and improving biological compatibility.[5] Critically, for mercury-containing preparations, this transformation converts raw, highly toxic elemental mercury into the thermodynamically stable crystalline α-HgS (cinnabar) phase.[6] The resulting structure exhibits extremely low aqueous solubility (Ksp ~4×10^{-54}) and high covalent Hg–S bond strength, fundamentally limiting the release of ionic or organic mercury species.[7] This speciation-dependent safety profile is the scientific basis upon which mercury-based Ayurvedic preparations are distinguished from raw mercury—a distinction that must be assessed through chemical speciation and bioavailability data rather than total metal content alone.[6,8]

Suvarnasindoor is a classical herbomineral preparation composed of purified mercury, sulfur, and gold (16:16:1) processed via the *Kupipakwa* method.[9] Classified as a Rasayana, it has been traditionally indicated for immunomodulation, respiratory weakness, and conditions characterized by airway inflammation.[4,5] Analytical characterization using X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), extended X-ray absorption fine structure (EXAFS), and inductively coupled plasma atomic emission spectrometry (ICP-AES) has confirmed >99% α-HgS phase purity.[6,7,10] Despite its documented traditional use, to the best of our knowledge, no prior study has attempted to engineer Suvarnasindoor into an inhalable DPI system—a gap this investigation addresses for the first time. Curcumin and piperine were selected as co-formulants based on a clearly defined pharmacological and physicochemical rationale. Curcumin, a polyphenol from *Curcuma longa*, inhibits NF-κB signaling, suppresses pro-inflammatory cytokine production, and scavenges reactive oxygen species—directly targeting the molecular mechanisms implicated in inflammatory airway disease.[11,12] Clinical evidence further supports its anti-inflammatory activity in respiratory conditions, including asthma.[13] Piperine, an alkaloid from Piper nigrum, augments membrane permeability and inhibits hepatic and intestinal glucuronidation, significantly enhancing the systemic bioavailability of co-administered curcumin.[14] Recent HgS nanoparticle-based formulations have demonstrated acceptable safety profiles in preclinical anti-inflammatory models, albeit via a different route and indication.[15] Furthermore, curcumin's β-diketone moiety facilitates stable metal–ligand chelation,[16] which may support physicochemical interaction with the gold-containing Suvarnasindoor matrix within the pulmonary microenvironment.

The pulmonary route was selected to exploit the lung's large absorptive surface area (~70–100 m^2), ultra-thin alveolar epithelium, and extensive vasculature, enabling rapid local drug action at substantially lower doses while avoiding first-pass metabolism.[17] Among pulmonary delivery devices, DPIs offer superior drug stability in the solid state, absence of propellants, and patient convenience.[17,18] Effective pulmonary deposition requires particles with a mass median aerodynamic diameter (MMAD) of 1–5 μm and minimal interparticulate cohesion to ensure efficient de-agglomeration under inspiratory airflow.[18] Conventional top-down milling of herbomineral preparations typically yields irregular, agglomerated particles with poor flowability and limited aerosolization efficiency.[18,19] Spray-drying technology overcomes these limitations through a bottom-up approach, producing composite microparticles with controlled spherical morphology, tunable aerodynamic diameter, and optimized surface properties in a single processing step.[19,20] Incorporation of mannitol as a stabilizing carrier further enhances moisture protection and ensures consistent powder dispersion at clinically relevant inspiratory flow rates.[20]

The primary objective of the present study was therefore to develop and characterize, for the first time, a respirable DPI formulation of Suvarnasindoor co-formulated with curcumin and piperine using spray-drying (F10); to compare its physicochemical properties, mercury speciation integrity, and aerosolization performance against a conventional ball-milled formulation (F6); and to evaluate its acute pulmonary tolerability in rats via the intratracheal route. By integrating modern particle engineering, orthogonal analytical characterization, and structured toxicological assessment, this investigation seeks to provide a scientifically grounded and regulatory-informed basis for the pulmonary delivery of this classical herbomineral preparation.[18,19,21]

Materials and Methods

Materials

Curcumin and piperine were sourced from Akay Natural Ingredients (Cochin) and Sigma–Aldrich (USA). Suvarnasindoor (3–5 μm, classical Kupipakwa), Inhalac® 300 lactose (Meggle, Germany), mannitol IP, and magnesium stearate (DPI-grade, SAVA Healthcare) were used as received.

Formulation Preparation

Composition

Each unit dose capsule of formulations F01 to F06 contained curcumin (5.0 mg), piperine (1.0 mg), Suvarnasindoor (1.56 mg), lactose monohydrate/Inhalac® 300 (16.94 mg), and magnesium stearate (0.5 mg), yielding a total fill weight of 25.0 mg per capsule. Each unit dose capsule of formulations F07–F10 contained curcumin (5.0 mg), piperine (1.0 mg), Suvarnasindoor (1.56 mg), mannitol IP (26.94 mg), and magnesium stearate (0.5 mg), yielding a total fill weight of 35.0 mg per capsule.

Ball-milled Formulation (F6)

Curcumin, piperine, lactose, and magnesium stearate were processed in a planetary ball mill (900 rpm, 90 min) using a 10:1 ball-to-powder mass ratio—selected based on established pharmaceutical milling protocols providing sufficient energy for dense mineral particles without thermal degradation of heat-sensitive actives.[22,23] Suvarnasindoor (1.56 mg) was incorporated post-milling by geometric mixing to preserve the α-HgS phase. The optimization trials, as well as the results, are presented in Table 1.

