



Original Research Paper

FORMULATION AND EVALUATION OF QUERCETIN-LOADED INTRANASAL NANOPARTICLES FOR NEUROPROTECTION AND ENHANCED BRAIN TARGETING IN ALZHEIMER'S DISEASE

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Abstract

Alzheimer's disease (AD) represents a formidable neurodegenerative disorder characterized by complex pathophysiology involving amyloid- β aggregation, tau hyperphosphorylation, oxidative stress, and neuroinflammation, with current therapeutic interventions offering only modest symptomatic relief. Quercetin, a naturally occurring flavonoid with potent multi-target neuroprotective properties, has emerged as a promising therapeutic candidate; however, its clinical translation is severely hampered by poor aqueous solubility, extensive first-pass metabolism, rapid elimination, and negligible blood-brain barrier permeability. This study aimed to develop and evaluate quercetin-loaded nanostructured lipid carriers (QT-NLCs) for intranasal administration to enhance brain targeting and neuroprotective efficacy in Alzheimer's disease. The formulation was optimized using Box-Behnken design, with the optimal composition comprising 5.2% w/w glyceryl monostearate, 2.8% w/w oleic acid, 3.5% w/w Tween 80, and a drug-to-lipid ratio of 1:10, prepared by high-pressure homogenization followed by ultrasonication. The optimized QT-NLCs exhibited a mean particle size of 128.4 ± 4.2 nm, polydispersity index of 0.194 ± 0.021 , zeta potential of -32.6 ± 2.8 mV, entrapment efficiency of $87.3 \pm 3.1\%$, and drug loading of $8.7 \pm$

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0.4%. In vitro drug release demonstrated biphasic sustained release with $78.4 \pm 3.6\%$ cumulative release at 72 hours, following Korsmeyer-Peppas kinetics ($R^2 = 0.982$). Ex vivo nasal permeation studies revealed 2.3-fold enhanced permeation flux ($2.84 \pm 0.21 \mu\text{g}/\text{cm}^2/\text{h}$) and significantly improved mucoadhesion with residence time of 4.2 ± 0.5 hours. In vitro cytotoxicity assays using PCS-200-014 neuronal cells demonstrated reduced cytotoxicity ($\text{IC}_{50} = 124.6 \pm 8.7 \mu\text{g}/\text{mL}$) and 3.5-fold higher cellular uptake for QT-NLCs compared to free quercetin. In vivo pharmacokinetic studies in Wistar rats following intranasal administration demonstrated significantly enhanced brain targeting with drug targeting efficiency of 342.6% and drug targeting percentage of 72.8%, indicating preferential brain delivery. Pharmacodynamic evaluation in trimethyltin-induced neurodegeneration model revealed significant cognitive improvement (42.8% reduction in escape latency, 38.6% increase in recognition index), reduction in oxidative stress (SOD: +38.7%, GSH: +42.3%, MDA: -51.2%), and attenuation of neuroinflammation. Histopathological and immunohistochemical analyses confirmed reduced neuronal damage, glial activation, amyloid- β plaque deposition, and phosphorylated tau expression. These findings demonstrate that intranasal administration of QT-NLCs offers a promising strategy to overcome quercetin's bioavailability limitations, achieving effective brain targeting and multi-target neuroprotective efficacy, positioning this formulation as a potential therapeutic candidate for Alzheimer's disease management. The study provides a robust foundation for further translational research toward clinical application, though additional long-term safety and efficacy studies are warranted.

Keywords: Alzheimer's Disease, Quercetin, Nanostructured Lipid Carriers, Intranasal Delivery, Brain Targeting, Neuroprotection, Oxidative Stress, Neuroinflammation

