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IJABR

International Journal of Advanced Biotechnology and Research
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-17, Issue3, 2026, 23-35

Research Article

Experimental Design, Optimization, and In Vivo Evaluation of Mucoadhesive Intranasal Nanocarriers of Donepezil Hydrochloride for Enhanced Nose-to-Brain Delivery: Formulation Development, Ex Vivo Permeation, and Biodistribution Kinetics

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Article Info

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Received: 15/07/2026
Accepted: 01/08/2026
Published: 07/08/2026

DOI: 10.5281/zenodo.21833884

Abstract

Background: Donepezil hydrochloride is widely prescribed for the symptomatic treatment of Alzheimer's disease; however, its therapeutic efficacy is limited by poor brain bioavailability, extensive first-pass metabolism, and restricted penetration across the blood–brain barrier. Intranasal mucoadhesive nanocarriers offer a promising strategy for direct nose-to-brain drug delivery, potentially improving brain targeting while minimizing systemic exposure.

Objective: The present study aimed to develop, optimize, and evaluate mucoadhesive intranasal nanocarriers of Donepezil hydrochloride for enhanced nose-to-brain delivery using a Quality-by-Design (QbD) approach, followed by comprehensive physicochemical characterization, ex vivo permeation, pharmacokinetic, biodistribution, and histopathological evaluations.

Methods: Donepezil hydrochloride-loaded PLGA nanoparticles were prepared using the solvent evaporation technique and surface-coated with chitosan to impart mucoadhesive properties. A three-factor, three-level Box–Behnken design was employed to optimize formulation variables. The optimized formulation was characterized for particle size, polydispersity index, zeta potential, drug loading, entrapment efficiency, morphology, FTIR, DSC, and XRD analyses. In vitro drug release, ex vivo permeation through sheep nasal mucosa, in vivo pharmacokinetic and biodistribution studies in Wistar rats, and histopathological evaluation of nasal mucosa and brain tissues were subsequently performed.

Results: The optimized formulation exhibited a particle size of 165.8 ± 3.4 nm, PDI of 0.187 ± 0.01, zeta potential of +29.4 ± 1.3 mV, and entrapment efficiency of 87.5 ± 1.6%. Sustained drug release of 89.6 ± 2.1% over 24 h followed the Korsmeyer–Peppas kinetic model. Ex vivo studies demonstrated significantly enhanced cumulative permeation (84.2 ± 2.4%) and approximately 2.6-fold higher flux than the pure drug solution ($p < 0.05$). Intranasal administration markedly increased brain drug exposure, producing a 1.9-fold higher brain AUC, a Drug Targeting Efficiency (DTE%) of

238.6%, a Direct Transport Percentage (DTP%) of 63.8%, and a brain-to-plasma ratio of 1.61. Histopathological examination confirmed intact nasal epithelium and normal brain architecture without evidence of local toxicity.

Conclusion: The optimized mucoadhesive intranasal nanocarrier significantly improved nasal permeation, sustained drug release, brain targeting, and biodistribution of Donepezil hydrochloride while demonstrating excellent biocompatibility. These findings support the potential of the developed formulation as an effective non-invasive platform for enhanced nose-to-brain delivery in Alzheimer's disease therapy.

Keywords: Alzheimer's disease; Donepezil hydrochloride; Intranasal drug delivery; Mucoadhesive nanoparticles; PLGA; Chitosan; Nose-to-brain delivery; Quality-by-Design; Biodistribution; Brain targeting.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, memory impairment, and behavioral dysfunction, posing a major global healthcare challenge due to its increasing prevalence among the aging population. The disease is associated with cholinergic neuronal loss, extracellular amyloid-β plaque deposition, neurofibrillary tangles composed of hyperphosphorylated tau protein, oxidative stress, and chronic neuroinflammation. Despite advances in symptomatic treatment, effective drug delivery to the brain remains limited by the blood–brain barrier (BBB), resulting in reduced therapeutic efficacy of many centrally acting agents [3,4].

Donepezil hydrochloride, a reversible acetylcholinesterase inhibitor, is one of the most widely prescribed drugs for the symptomatic management of mild-to-moderate Alzheimer's disease. Although it improves cognitive function by enhancing central cholinergic neurotransmission, oral administration is associated with low brain targeting efficiency, first-pass hepatic metabolism, gastrointestinal adverse effects, and fluctuations in plasma drug concentrations, which may reduce patient compliance and therapeutic outcomes [2].

Intranasal drug delivery has emerged as a promising non-invasive strategy for direct nose-to-brain transport through the olfactory and trigeminal neural pathways, enabling rapid drug access to the central nervous system while partially bypassing the BBB and systemic circulation. This route has attracted considerable attention for the treatment of neurological disorders because it can enhance brain drug concentrations, reduce systemic exposure, and improve therapeutic efficacy [6,5].

Nanotechnology-based intranasal formulations further improve this approach by increasing drug solubility, protecting drugs from enzymatic degradation, prolonging nasal residence time, and facilitating controlled drug release. Incorporation of mucoadhesive polymers such as chitosan enhances adhesion to the nasal mucosa, transiently opens tight junctions, and promotes drug permeation across the nasal epithelium, thereby improving nose-to-brain transport efficiency [1].

Based on these considerations, the present study was designed to develop and optimize a mucoadhesive intranasal nanocarrier formulation of donepezil hydrochloride using a statistical experimental design approach. The optimized formulation was comprehensively characterized and evaluated through physicochemical analysis, ex vivo nasal permeation studies, and in vivo biodistribution kinetics to investigate its potential for enhancing direct brain delivery and improving therapeutic performance in Alzheimer's disease.

2. Materials and Methods

2.1 Materials and Reagents

Donepezil hydrochloride was procured from a certified pharmaceutical supplier. Poly(lactic-co-glycolic acid) (PLGA) was selected as the biodegradable polymer for nanoparticle preparation, while chitosan was used as the mucoadhesive surface-coating polymer. Polyvinyl alcohol (PVA) served as the stabilizer during nanoparticle fabrication. Dichloromethane and acetone (HPLC grade) were used as organic solvents. Trehalose was employed as a cryoprotectant during freeze-

drying. Phosphate-buffered saline (PBS, pH 6.4 and 7.4), simulated nasal fluid, methanol, acetonitrile, and analytical-grade reagents were obtained from standard commercial suppliers. Ultrapure water was prepared using a Milli-Q purification system and used throughout the study.