Spray-dried Microparticles (F10)

Curcumin and piperine were dissolved in ethanol; mannitol was dissolved in purified water. Spray-drying (Büchi B-290): inlet 120°C–140°C, outlet 65°C–80°C, feed rate 2–5 mL/min, aspiration 100%. The inlet temperature range maintained a >50°C safety margin below curcumin's differential scanning calorimeter (DSC)-confirmed degradation onset (~190°C), preserving phytoconstituent integrity.[19] Suvarnasindoor was added post-spray-drying by geometric dilution; XRD confirmed no phase change. Stored in airtight containers at room temperature (<25°C, <60% RH)

Pre-formulation Characterization

Identity, phase purity, and structural integrity of Suvarnasindoor, curcumin, and piperine were confirmed by laser diffraction, XRD, Raman spectroscopy, ICP-AES, XPS, EXAFS at RRCAT, Indore, India and Karl Fischer titration.[6,7,24,25]

Formulation Study

Appearance, pH, bulk/tapped density, moisture content, yield, particle size distribution, scanning electron microscopy (SEM) morphology, zeta potential, and flowability were assessed for F6 and F10. Mannitol was preferred over lactose for its lower hygroscopicity and reduced capsule residue.[20,26]

HPLC and ICP-AES Analysis

Curcumin and piperine were quantified by RP-HPLC (Vanquish; 250 × 4.6 mm, 5 µm; mobile phase: KH_2PO_4 pH 3.5/acetonitrile 40:60 v/v; 1.0 mL/min; 353 nm). Mercury and sulfur in Suvarnasindoor were quantified by ICP-AES at SAIF, IIT Powai, Bombay.[24]

In Vitro ACI

Aerosolization was evaluated using an aerodynamic performance (ACI) (Copley Scientific TPK2100) per USP <601>. Ten capsules were actuated individually through a HandiHaler® at 90 L/min for 4 s, resulting in a total sampled volume of 6 L, USP <601> compliant.[27,28] The flow rate of 90 L/min was selected to ensure complete de-aggregation of high-density α-HgS particles (ρ = 8.1 g/cm³), consistent with validated ACI protocols for high-dose DPIs.[29,30] ED, FPF, and MMAD were calculated using CITDAS software.[31,32]

Formulation Characterization (F10)

SEM (JEOL JSM 6360A with EDX), ultraviolet–visible spectroscopy (UV–vis) spectroscopy (200–800 nm), DSC (Mettler Toledo; 30°C–450°C at 10°C/min, N_2), XPS (XPS analysis was performed at NCL Pune (Thermo Scientific K-Alpha); results were independently verified by ICP-AES at SAIF, IIT Bombay,[24] and liquid chromatography–mass spectrometry (LC–MS) (reverse-phase, ESI+; *m/z* identification of curcumin and piperine against standards)[33] were performed at Venture laboratory at Pune.

Table 1. Optimization Trials for Ball-milling and Spray-drying Parameters in DPI Formulation Development.

Ball-milling Trials (F1–F6)					
Trial	Carrier	Speed	Grinding Time		Result Based on Particle Size
F1	Lactose	500 rpm	15 min		MMAD 5.8 µm (too large)
F2	Lactose	500 rpm	30 min		MMAD 5.2 µm (still large)
F3	Lactose	700 rpm	45 min		MMAD 4.8 µm (better)
F4	Lactose	700 rpm	60 min		MMAD 4.5 µm (good)
F5	Lactose	900 rpm	90 min		MMAD 4.1 µm (optimal) without Suvarnasindoor
F6	Lactose	900 rpm	90 min		Final formulation prepared using optimized F5 conditions with Suvarnasindoor (1.56 mg) added post-milling by geometric dilution.
Spray-drying Trials (F7–F10)					
Trial	Carrier	Inlet Temp	Yield (%)	Moisture (%)	Result
F7	Lactose	120°C	45	6.2	High moisture
F8	Lactose	140°C	52	5.1	Still high
F9	Mannitol	140°C	68	2.8	Optimal
F10	Mannitol	120°C	68	2.1	Final formulation

Note: For formulations F5 and F6, identical milling parameters (900 rpm, 90 min) were intentionally maintained; the only distinction was the post-milling incorporation of Suvarnasindoor in F6 to preserve the crystalline integrity of α-HgS.

In Vivo Toxicity

Acute toxicity of F10 was assessed in rats (IAEC: SC/IAEC/2025/026).[34,35] Human equivalent dose (6.3 mg/kg in 0.1 mL saline) was administered intratracheally under anesthesia; following an institutional acute intratracheal tolerability protocol guided by Driscoll et al.[34] The 14-day observation period was aligned with the post-exposure monitoring for acute pulmonary toxicity studies. Two-way ANOVA with Tukey's post-test was used. Statistical analysis was performed using GraphPad Prism v9.0 (GraphPad Software, USA).

Stability Studies

Stability was evaluated per ICH Q1A (R2)[36] at long-term (25°C ± 2°C, 60% ± 5% RH) and accelerated (40°C ± 2°C, 75% ± 5% RH) conditions for 6 months, assessing assay recovery, pH, moisture content, particle size, and zeta potential.

Results and Discussion

Pre-formulation

Laser diffraction showed Suvarnasindoor D90 = 4.2 µm, confirming micronized suitability for inhalation (Table 2). ICP-AES confirmed 47.9% w/w Hg and 45.1% w/w S, corresponding to an approximate Hg:S weight ratio of 1.06:1 (approximately 1:1, w/w). XRD confirmed >99% α-HgS phase purity (Table 2). Raman spectroscopy showed characteristic Hg–S vibrations at 254.18, 289.85, and 343.25 cm^{-1} (Figure 1a). XPS and EXAFS verified Hg^{2+}–S^{2-} bonding, with no evidence of elemental Hg^0 or organic mercury (Figures 1c and 1d; Table 3).[6,7] The identification of the α-HgS phase is therefore supported by the complementary XRD, Raman, XPS, and EXAFS findings. EDX is a semi-quantitative, surface-sensitive technique and may be influenced by atomic-number-related matrix effects, whereas ICP-AES provides quantitative bulk elemental composition. Therefore, differences between the EDX and ICP-AES values may reflect the different analytical principles and sampling characteristics of the two techniques.