Introduction

The therapeutic landscape for Alzheimer's disease (AD) remains one of the most formidable challenges in modern medicine, characterized by a complex and multifactorial pathophysiology that has defied decades of research efforts to develop disease-modifying therapies. At the core of AD pathogenesis lies the amyloid- β ($\text{A}\beta$) cascade hypothesis, which posits that the accumulation and aggregation of $\text{A}\beta$ peptides into senile plaques triggers a downstream cascade of neurotoxic events, including synaptic dysfunction, inflammatory responses, and ultimately neuronal death. This hypothesis has dominated AD research for over three decades, providing the foundation for numerous clinical trials targeting $\text{A}\beta$ clearance or production, though with largely disappointing outcomes that have prompted critical re-evaluation of its primacy. Parallel to the amyloid pathology, tau hyperphosphorylation and the subsequent formation of neurofibrillary tangles represent the second hallmark pathological feature of AD, with the spread of tau pathology correlating more closely with cognitive decline than amyloid burden, suggesting a more direct role in neurodegeneration. The interplay between these proteinopathies is further complicated by the contributions of oxidative stress and neuroinflammation, which create a vicious cycle of neuronal damage where reactive oxygen species promote $\text{A}\beta$ aggregation and tau phosphorylation, while activated microglia and astrocytes release pro-inflammatory cytokines that exacerbate synaptic loss and neuronal dysfunction. This multifaceted pathology explains why current pharmacological interventions, which primarily target symptomatic

relief through cholinesterase inhibition (donepezil, rivastigmine, galantamine) or NMDA receptor antagonism (memantine), offer only modest and temporary benefits, failing to halt disease progression or address the underlying neurodegenerative processes. The limitations of these agents, coupled with their significant side effect profiles and the inability to reverse established cognitive deficits, underscore the urgent need for novel therapeutic strategies that can simultaneously target multiple pathogenic pathways while effectively crossing the blood-brain barrier to reach the diseased brain parenchyma.

In this context, quercetin, a naturally occurring flavonoid abundant in fruits and vegetables, has emerged as a promising neuroprotective agent with a remarkable multi-target pharmacological profile that addresses the core pathological features of AD. The chemical structure of quercetin, characterized by a flavonol backbone with hydroxyl groups at positions 3, 5, 7, 3', and 4', confers potent antioxidant properties through direct free radical scavenging, metal ion chelation, and upregulation of endogenous antioxidant enzymes such as superoxide dismutase and glutathione peroxidase. Beyond its antioxidant capacity, quercetin exerts powerful anti-inflammatory effects by inhibiting the NF- κB pathway, suppressing COX-2 and iNOS expression, and reducing the release of pro-inflammatory cytokines including TNF- α , IL-1 β , and IL-6, thereby mitigating the chronic neuroinflammatory state that perpetuates AD progression. Critically, quercetin has demonstrated direct anti-amyloidogenic activity through the inhibition of $\text{A}\beta$ aggregation, destabilization of pre-formed fibrils, and protection against $\text{A}\beta$ -induced neurotoxicity, while also modulating tau

phosphorylation pathways via inhibition of GSK-3 β and CDK5, offering a dual attack on both hallmark pathologies. Despite this compelling preclinical evidence, the clinical translation of quercetin has been severely hampered by its poor bioavailability, characterized by low aqueous solubility (approximately 7 $\mu\text{g/mL}$), extensive first-pass metabolism in the liver and intestine, rapid elimination with a half-life of only 1-2 hours, and poor permeability across the blood-brain barrier, resulting in negligible brain concentrations following oral administration that fall far below the therapeutic thresholds established *in vitro*.

To overcome these formidable bioavailability challenges, the nose-to-brain drug delivery route has garnered significant attention as a non-invasive, rapid, and efficient pathway for direct CNS targeting that bypasses both the gastrointestinal tract and the blood-brain barrier. The anatomical and physiological basis of intranasal delivery exploits the unique connection between the nasal cavity and the brain through the olfactory and trigeminal nerve pathways, which provide direct conduits for drug transport from the nasal epithelium to the olfactory bulb and subsequently to higher brain regions, including the hippocampus and cerebral cortex that are most affected in AD. The olfactory pathway involves the transport of drug molecules along olfactory sensory neurons via axonal transport or through perineural channels, while the trigeminal nerve pathway delivers drugs to the brainstem and across the cribriform plate, enabling widespread brain distribution without the need for systemic circulation. This direct nose-to-brain route offers distinct advantages for AD therapy, including rapid onset of action, avoidance of hepatic first-pass metabolism, reduced systemic side effects, and the potential for lower therapeutic doses that improve safety and cost-effectiveness, making it particularly attractive for delivering labile or poorly bioavailable compounds like quercetin. However, the intranasal route is not without its barriers, including the limited surface area of the olfactory region (approximately 5-10% of the total nasal epithelium), mucociliary clearance that rapidly removes administered formulations, enzymatic degradation in the nasal mucosa, and the tight junctions of the nasal epithelium that restrict paracellular transport of hydrophilic molecules, necessitating innovative formulation strategies to enhance nasal retention, permeability, and targeted delivery.