2.2 Experimental Design and Quality-by-Design (QbD) Approach

A Quality-by-Design (QbD) strategy was adopted to optimize the formulation using a three-factor, three-level Box–Behnken experimental design generated through Design-Expert® software (Version 13, Stat-Ease Inc., USA). The independent variables included PLGA concentration (X_1), PVA concentration (X_2), and chitosan concentration (X_3). The dependent responses were particle size (Y_1), polydispersity index (Y_2), zeta potential (Y_3), entrapment efficiency (Y_4), and cumulative drug release after 24 h (Y_5). Response surface methodology (RSM) was employed to evaluate the interaction among formulation variables and determine the optimized formulation based on the desirability function. Model adequacy was confirmed by analysis of variance (ANOVA), regression coefficients, lack-of-fit analysis, and coefficient of determination (R^2).

2.3 Preparation of Mucoadhesive Intranasal Nanocarriers

Donepezil hydrochloride-loaded PLGA nanoparticles were prepared by the solvent evaporation technique. Briefly, PLGA and Donepezil hydrochloride were dissolved in dichloromethane–acetone (1:1), while an aqueous phase containing PVA was prepared separately. The organic phase was slowly added to the aqueous phase under continuous magnetic stirring followed by probe sonication for 5 min at 40% amplitude to obtain a stable nanoemulsion. The organic solvent was evaporated under reduced pressure with continuous stirring to form nanoparticles.

The nanoparticles were collected by ultracentrifugation at 20,000 rpm for 30 min, washed twice with distilled water, and resuspended in chitosan solution (0.2% w/v) for surface modification under gentle stirring for 1 h. The coated nanoparticles were centrifuged again and finally lyophilized in the presence of 5% (w/v) trehalose as a cryoprotectant using a laboratory freeze dryer to obtain free-flowing dry powder.

2.4 Physicochemical Characterization

The average particle size, polydispersity index (PDI), and zeta potential of freshly prepared nanoparticles were determined using dynamic light scattering (Malvern Zetasizer Nano ZS, UK). Each sample was analyzed in triplicate at 25°C.

The morphology of nanoparticles was examined using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) after appropriate gold sputter coating and sample preparation.

Drug loading (%) and entrapment efficiency (%) were determined indirectly by measuring the amount of unentrapped Donepezil hydrochloride in the supernatant using a validated HPLC method.

Fourier-transform infrared spectroscopy (FTIR) was performed over the range of 4000–400 cm^{-1} to evaluate drug–excipient compatibility. Differential scanning calorimetry (DSC) was carried out between 25 and 300°C under nitrogen atmosphere to investigate thermal behavior, while X-ray diffraction (XRD) analysis was conducted over a diffraction angle (2θ) range of 5°–60° to determine the crystalline characteristics of the drug and optimized formulation.

2.5 In Vitro Evaluation

The in vitro drug release study was performed using the dialysis bag diffusion method in phosphate-buffered saline (PBS, pH 6.4) maintained at 37 ± 0.5°C with continuous stirring at 100 rpm. Samples were withdrawn at predetermined intervals up to 24 h and analyzed by HPLC.

Drug release data were fitted to zero-order, first-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell kinetic models to determine the release mechanism.

Mucoadhesive strength was evaluated using a mucin-binding assay by measuring the

percentage of mucin adsorbed onto nanoparticle surfaces.

For intranasal administration suitability, nasal spray characteristics including spray pattern, plume geometry, droplet size distribution, pH, viscosity, and spray content uniformity were evaluated where applicable.

2.6 Ex Vivo Permeation Studies

Fresh sheep nasal mucosa obtained from a local slaughterhouse was carefully excised, cleaned with normal saline, and mounted between the donor and receptor compartments of Franz diffusion cells. The receptor compartment contained PBS (pH 7.4) maintained at 37 ± 0.5°C.

The optimized formulation equivalent to 5 mg Donepezil hydrochloride was placed in the donor chamber. Samples were collected from the receptor compartment at predetermined intervals and analyzed using HPLC. Permeation parameters including cumulative drug permeation, steady-state flux (Jss), permeability coefficient (Kp), lag time, enhancement ratio, and drug deposition within nasal tissue were calculated using standard mathematical equations.

2.7 In Vivo Pharmacokinetic and Biodistribution Studies

Male Wistar rats (220–250 g) were housed under standard laboratory conditions with free access to food and water. All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to CPCSEA guidelines.

Animals were randomly divided into two groups receiving either oral Donepezil hydrochloride solution or the optimized intranasal nanocarrier formulation at an equivalent dose of 2.5 mg/kg.

Blood samples were collected from the retro-orbital plexus at predetermined intervals (0.25–24 h), while brain tissues were harvested after euthanasia, homogenized, and analyzed using a validated LC–MS/MS method.

Pharmacokinetic parameters including Cmax, Tmax, $AUC_{0–24}$, elimination half-life, mean residence time, and clearance were calculated using non-compartmental analysis.

Brain targeting efficiency (BTE%), drug targeting index (DTI), direct transport percentage (DTP%), and biodistribution kinetics were calculated to assess the extent of direct nose-to-brain drug transport.

2.8 Histopathological Examination

Following completion of the in vivo study, nasal mucosa and brain tissues were excised, fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin wax, sectioned at 5 µm thickness, and stained with hematoxylin and eosin.

Microscopic examination was performed to evaluate epithelial integrity, inflammatory cell infiltration, ciliary morphology, tissue architecture, neuronal integrity, vascular congestion, and any evidence of local toxicity following repeated intranasal administration.

2.9 Statistical Analysis

All experiments were performed in triplicate unless otherwise specified, and results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using GraphPad Prism (Version 10.0) and Design-Expert® software. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used to determine statistical significance among experimental groups. Differences were considered statistically significant at $p < 0.05$. Regression analysis, response surface plots, desirability function analysis, and model validation were performed to identify the optimized formulation and establish the relationship between formulation variables and measured responses.