Table 2. Pre-formulation Study Parameters (Suvarnasindoor α-HgS).

Analysis	Method	Result
Particle size (D90)	Laser diffraction	4.2 µm
Mercury content	ICP-AES	47.9% w/w
Sulfur content	ICP-AES	45.1% w/w
Hg:S weight ratio (approx.)	ICP-AES	~1:1 w/w
Phase purity	XRD	>99% α-HgS
Hg oxidation state	XPS	Hg^{2+} only
Hg–S bond length	EXAFS	2.37 Å
Raman peaks (cm^{-1})	Raman	254.18, 289.85, 343.25
Moisture content	Karl Fischer	2.1% w/w

Note: *n* = 3; mean values reported.

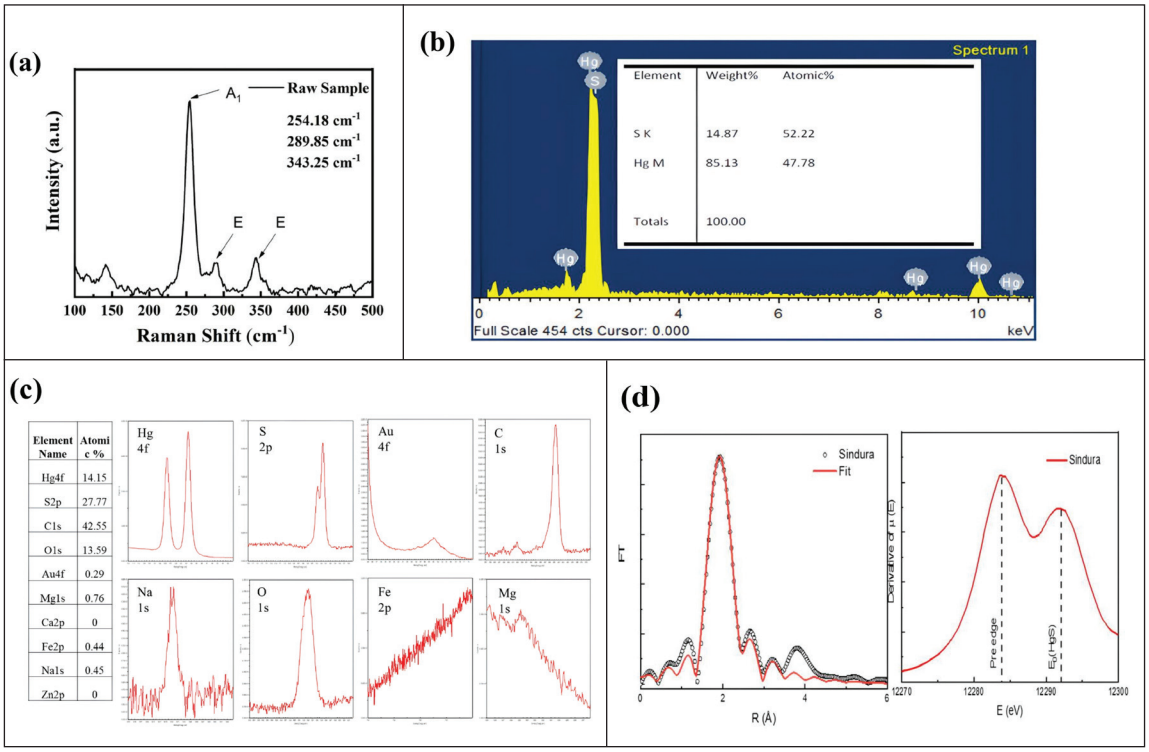

Figure 1. Physicochemical characterization of Suvarnasindoor (α-HgS): (a) Raman spectrum, (b) EDX spectrum showing semi-quantitative surface elemental composition, (c) XPS spectra, and (d) EXAFS spectra. Bulk quantitative Hg and S contents were determined separately by ICP-AES.

Formulation Study

Spray-drying (F10) improved micromeritic properties compared to ball milling (F6) (Table 4). F10 exhibited lower bulk/tapped density, 2.1% moisture, zeta potential of −20 mV, and spherical particle size range of 2–5 µm as confirmed by SEM (Section "SEM and UV–Vis"), with a minority of particles extending to 8 µm as observed by dynamic light scattering. F6 produced irregular agglomerates with broader size distribution and higher moisture content.

Content Uniformity

F10 demonstrated %RSD of 0.88%, 0.92%, and 0.96% for curcumin, piperine, and Suvarnasindoor—all within USP <905> (<5% RSD).[37] F6 showed %RSD of 4.05%, 5.67%, and 3.10%; piperine exceeded the threshold due to differential attrition of soft organic versus hard inorganic particles during ball milling. One-way ANOVA confirmed significantly superior content uniformity for F10 ($p < .0001$, >5-fold improvement). RP-HPLC and ICP-AES validation showed $r^2 \geq 0.999$, acceptable linearity, repeatability, and recovery across all analytes (Tables 5 and 6). Representative HPLC chromatograms of F6. Mixed Standard, and F10 are shown in figure 2, confirming chromatographic resolution and specificity.[24]

Aerodynamic Performance

F10 demonstrated markedly superior ACI versus F6 per USP <601>[37] (Table 7 and Figure 3). F10 FPF values—38.56%

Table 3. Comparative Hg–S Bond Lengths (Å): EXAFS Analysis.

Sample	Hg–S1 (Å)	Hg–S2 (Å)	Hg–S3 (Å)
α-HgS theoretical	2.36	3.10	3.30
Red α-HgS (lab)	2.36	3.10	3.30
Rasasindoora	2.34	3.02	3.31
Suvarnasindoor	2.37	3.14	3.33

Table 4. Formulation Study Parameters: F6 Versus F10.