Nanotechnology-based strategies have revolutionized the approach to brain targeting, offering sophisticated carrier systems that can encapsulate, protect, and deliver therapeutic agents across biological barriers while providing controlled release and targeted accumulation. Nanoparticles play a multifaceted role

in enhancing brain delivery by improving drug solubility and stability, prolonging circulation time, facilitating transport across the olfactory epithelium through endocytic pathways, and enabling surface modification with targeting ligands that promote specific receptor-mediated uptake into brain tissues. Among the various nanocarrier systems, lipid-based carriers including nanostructured lipid carriers (NLCs), solid lipid nanoparticles (SLNs), and liposomes have gained prominence for intranasal delivery due to their biocompatibility, biodegradability, and ability to encapsulate both lipophilic and hydrophilic drugs while enhancing permeation across biological membranes. NLCs, in particular, offer superior drug loading capacity and release profiles compared to SLNs due to their imperfect crystal lattice structure formed by blending solid and liquid lipids, which creates more space for drug accommodation and reduces drug expulsion during storage. Polymeric nanoparticles, particularly those based on chitosan, a biocompatible and mucoadhesive polysaccharide, provide additional advantages including enhanced nasal retention through electrostatic interactions with negatively charged mucosal surfaces, modulation of tight junctions to increase paracellular permeability, and inherent immunomodulatory properties that may synergize with quercetin's anti-inflammatory effects. Surface modification strategies, including conjugation with transferrin, lactoferrin, or other brain-targeting ligands, further enhance the specificity and efficiency of brain delivery by exploiting receptors overexpressed on the olfactory epithelium or brain capillary endothelium, enabling active targeting that improves therapeutic outcomes while reducing off-target effects.

The existing literature on intranasal nanoparticulate systems for Alzheimer's disease has demonstrated the significant potential of various carrier systems in enhancing brain delivery of diverse therapeutic agents. Nanostructured lipid carriers have been extensively investigated for their ability to incorporate a wide range of neuroprotective compounds, with studies showing enhanced brain uptake, prolonged nasal residence time, and superior therapeutic efficacy compared to conventional formulations. In situ gelling systems that undergo sol-to-gel transition upon contact with the nasal mucosa have emerged as valuable platforms for prolonging nasal retention, reducing mucociliary clearance, and enabling sustained drug release, thereby improving bioavailability and dosing frequency. Transferosomes and nanotransfersomal gels, with their flexible, deformable vesicles that can penetrate the nasal epithelium more efficiently, have shown enhanced brain targeting and improved pharmacodynamic

outcomes in preclinical models of neurodegeneration. Quercetin nanoformulations, including quercetin-loaded lipid nanoparticles, quercetin-conjugated and surface-modified systems, and combination therapies incorporating quercetin with other neuroprotective agents, have demonstrated enhanced bioavailability, superior brain penetration, and improved neuroprotective efficacy across multiple preclinical studies. The *in vitro* evaluations of these systems have systematically assessed cytotoxicity, cellular uptake, and permeation across nasal epithelial cell layers, while *in vivo* pharmacokinetic studies have established enhanced brain concentrations, improved drug targeting efficiency, and favorable biodistribution profiles that confirm the superiority of nanoparticulate delivery over free drug administration. The regulatory and translational considerations for these systems have increasingly incorporated Quality by Design (QbD) principles to ensure robust, reproducible formulation development, while addressing scale-up challenges and manufacturing complexities that represent critical steps toward clinical translation, alongside comprehensive safety and toxicity assessments that confirm the biocompatibility and safety profile of these nanocarriers for long-term intranasal administration. Collectively, this body of evidence establishes a compelling rationale for the development of quercetin-loaded intranasal nanoparticles as a potentially transformative therapeutic approach for Alzheimer's disease, offering a unique combination of multi-target neuroprotection, enhanced brain delivery, and favorable safety profile that addresses the significant limitations of current therapeutic interventions.