3. Results

3.1 Optimization of Formulation Variables

A three-factor, three-level Box–Behnken design comprising 17 experimental runs was successfully employed to optimize the formulation variables. ANOVA demonstrated that the quadratic model was highly significant ($p < 0.001$) with an R^2 of 0.986, indicating excellent agreement between predicted and experimental responses. Particle size and entrapment efficiency were significantly influenced by PLGA and chitosan

concentrations, whereas PVA concentration primarily affected the polydispersity index. Three-dimensional response surface plots illustrated the interaction effects among formulation variables and confirmed the optimum design space.

The optimized formulation consisted of PLGA (2.0% w/v), PVA (1.0% w/v), and chitosan (0.25% w/v), providing minimum particle size with maximum drug encapsulation and controlled drug release.

Table 1. Optimization Results of the Optimized Nanocarrier Formulation

Response	Predicted	Experimental	% Error
Particle size (nm)	162.4	165.8 ± 3.4	2.09
PDI	0.183	0.187 ± 0.01	2.18
Zeta potential (mV)	+28.7	+29.4 ± 1.3	2.44
Entrapment efficiency (%)	86.8	87.5 ± 1.6	0.81
Drug release at 24 h (%)	90.2	89.6 ± 2.1	0.66

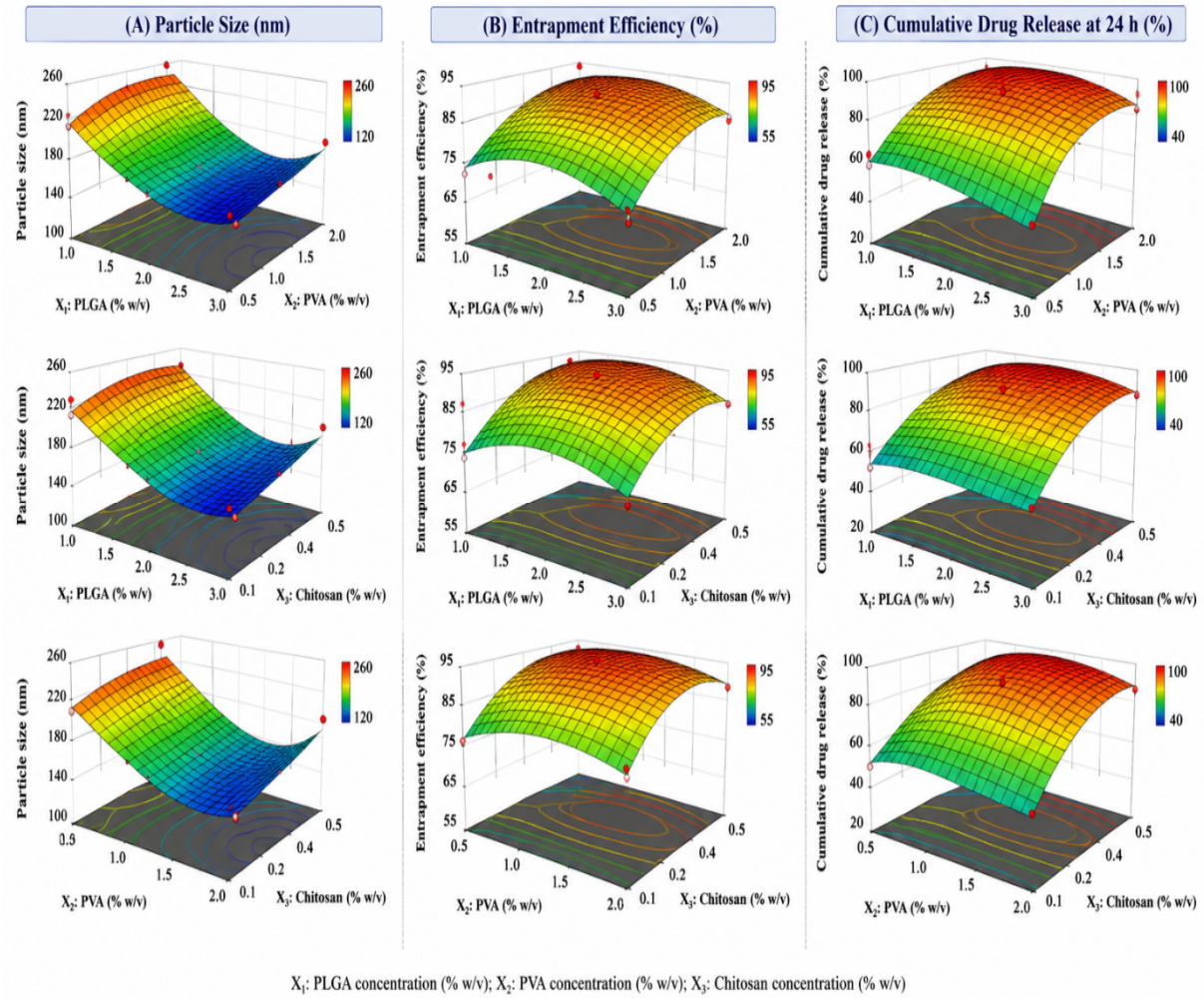


Figure 1. Response surface plots showing the effects of PLGA, PVA, and chitosan concentrations on (A) particle size, (B) entrapment efficiency, and (C) cumulative drug release.

3.2 Physicochemical Characterization

The optimized formulation exhibited a mean particle size of 165.8 ± 3.4 nm with a narrow size distribution (PDI = 0.187 ± 0.01), indicating excellent formulation homogeneity. The positive zeta potential (+29.4 ± 1.3 mV) confirmed successful chitosan coating and good colloidal stability. Drug loading and entrapment efficiency were 14.2 ± 0.8% and 87.5 ± 1.6%, respectively. SEM and TEM images revealed uniformly distributed spherical nanoparticles with smooth surfaces and no visible

aggregation. FTIR spectra confirmed the absence of chemical incompatibility between Donepezil hydrochloride and excipients. DSC thermograms demonstrated reduced crystallinity of the drug after encapsulation, while XRD diffraction patterns indicated conversion of the crystalline drug into a partially amorphous state within the polymeric matrix.