Parameter	F6 (Ball-milled)	F10 (Spray-dried)
Appearance	Orange fine powder	Spherical orange soft powder
pH	7.8	7.6
Bulk density (g/cm³)	1.01	0.76
Tapped density (g/cm³)	1.25	0.81
Moisture (%)	2.9	2.1
Zeta potential (mV)	−16	−20
Polydispersity index (PDI)	0.1317	0.1286
Assay: Curcumin/Piperine/SS (%)	103.97/120.71/105.64	99.13/98.52/98.86
%RSD: Curcumin/Piperine/SS	4.05/5.67/3.10	0.88/0.92/0.96

Notes: SS: Suvarnasindoor. F6 piperine assay 120.71% fails 90%–110% USP criterion. *F10 Suvarnasindoor at 98.86% is within the pharmacopoeial 90%–110% criterion; slightly elevated values reflect inherent mineral phase density variability and ICP-AES back-calculation bias for α-HgS stoichiometric units.[24]

Table 5. RP-HPLC Analytical Method Validation: Curcumin and Piperine.

Parameter	Criteria	Curcumin	Piperine
Linearity (r^2)	≥0.99	0.999	0.9996
LOD/LOQ (µg/mL)	–	0.40/1.21	1.61/4.87
Repeatability (%RSD)	<2%	0.10%	0.09%
Interday precision (%RSD)	<2%	0.23%	0.22%
Specificity (peak purity)	1.000	1.000	1.000
Assay (% label claim)	90%–110%	98.64%	99.70%

Table 6. ICP-AES Validation: Suvarnasindoor (Hg and S).

Parameter	Specification	Hg	S
Calibration range	0.1–10 mg/L	R^2 = 0.9998	R^2 = 0.9997
Recovery (%)	95%–105%	98.5%	99.2%
%RSD (n = 3)	<5%	2.1%	2.3%
LOD/LOQ	<0.1/0.5 µg/L	0.08/0.25 µg/L	0.09/0.28 µg/L

Table 7. ACI aerodynamic performance comparison of F6 and F10.

	Curcumin		Piperine		Suvarnasindoor	
Metric	F6	F10	F6	F10	F6	F10
% FPF (≤5.0 µm)	16.89	38.56	1.66	38.86	10.20	31.85
MMAD (µm)	5.609	5.593	7.235	5.556	5.504	3.971
Assay (%)	103.97	99.13	120.71	98.52	105.64	98.86
Device residue%	55.19	42.90	11.74	8.81	44.80	21.08
Geometric standard deviation (GSD)	–	1.12	–	1.18	–	1.16
Mass balance (%)	–	88.86	–	75.9	–	87.7

Note: Single determination under standardized conditions per USP <601>.

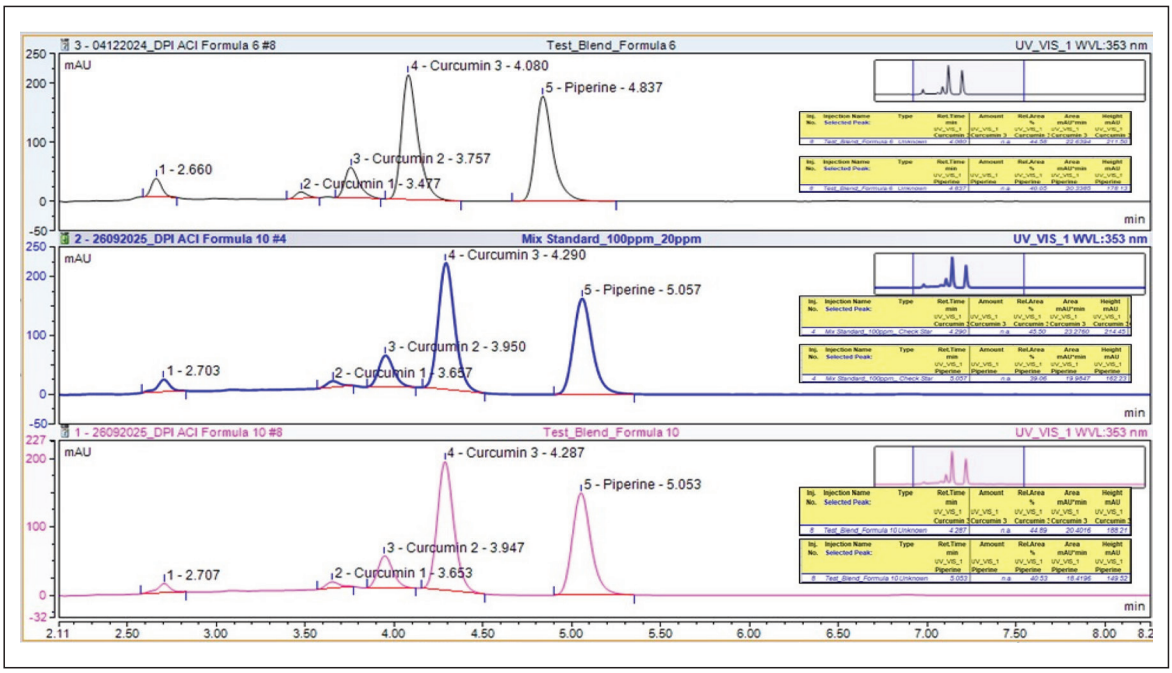

Figure 2. Comparative HPLC Chromatograms of Formulation F6, Mixed Standard (100 ppm/20 ppm), and Formulation F10.