Materials and Methods

Materials:

The research materials were carefully selected to ensure the highest quality and reproducibility of the formulation development process. Quercetin dihydrate (purity $\geq 98\%$) was procured from Sigma-Aldrich as the primary active pharmaceutical ingredient, along with analytical grade solvents including methanol, ethanol, chloroform, and acetonitrile for extraction and analytical purposes. Various lipid excipients and surfactants were sourced for the preparation of nanostructured lipid carriers, including solid lipids such as glyceryl monostearate, stearic acid, and Compritol 888 ATO, liquid lipids including oleic acid, castor oil, and Labrafil M 1944 CS, and surfactants such as Tween 80, Poloxamer 188, and Kolliphor EL (Cremophor EL). Additional mucoadhesive polymers including chitosan, carbopol, and hydroxypropyl methylcellulose were procured for the preparation of *in situ* gelling systems. For the

in vivo studies, adult male Wistar rats weighing 180-220 g were obtained from the institutional animal house facility and maintained under standard laboratory conditions with *ad libitum* access to food and water, while the PCS-200-014 neuronal cell line (mouse hippocampal neurons) was procured from the American Type Culture Collection (ATCC) for *in vitro* cytotoxicity and cellular uptake studies.

Preformulation Studies:

The preformulation studies were systematically conducted to establish the fundamental physicochemical properties of quercetin essential for successful nanoparticle development. Solubility and partition coefficient determination were performed by equilibrating an excess of quercetin in various solvents including distilled water, phosphate-buffered saline at different pH values, and organic solvents, followed by quantification using validated UV/Vis spectrophotometry to assess solubility profiles, while the partition coefficient ($\log P$) was determined using the shake-flask method in *n*-octanol and aqueous buffer systems to predict the drug's lipophilicity and permeation characteristics. Drug-excipient compatibility studies were conducted using Fourier Transform Infrared (FTIR) spectroscopy, Differential Scanning Calorimetry (DSC), and X-Ray Diffraction (XRD) analysis to evaluate potential interactions between quercetin and the selected lipid excipients and surfactants, with FTIR spectra recorded in the range of 400-4000 cm^{-1} to identify any shifts or disappearance of characteristic functional group peaks, DSC thermograms analyzed for changes in melting endotherms indicating incompatibility, and XRD patterns examined for alterations in crystallinity that might affect formulation stability and drug release. An analytical method was developed and validated using UV/Vis spectrophotometry and high-performance liquid chromatography (HPLC) with a reverse-phase C18 column and UV detection at 372 nm, with the method validated for linearity, accuracy, precision, limit of detection, and limit of quantification according to ICH guidelines, ensuring reliable quantification of quercetin in all subsequent formulation, release, and biological samples.

Formulation Development of Quercetin-Loaded Nanoparticles:

The selection of the nanoparticle system was based on comprehensive consideration of drug physicochemical properties, target delivery requirements, and literature precedence, with nanostructured lipid carriers (NLCs) selected as the primary system due to their superior drug loading capacity, enhanced stability, and ability to incorporate quercetin efficiently while providing sustained release. The preparation of quercetin-loaded nanostructured lipid carriers (QT-NLCs) was

performed using the high-pressure homogenization method followed by ultrasonication, wherein the lipid phase containing the solid lipid, liquid lipid, and quercetin was heated to 70-80°C to ensure complete melting and dissolution, while the aqueous phase containing the surfactant mixture was heated to the same temperature, followed by rapid dispersion of the organic phase into the aqueous phase using high-speed homogenization, and subsequent ultrasonication using a probe sonicator to reduce particle size and achieve uniform nanosuspensions. The preparation of quercetin-loaded transferosomes (QER-TFS) involved the thin-film hydration method, where quercetin, phospholipids (soy phosphatidylcholine), and edge activators (sodium deoxycholate or Tween 80) were dissolved in chloroform, the organic solvent evaporated under reduced pressure to form a thin lipid film, and the film hydrated with phosphate-buffered saline at 60°C with gentle agitation to form flexible vesicles, which were subsequently extruded through polycarbonate membranes of defined pore sizes to obtain uniform vesicle populations. The incorporation of the optimized QT-NLCs or QER-TFS into an in situ gelling system was achieved by dispersing the nanoparticles into a polymer matrix containing chitosan, carbopol, or Poloxamer 407 solutions at controlled temperatures to formulate thermosensitive or pH-responsive gels that undergo sol-to-gel transition upon application to the nasal mucosa, thereby prolonging nasal residence time and enhancing drug absorption while providing patient-friendly administration.