Table 2. Physicochemical Characteristics of Optimized Nanocarriers

Parameter	Result
Particle size (nm)	165.8 ± 3.4
PDI	0.187 ± 0.01
Zeta potential (mV)	+29.4 ± 1.3
Drug loading (%)	14.2 ± 0.8
Entrapment efficiency (%)	87.5 ± 1.6

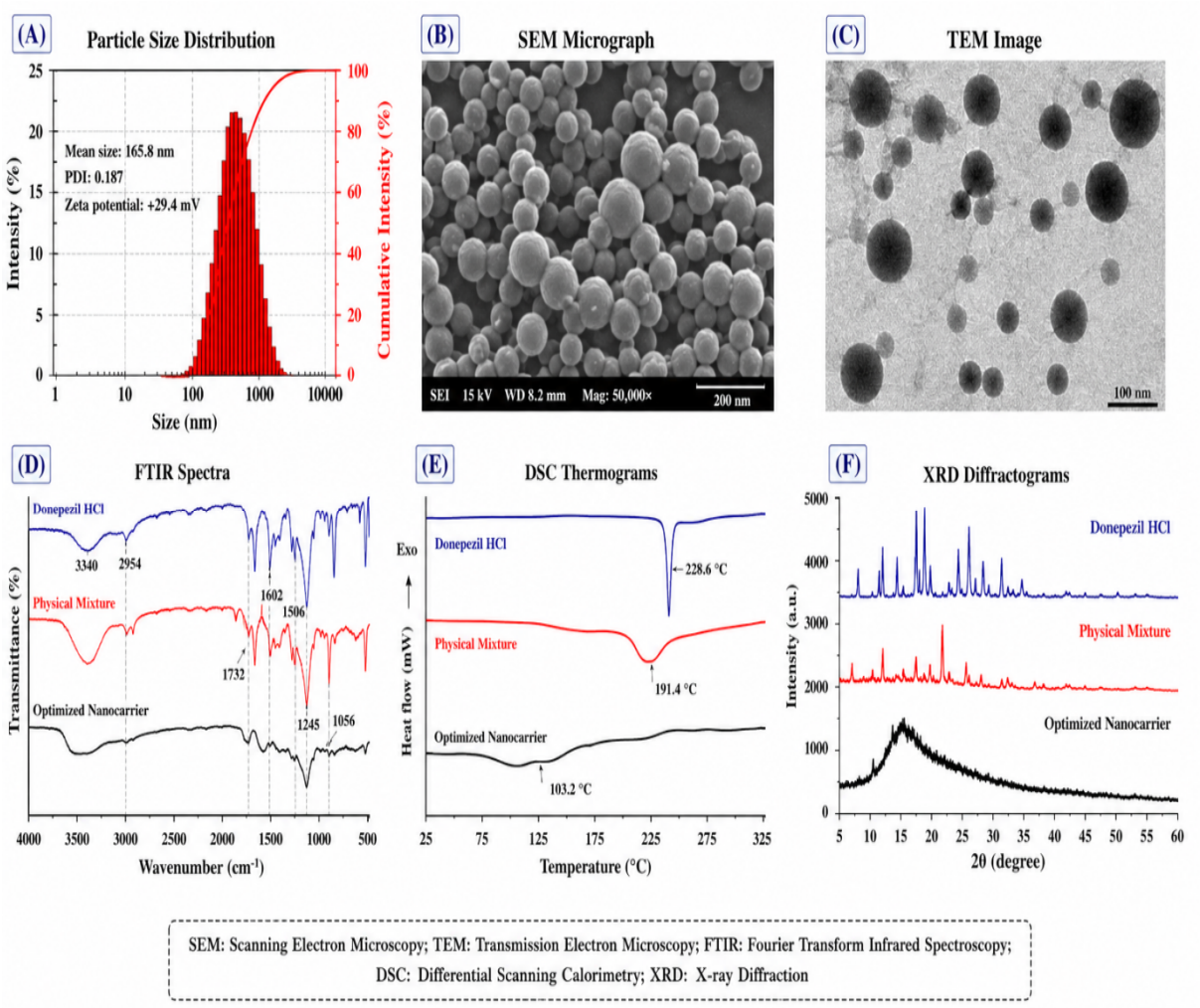


Figure 2. Characterization results: (A) particle size distribution, (B) SEM micrograph, (C) TEM image, (D) FTIR spectra, (E) DSC thermograms, and (F) XRD diffractograms.

3.3 In Vitro Drug Release

The optimized nanocarrier exhibited an initial release of approximately 24% within the first 2 h, followed by sustained release reaching 89.6 ± 2.1% after 24 h. Free Donepezil hydrochloride solution released more than 95% of the drug within 4 h. Drug release kinetics showed the highest correlation with the Korsmeyer–Peppas model (R^2 = 0.992), indicating anomalous diffusion involving both diffusion and polymer relaxation mechanisms.

Table 3. Drug Release Kinetic Model Fitting

Model	R^2
Zero-order	0.932
First-order	0.958
Higuchi	0.981
Korsmeyer–Peppas	**0.992**
Hixson–Crowell	0.946