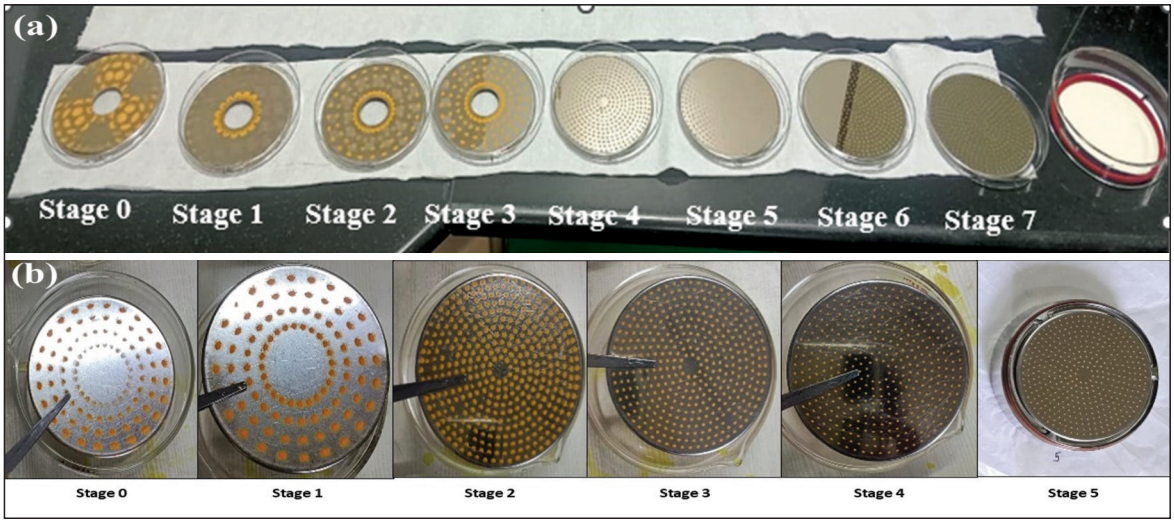

Figure 3. Aerosolization Performance Comparison Between (a) Formulation F6 and (b) Formulation F10 Across Various Stages.

(curcumin), 38.86% (piperine), 31.85% (Suvarnasindoor)—indicating good aerosolization performance consistent with spray-dried DPI formulation.[38] The MMAD of Suvarnasindoor (3.97 µm) falls within the optimal 1–5 µm range for alveolar deposition, while curcumin (5.59 µm) and piperine (5.56 µm) MMADs are marginally above 5 µm. Particles in the 5–7 µm range deposit in bronchial and central airways—therapeutically relevant targets for anti-inflammatory agents in COPD and asthma—and the conducting airway remains a primary site of NF-κB-mediated inflammation.[28,38,39] Co-localization of curcumin and piperine at near-identical aerodynamic diameters ensures synchronized deposition and preservation of the bioavailability enhancement at the site of action.[14] Device residue of 21.08% for Suvarnasindoor reflects the high density of the α-HgS mineral phase (ρ = 8.1 g/cm³), which increases gravitational settling and device adhesion relative to organic phytoconstituents, consistent with published data on high-density inorganic particles in capsule-based DPIs.[40,41] Although F10 produced an FPF of 31.85% for Suvarnasindoor, the piperine mass balance was lower (75.9%) than that of curcumin (88.86%) and Suvarnasindoor (87.7%). This lower recovery may be attributable to the cohesive and hydrophobic nature of the multi-constituent herbo-mineral formulation and possible retention within the device/impactor assembly. Therefore, the piperine ACI recovery should be interpreted cautiously, and further method optimization with replicate ACI recovery assessment is warranted.[31,32]

SEM and UV–Vis Spectroscopy

SEM confirmed spherical microparticles of 2–5 µm with smooth surfaces for F10, compared to irregular angular agglomerates in F6 (Figure 4a–4h). Uniform particle formation during spray-drying minimizes surface irregularities and maximizes dispersion properties.[19,21,27] UV-vis analysis showed a slight band gap increase from 2.00 eV to 2.04 eV after ball milling (Figure 4i), confirming particle size reduction.

Differential Scanning Calorimeter

Pure curcumin exhibited a sharp endothermic melting peak at 178°C–183°C; pure piperine at 128°C–132°C, confirming crystallinity (Figure 5). F10 showed peaks at 130°C (piperine), 167°C (mannitol), and 180°C (curcumin) with slight shifts and reduced intensity, indicating partial amorphous character. No degradation events were detected, confirming appropriate spray-drying temperature selection.[19]