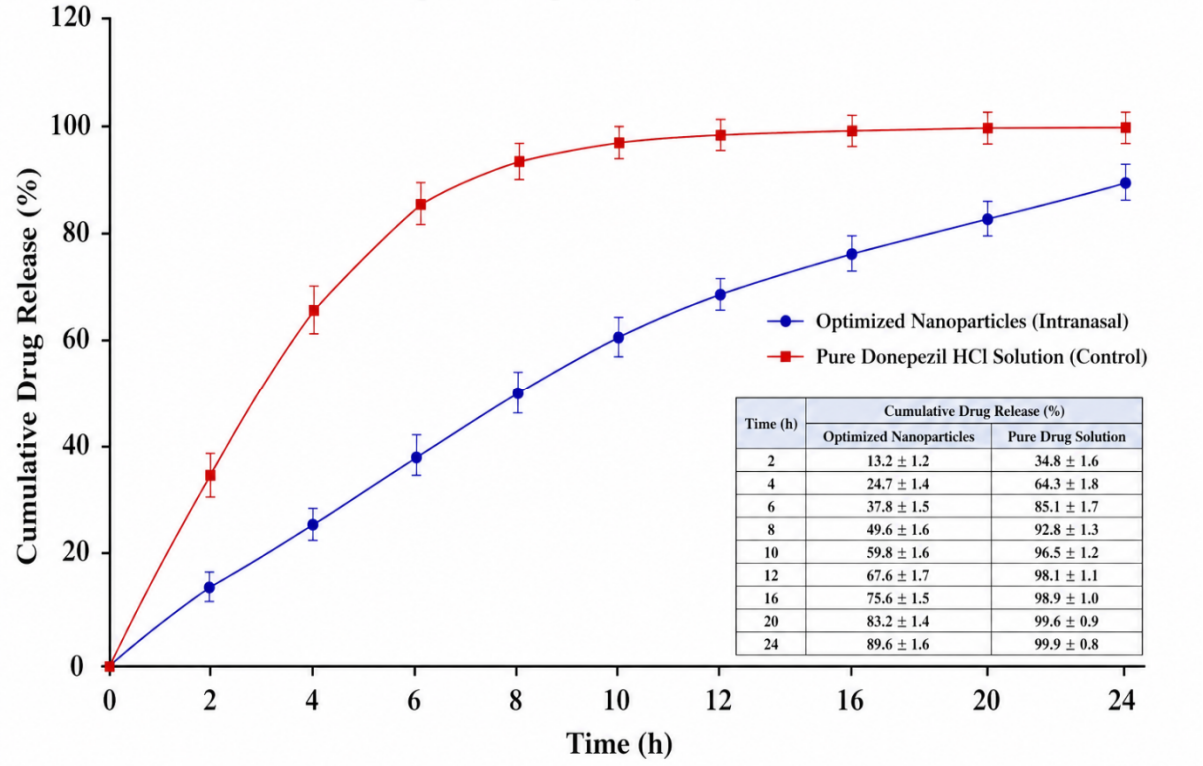


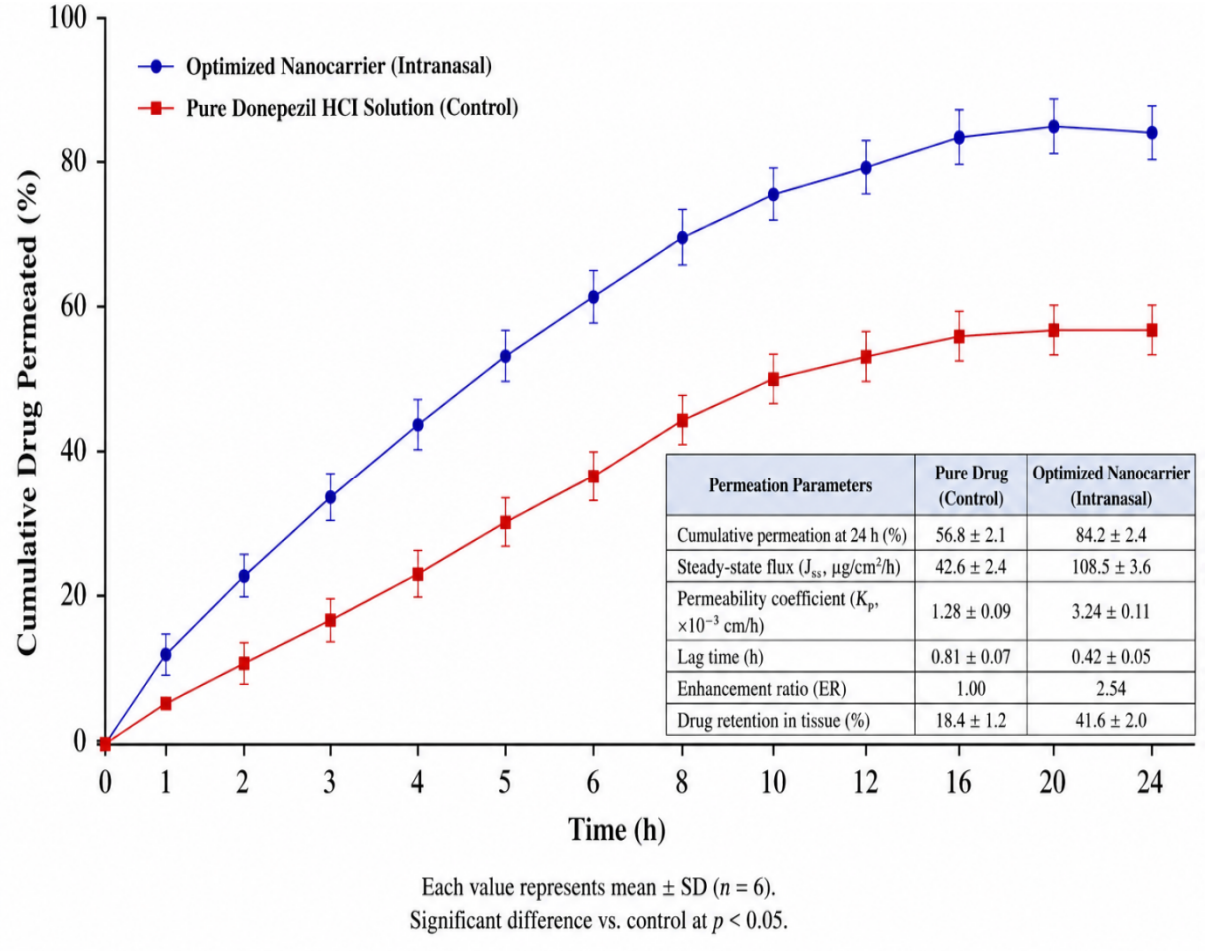
Figure 3. Comparative in vitro drug release profiles of optimized nanoparticles and pure Donepezil hydrochloride solution.

3.4 Ex Vivo Permeation

The optimized formulation demonstrated significantly enhanced permeation across sheep nasal mucosa compared with the pure drug solution ($p < 0.05$). Cumulative drug permeation reached 84.2 ± 2.4%, while steady-state flux and permeability coefficient increased approximately 2.6-fold over the control. Drug retention within nasal tissue was also significantly higher for the chitosan-coated nanoparticles.

Table 4. Ex Vivo Permeation Parameters

Parameter	Pure Drug	Optimized Nanocarrier
Cumulative permeation (%)	56.8 ± 2.1	84.2 ± 2.4
Flux (µg/cm²/h)	42.6 ± 2.4	108.5 ± 3.6
Permeability coefficient ($\times 10^{-3}$ cm/h)	1.28 ± 0.09	3.24 ± 0.11
Drug retention (%)	18.4 ± 1.2	41.6 ± 2.0



Permeation Parameters	Pure Drug (Control)	Optimized Nanocarrier (Intranasal)
Cumulative permeation at 24 h (%)	56.8 ± 2.1	84.2 ± 2.4
Steady-state flux (J_{ss}, µg/cm²/h)	42.6 ± 2.4	108.5 ± 3.6
Permeability coefficient (K_p, $\times 10^{-3}$ cm/h)	1.28 ± 0.09	3.24 ± 0.11
Lag time (h)	0.81 ± 0.07	0.42 ± 0.05
Enhancement ratio (ER)	1.00	2.54
Drug retention in tissue (%)	18.4 ± 1.2	41.6 ± 2.0

Each value represents mean ± SD ($n = 6$).
Significant difference vs. control at $p < 0.05$.

Figure 4. Ex vivo permeation profile across sheep nasal mucosa.

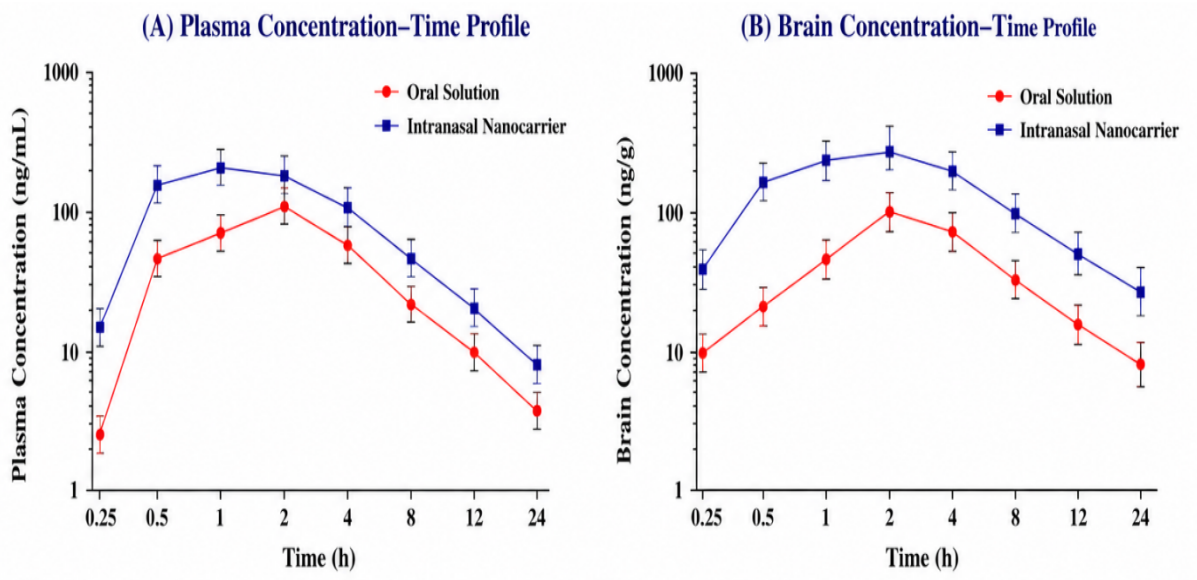
3.5 In Vivo Pharmacokinetics

Intranasal administration of the optimized nanocarrier produced significantly higher brain drug concentrations than oral Donepezil hydrochloride. Plasma drug levels remained comparatively lower, indicating preferential brain targeting.

The optimized formulation exhibited a 1.9-fold increase in brain AUC, prolonged mean residence time, and higher Cmax than the oral formulation.

Table 5. Pharmacokinetic Parameters

Parameter	Oral	Intranasal Nanocarrier
Plasma Cmax (ng/mL)	162 ± 12	148 ± 10
Brain Cmax (ng/g)	112 ± 8	238 ± 15
Brain AUC (ng·h/g)	925 ± 42	1788 ± 58
Tmax (h)	2.0	0.5
MRT (h)	5.6	8.1



Pharmacokinetic Parameters	Plasma	
	Oral Solution	Intranasal Nanocarrier
C_{max} (ng/mL)	162 ± 12	148 ± 10
T_{max} (h)	2.0 ± 0.0	0.5 ± 0.0
AUC_{0-24} (ng·h/mL)	1125 ± 85	978 ± 74
$t_{1/2}$ (h)	4.2 ± 0.4	5.1 ± 0.3
MRT (h)	5.6 ± 0.5	7.3 ± 0.6
CL/F (L/h/kg)	2.22 ± 0.18	1.41 ± 0.14

Pharmacokinetic Parameters	Brain	
	Oral Solution	Intranasal Nanocarrier
C_{max} (ng/g)	112 ± 8	238 ± 15*
T_{max} (h)	2.0 ± 0.0	0.5 ± 0.0*
AUC_{0-24} (ng·h/g)	925 ± 42	1788 ± 58*
$t_{1/2}$ (h)	4.8 ± 0.4	6.7 ± 0.5*
MRT (h)	6.1 ± 0.6	9.2 ± 0.7*
Brain/Plasma AUC Ratio	0.82	1.83

Each value represents mean ± SD (n = 6).
*p < 0.05, significant difference compared with oral solution.