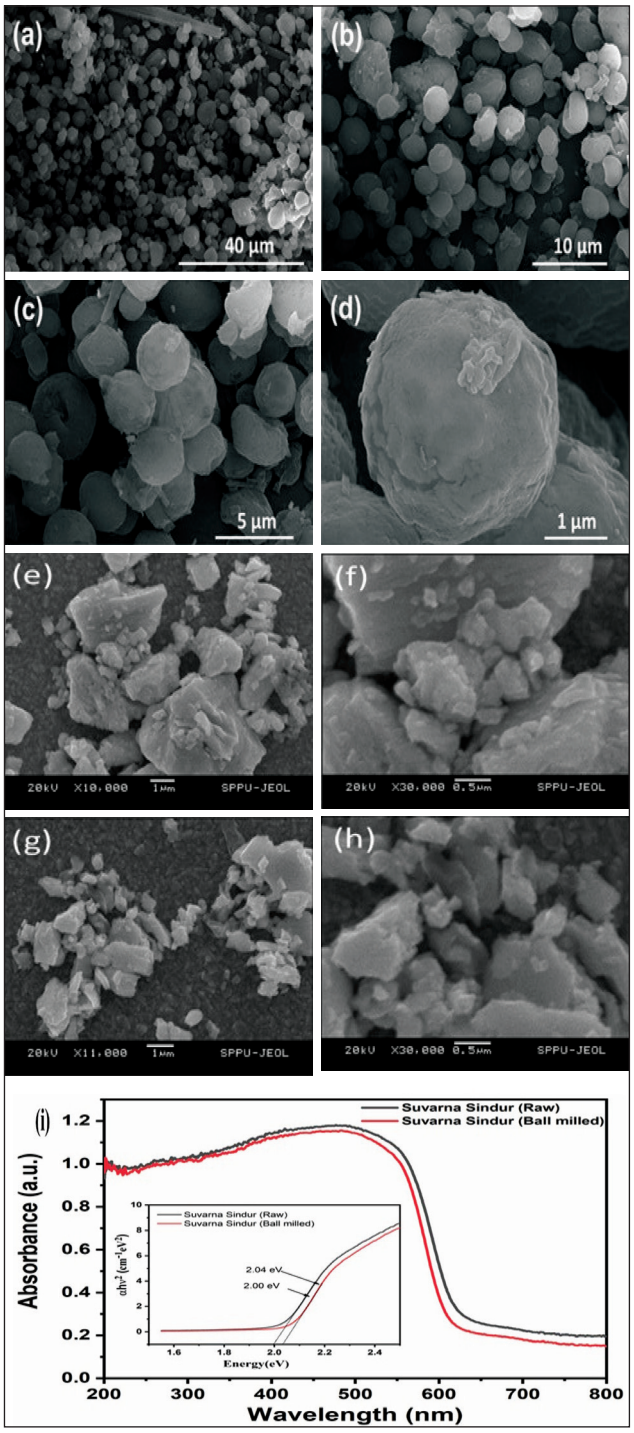

Figure 4. SEM Micrographs of (a–d) Spray-Dried and (e–h) Ball-Milled Particles, with (i) Corresponding UV–Visible Spectra.

XPS Analysis

XPS confirmed Hg^{2+} as the exclusive mercury species in F10: the Hg 4f spectrum displayed a characteristic spin-orbit

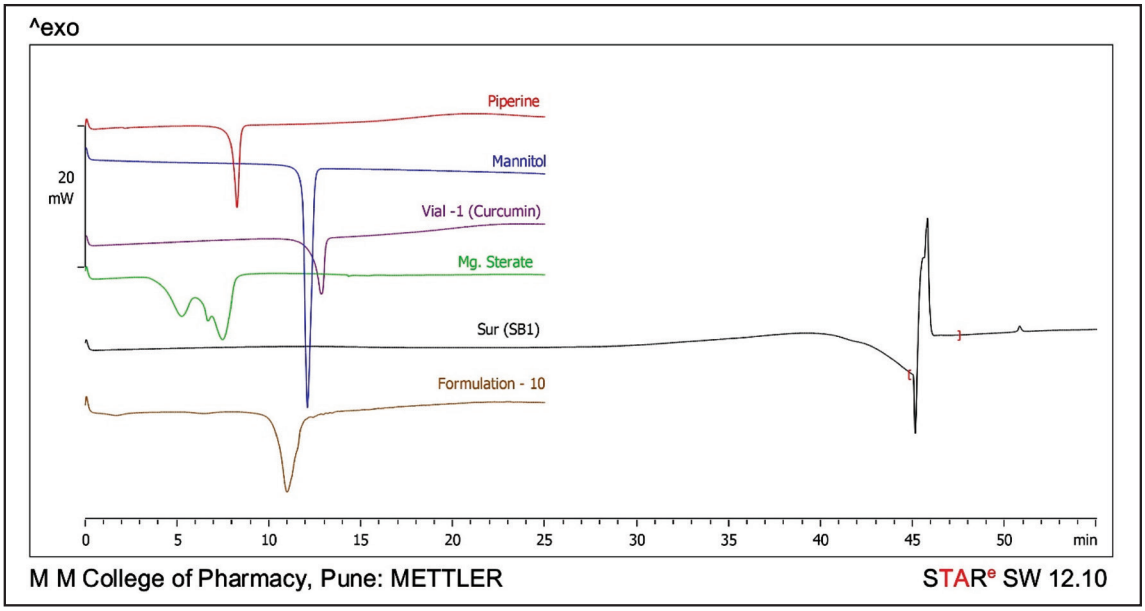

Figure 5. Overlay of DSC Thermograms for Piperine, Mannitol, Curcumin, Mg Stearate, Suvarnasindoor (SB1), and Formulation 10.

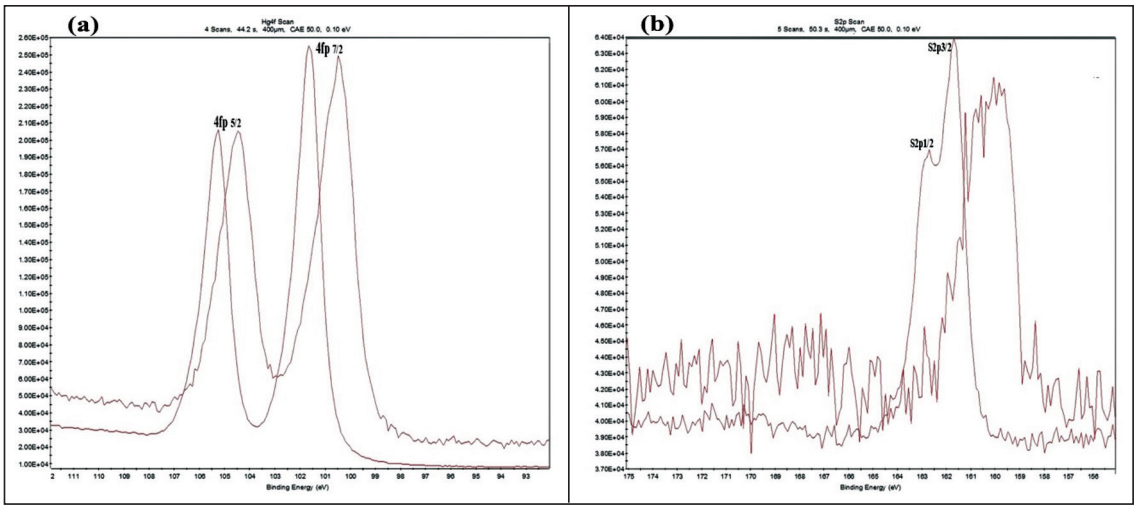

Figure 6. High-resolution XPS Spectra of (a) Hg 4f and (b) S 2p Regions with Associated Spin-orbit Doublets.