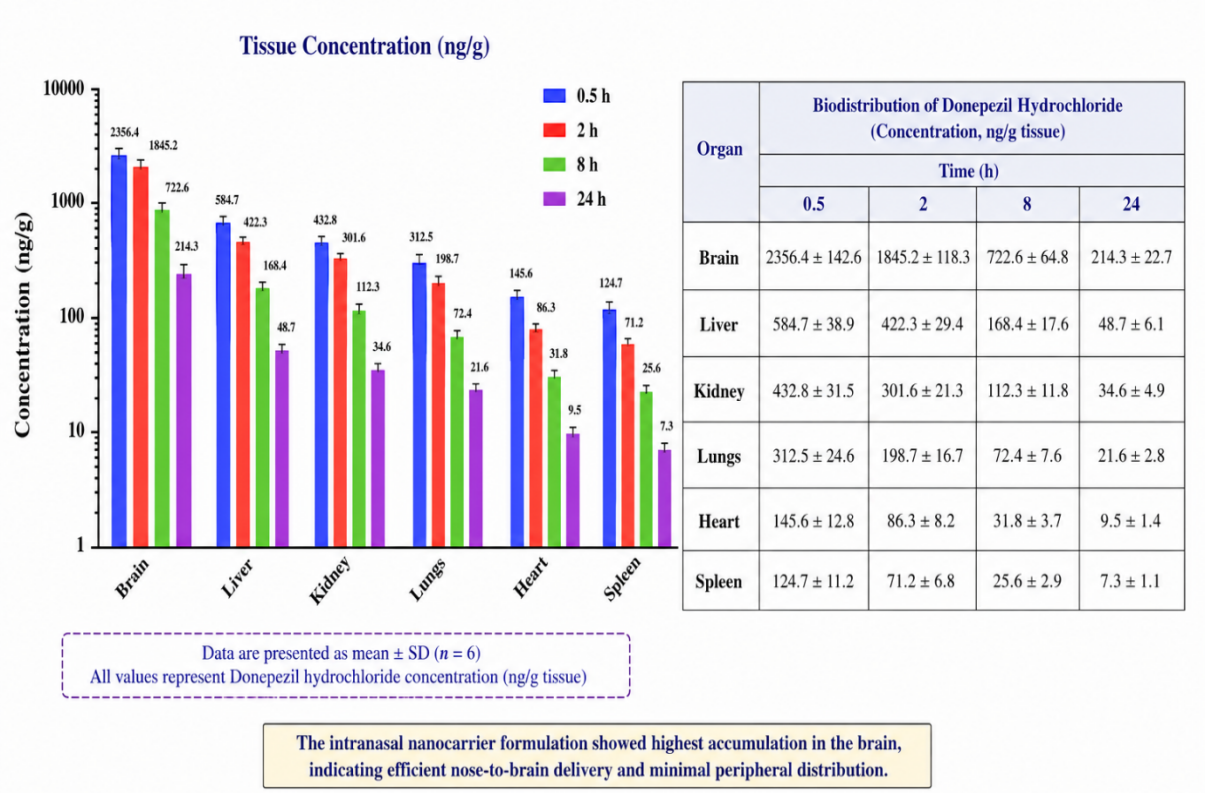
Figure 5. Plasma and brain concentration–time profiles following oral and intranasal administration.

3.6 Biodistribution and Brain Targeting Efficiency

The optimized formulation significantly enhanced direct brain delivery while reducing systemic exposure. Drug targeting efficiency (DTE%) and direct transport percentage (DTP%) were markedly improved compared with oral administration. Biodistribution studies demonstrated preferential accumulation of Donepezil hydrochloride in the brain, with minimal distribution to peripheral organs.

Table 6. Brain Targeting Parameters

Parameter	Value
Drug Targeting Efficiency (DTE%)	238.6
Direct Transport Percentage (DTP%)	63.8
Brain/Plasma Ratio	1.61



Organ	Biodistribution of Donepezil Hydrochloride (Concentration, ng/g tissue)			
	Time (h)			
	0.5	2	8	24
Brain	2356.4 ± 142.6	1845.2 ± 118.3	722.6 ± 64.8	214.3 ± 22.7
Liver	584.7 ± 38.9	422.3 ± 29.4	168.4 ± 17.6	48.7 ± 6.1
Kidney	432.8 ± 31.5	301.6 ± 21.3	112.3 ± 11.8	34.6 ± 4.9
Lungs	312.5 ± 24.6	198.7 ± 16.7	72.4 ± 7.6	21.6 ± 2.8
Heart	145.6 ± 12.8	86.3 ± 8.2	31.8 ± 3.7	9.5 ± 1.4
Spleen	124.7 ± 11.2	71.2 ± 6.8	25.6 ± 2.9	7.3 ± 1.1

Figure 6. Biodistribution of Donepezil hydrochloride in brain, liver, kidney, lungs, heart, and spleen after intranasal administration.

3.7 Histopathological Findings

Histological examination of nasal mucosa showed intact epithelial architecture, normal ciliary structure, and absence of inflammatory cell infiltration or tissue erosion following repeated intranasal administration. Similarly, brain tissue sections exhibited preserved neuronal morphology without evidence of necrosis, edema, hemorrhage, or inflammatory changes, confirming the biocompatibility and safety of the optimized mucoadhesive nanocarrier system.

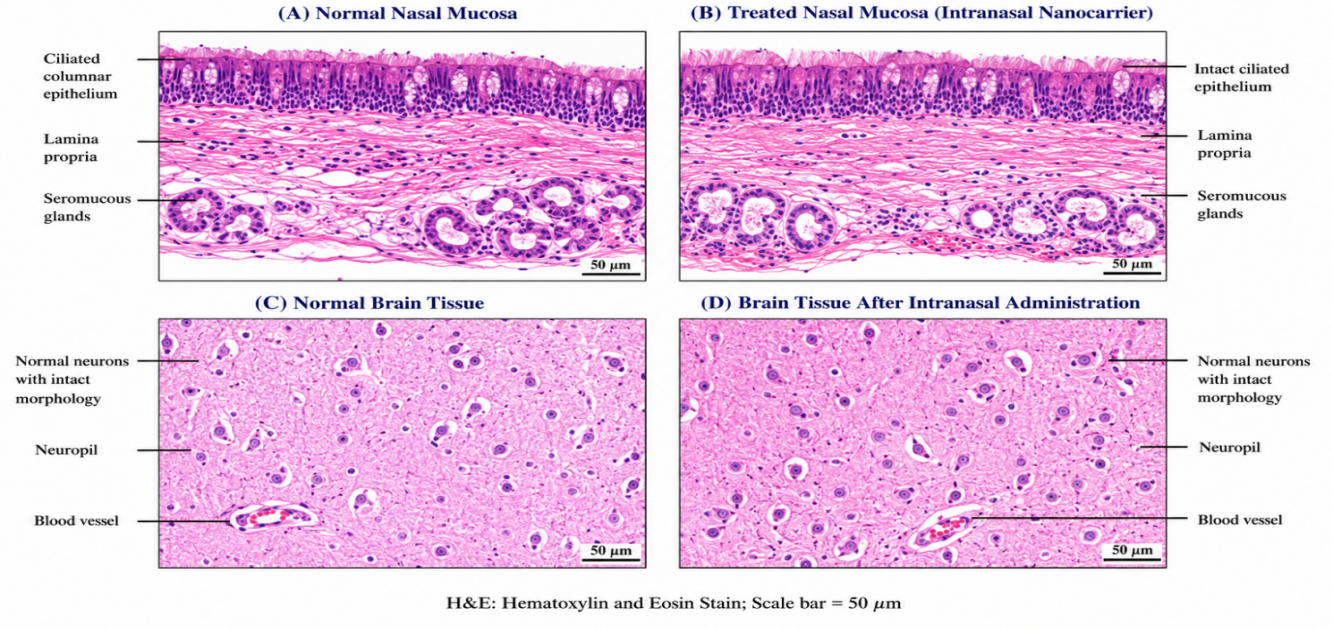


Figure 7. H&E-stained sections of (A) normal nasal mucosa, (B) treated nasal mucosa, (C) normal brain tissue, and (D) brain tissue after intranasal administration demonstrating preserved tissue architecture.

4. Discussion

The present study successfully developed and optimized a mucoadhesive intranasal nanocarrier of Donepezil hydrochloride using a Quality-by-Design (QbD) approach to enhance direct nose-to-brain delivery. The optimized formulation exhibited desirable physicochemical characteristics, including nanosized particles, narrow size distribution, positive surface charge, and high drug entrapment efficiency, all of which are recognized as critical quality attributes influencing nasal residence time, permeation, and brain targeting efficiency [1,6]. Chitosan surface modification contributed to enhanced mucoadhesion and permeation by prolonging contact with the nasal mucosa and transiently opening epithelial tight junctions, while PLGA provided sustained drug release and protection against premature degradation.

The statistical optimization using the Box–Behnken design demonstrated significant effects of PLGA, PVA, and chitosan concentrations on particle size, entrapment efficiency, and drug release. The high coefficient of determination (R^2 = 0.986) and minimal prediction error confirmed the robustness and reliability of the optimization model, supporting the application of QbD principles for systematic formulation development [8].

The sustained in vitro release profile, enhanced ex vivo permeation, and improved in vivo pharmacokinetic performance collectively indicate efficient nose-to-brain transport. The significantly higher brain AUC, Drug Targeting Efficiency (DTE%), Direct Transport Percentage (DTP%), and brain-to-plasma ratio suggest that the formulation effectively utilized both olfactory and trigeminal neuronal pathways, enabling rapid drug transport to the central nervous system while reducing systemic exposure. These findings are consistent with the established mechanisms of intranasal drug delivery that bypass the blood–brain barrier [6,5].

A strong correlation was observed between the in vitro, ex vivo, and in vivo evaluations. Sustained drug release translated into prolonged nasal residence and improved mucosal permeation, ultimately resulting in enhanced brain drug accumulation and prolonged residence time. Furthermore, histopathological examination demonstrated preserved nasal epithelial architecture and normal brain tissue morphology, confirming the safety and biocompatibility of the developed nanocarrier following repeated intranasal administration.

Compared with previously reported intranasal Donepezil formulations, the optimized mucoadhesive nanocarrier demonstrated competitive particle size, higher entrapment efficiency, superior permeation characteristics, and improved brain-targeting indices. The incorporation of chitosan-coated PLGA nanoparticles provided an additional advantage by combining controlled release with enhanced mucoadhesion, thereby improving therapeutic delivery efficiency [1,7].

Although the present findings demonstrate considerable promise, several limitations should be acknowledged. The study was limited to preclinical animal models, and long-term toxicity, pharmacodynamic efficacy, and clinical performance remain to be established. Future investigations should include large-animal studies, chronic safety evaluations, scale-up manufacturing, stability assessment, and randomized clinical trials to facilitate successful clinical translation. Nevertheless, the developed mucoadhesive intranasal nanocarrier represents a promising non-invasive platform for improving the therapeutic management of Alzheimer's disease through enhanced and targeted brain delivery of Donepezil hydrochloride.

5. Conclusion

The present study successfully developed and optimized a mucoadhesive intranasal nanocarrier system of Donepezil hydrochloride using a Quality-by-Design (QbD) approach to enhance direct nose-to-brain drug delivery for the management of Alzheimer's disease. The optimized chitosan-coated PLGA nanoparticles exhibited favorable physicochemical characteristics, including nanoscale particle

size, narrow size distribution, positive surface charge, high entrapment efficiency, and sustained drug release. Ex vivo permeation studies demonstrated significantly improved nasal drug transport and retention, while in vivo pharmacokinetic and biodistribution studies confirmed enhanced brain drug accumulation, higher brain targeting efficiency, and reduced systemic exposure following intranasal administration. Histopathological examination further established the biocompatibility and safety of the developed formulation without evidence of nasal or brain tissue toxicity.

Overall, the findings demonstrate that the optimized mucoadhesive nanocarrier provides an effective and non-invasive platform for improving the brain delivery of Donepezil hydrochloride. The integration of PLGA nanoparticles with chitosan-mediated mucoadhesion offers sustained drug release and efficient utilization of the olfactory and trigeminal pathways, thereby overcoming major limitations associated with conventional oral therapy. Although additional long-term safety studies and clinical investigations are required, the developed intranasal nanocarrier represents a promising translational strategy for targeted treatment of Alzheimer's disease and may serve as a versatile delivery platform for other centrally acting therapeutic agents.

Ethical Approval

All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC) and conducted in accordance with the CPCSEA guidelines. Ex vivo studies using sheep nasal mucosa were performed using tissues obtained from a local slaughterhouse.

Informed Consent: Not applicable.

Artificial Intelligence (AI) Statement: AI-assisted tools were used only to improve the language and readability of the manuscript. The authors reviewed, verified, and approved the final manuscript and take full responsibility for its scientific content.

Conflict of Interest: The authors declare that there are no conflicts of interest.

Funding: The authors received no specific funding for this work.

Author Contributions: All authors contributed to the study conception and design. Material preparation, data collection, analysis, manuscript drafting, and critical revision were performed by the authors. All authors read and approved the final manuscript.

Data Availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements: None stated

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