doublet with no metallic Hg^0 peaks (Figure 6a). The S 2p spectrum confirmed S^{2-} with no oxidized sulfur peaks (168–170 eV) (Figure 6b). These findings establish that the α-HgS crystalline form is preserved post-formulation, supporting the safety profile.[6,42]

Liquid Chromatography–Mass Spectrometry

LC–MS confirmed curcumin (*m/z* 369.13, RT 11.94 min) and piperine (*m/z* 286.14, RT 11.95 min) in F10, matching standards. No interference from Suvarnasindoor or formulation additives was detected (Figure 7 and Table 8).[33]

In Vivo Toxicity

No mortality or clinical signs of toxicity were observed in control or F10-treated rats over 14 days following intratracheal administration at 6.3 mg/kg (SC/IAEC/2025/026).[34,35] Two-way ANOVA with Tukey's post-test confirmed no significant toxicity versus controls. Histopathological examination (H&E staining) of lung tissue revealed no evidence of inflammation, necrosis, or structural abnormality. The extremely low Ksp of α-HgS ($\sim 4 \times 10^{-54}$).[7] indicates minimal systemic mercury absorption at the tested dose; long-term fate of retained particles warrants sub-chronic investigation.

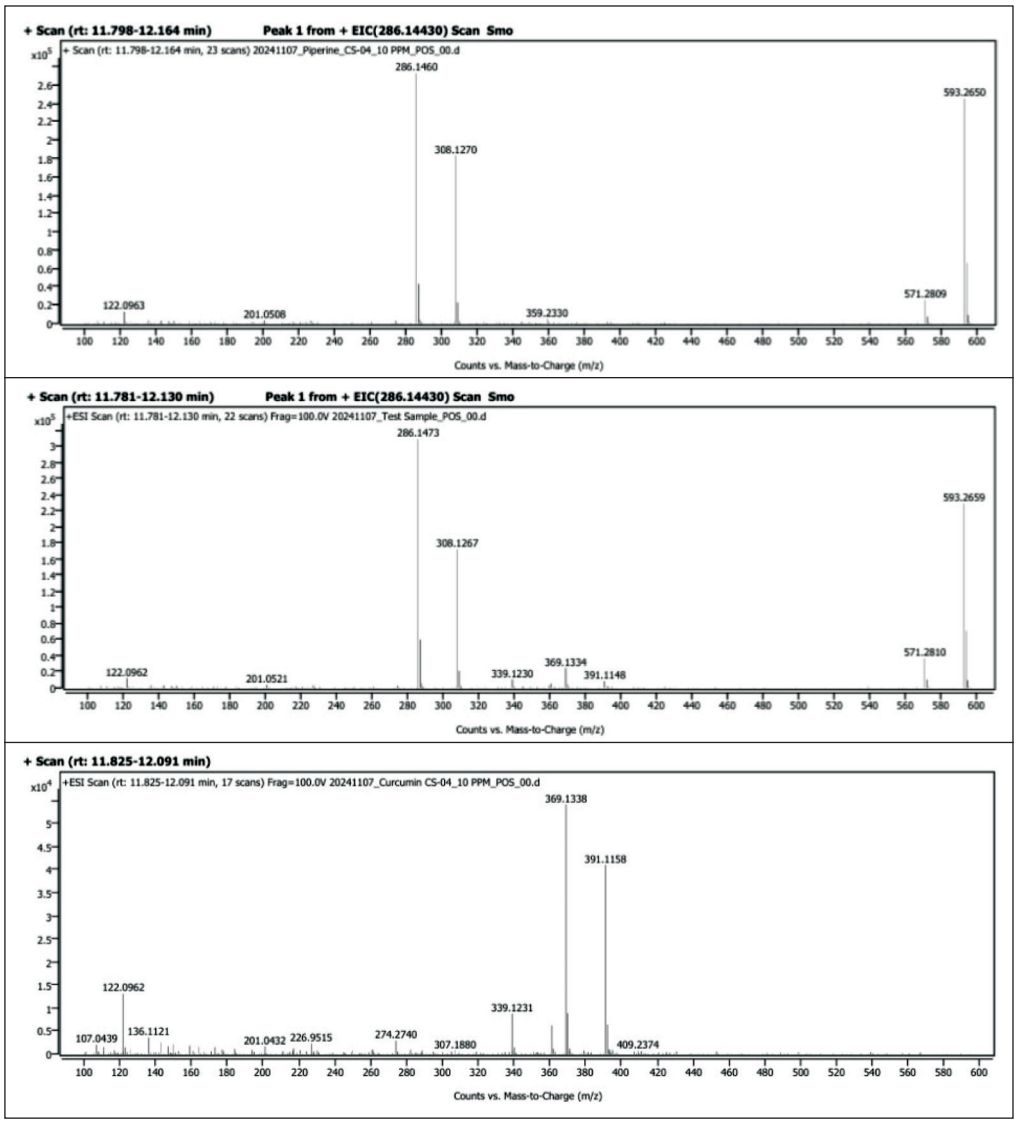

Figure 7. Liquid Chromatography–Mass Spectrometry (LC–MS) Spectra for Peak Identification and Sample Analysis.

Table 8. LC–MS Identification: Standards Versus F10 Capsule Formulation.

Parameter	Curcumin Std.	Piperine Std.	F10 Capsule
Retention time (min)	11.942	11.951	~11.94/~11.95
Mass (*m*/z)	369.1338	286.1443	~369.13/~286.14
Identity	Curcumin	Piperine	Both confirmed

Table 9. Stability Study Results: Formulation F10 (ICH Q1A R2).

Time Point	Curcumin (%)	Piperine (%)	Suvarnasindoor (%)	pH	Moisture (%)	PS (µm)	ZP (mV)
Initial	99.13 ± 0.87	98.52 ± 0.91	98.86 ± 1.04	7.65	2.1	4.2	−20
3M–RT	98.20 ± 0.31	99.20 ± 0.35	98.40 ± 0.47	7.69	2.1	4.2	−20
3M–AC	97.55 ± 0.31	98.53 ± 0.35	97.76 ± 0.47	7.61	2.2	4.3	−18
6M–RT	97.90 ± 0.31	98.81 ± 0.35	98.03 ± 0.47	7.67	2.2	4.2	−19
6M–AC	96.78 ± 0.31	97.78 ± 0.35	96.90 ± 0.47	7.66	2.2	4.1	−16

Note: RT: Real-time (25°C ± 2°C, 60% RH), AC: Accelerated (40°C ± 2°C, 75% RH), PS: Particle size, ZP: Zeta potential. *n* = 3, mean ± SD.

Stability

All assay values remained within 90%–110% across 6 months under both storage conditions (Table 9). Real-time: 97.90% (curcumin), 98.81% (piperine), 98.03% (Suvarnasindoor); accelerated: 96.78%, 97.78%, 96.90%. No significant changes were observed in assay, pH, moisture content, particle size, or zeta potential across all timepoints, confirming the physicochemical stability of F10 per ICH Q1A (R2).[36]

Conclusion

A stable and respirable dry powder inhaler formulation of Suvarnasindoor co-formulated with curcumin and piperine was successfully developed by spray-drying. The optimized formulation (F10) demonstrated uniform particle size distribution with an MMAD of 3.97 μm and improved fine particle fraction (31.85%), with aerodynamic properties meeting USP <601> requirements. Analytical characterization by LC–MS, XPS, and ICP-AES confirmed structural integrity of α-HgS, compatibility with co-formulants, and stability under both real-time and accelerated conditions. Acute intratracheal toxicity studies in rats revealed no mortality or overt signs of toxicity, suggesting preliminary tolerability.

This study highlights the necessity of advanced analytical tools in the evaluation of herbomineral formulations intended for local pulmonary delivery. The formulation may have potential for future evaluation as an adjuvant pulmonary delivery system for chronic inflammatory respiratory conditions such as COPD and asthma, considering the reported anti-inflammatory properties of its phytoconstituents. Moreover, this research serves as a translational blueprint for the modernization of other herbomineral Ayurvedic preparations, demonstrating that rigorous physicochemical characterization and modern inhalation engineering can be applied to traditional formulations to yield globally acceptable pharmaceutical products. To the best of the authors' knowledge, this represents the first reported attempt to convert Suvarnasindoor into a respirable DPI formulation, highlighting the novelty of this approach.

Certain limitations must be acknowledged. The long-term safety of inhaled α-HgS microparticles, including potential mercury accumulation in lung tissue following repeated dosing, remains to be investigated. In vitro drug release studies in simulated lung fluid were not performed due to the unique physicochemical nature of the formulation components: α-HgS is thermodynamically stable and practically insoluble in aqueous media (Ksp ~4×10^{-54}), rendering conventional dissolution testing uninformative. Furthermore, validated dissolution methods for inhaled phytoconstituents in simulated lung fluid lack pharmacopoeial standardization. A modified Franz diffusion cell method with Gamble's solution or a Transwell-based dissolution approach is proposed for subsequent studies. Future work should include sub-chronic inhalation toxicity evaluation (OECD TG 412), mercury speciation in lung tissue, and in vivo pharmacodynamic assessment in established disease models such as OVA-induced asthma or CSE-induced COPD.

Acknowledgement

The authors gratefully acknowledge Dr. Srinivasan Bhope (SAVA Healthcare, Bhosari) for providing samples, access to Copley software, and infrastructure support for DPI aerosol performance testing. The authors also thank Dr. Avinash and Dr. Bendale (Ayurved Rasayani) for supplying Suvarnasindoor API along with relevant Ayurvedic references. Special thanks are extended to Dr. Ravindra Purohit (RN Pharma Consulting, Pune), Dr. Sudhir Pandya, Dr. Vaibhav Vaidya, Dr. Shubangi Pharande and Mr. Maruf Shikalgar for their continuous guidance, valuable insights, and technical assistance throughout the study.

Authors' Contribution

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Data Availability Statement

All the data is available with the authors and shall be provided upon request.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Sciore Research Private Limited, Pune, Maharashtra, India (Company Registration Nos. 2113/PO/RcBi/S/2020/CPCSEA). The study was conducted in accordance with the approval granted under Proposal No. SC/IAEC/2025/026.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Informed Consent

Not applicable.

Use of Artificial Intelligence-assisted Tools

The authors declare that the AI-assisted tool (Grammarly) was used during manuscript preparation solely for English language enhancement, grammatical correction and reference formatting. No AI-assisted tool was used for the generation of scientific content, experimental data, research concepts, analysis, or interpretation of results. All edits suggested by the software were carefully reviewed, verified, and approved by the authors prior to submission. The authors assume full responsibility for the accuracy and integrity of the content presented in this work.

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