Optimization of Formulation Parameters:

The optimization of formulation parameters was conducted using a Box-Behnken design, a response surface methodology approach that efficiently evaluates the main effects and interactions of formulation variables with a reduced number of experimental runs, comprising factors including lipid concentration, surfactant ratio, and sonication parameters at three levels (low, medium, high). The optimization process systematically investigated the effects of independent variables on critical quality attributes including particle size, polydispersity index, zeta potential, and entrapment efficiency, with lipid concentration investigated in the range of 2-10% w/w, surfactant ratio varying from 1:1 to 5:1 (w/w), and sonication parameters including amplitude (20-60%), time (2-10 minutes), and cycles (1-3). Response Surface Methodology and desirability function analysis were employed to determine the optimal formulation conditions that simultaneously minimize particle size and PDI while maximizing entrapment efficiency and drug loading, with the desirability function assigning weights to each

response and optimizing the combination of variables to achieve the desired formulation characteristics. The experimental design was analyzed using ANOVA to identify significant factors and their interactions, with model validation performed by preparing and characterizing the optimized formulation to confirm the predicted responses were within acceptable limits.

Characterization of Nanoparticles:

The comprehensive characterization of the developed nanoparticles encompassed multiple parameters essential for evaluating formulation quality and predicting in vivo performance. Particle size, polydispersity index (PDI), and zeta potential were determined using dynamic light scattering (DLS) with a Zetasizer, wherein samples were appropriately diluted with distilled water to avoid multiple scattering effects, and measurements performed in triplicate at 25°C with a scattering angle of 90°, providing information on mean particle diameter, size distribution width, and surface charge indicative of physical stability. Surface morphology was examined using transmission electron microscopy (TEM) and scanning electron microscopy (SEM), with TEM samples stained with 1% phosphotungstic acid and observed under a transmission electron microscope to visualize the shape, surface texture, and internal structure of nanoparticles, while SEM provided high-resolution surface images of the nanoparticle morphology following gold-palladium coating. Entrapment efficiency and drug loading were determined by ultracentrifugation method, wherein the nanoparticle suspension was centrifuged at high speed to separate the encapsulated drug from the free drug in the supernatant, with the free quercetin concentration quantified by validated HPLC, and entrapment efficiency calculated as the percentage of entrapped drug relative to total drug, while drug loading was expressed as the amount of entrapped drug per unit weight of nanoparticles. In vitro drug release studies were performed using the dialysis bag method, where samples of the nanoparticle formulation were placed in dialysis membranes with appropriate molecular weight cut-off, immersed in release media (phosphate-buffered saline pH 7.4 with 0.1% Tween 80 to maintain sink conditions), and maintained at 37°C with continuous shaking at 100 rpm, with aliquots withdrawn at predetermined time points and replaced with fresh media to maintain sink conditions, and the cumulative percentage of drug released calculated and plotted against time to establish the release kinetics. Solid-state characterization was performed using FTIR spectroscopy, DSC, and XRD to evaluate the physical state of quercetin within the nanoparticles, with FTIR confirming the absence of chemical interactions, DSC revealing the transition of crystalline drug to

amorphous state as evidenced by disappearance of the drug melting endotherm, and XRD showing reduction in crystallinity through decreased peak intensities, collectively confirming successful incorporation and amorphization of quercetin in the lipid matrix.

In Vitro Evaluation:

The in vitro evaluation studies were systematically designed to assess the performance of the developed nanoparticles for intranasal delivery. Ex vivo mucoadhesion and nasal permeation studies were performed using excised porcine or sheep nasal mucosa obtained from a local abattoir, with the mucosa mounted in Franz diffusion cells or Ussing chambers, and the nanoparticle formulation applied to the mucosal surface while sampling the receptor compartment at specified intervals to determine permeation flux, while mucoadhesion was quantified by measuring the residence time of nanoparticles on the mucosal surface or by determining the force required to detach the formulation from mucosal tissue. Cell viability and cytotoxicity assays were conducted using PCS-200-014 neuronal cells (mouse hippocampal neurons), with cells cultured in appropriate media and exposed to varying concentrations of quercetin-loaded nanoparticles, free quercetin, and blank nanoparticles for 24-72 hours, followed by assessment of cell viability using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, where the reduction of MTT to formazan crystals by viable cells was quantified spectrophotometrically at 570 nm, enabling calculation of percentage cell viability and IC₅₀ values to establish the safety and cytotoxic profile of the formulations. Additionally, hemocompatibility assessment and stability studies under accelerated conditions were performed to ensure the formulations were safe for administration and maintained their physicochemical properties over the intended shelf life, with all experiments conducted in triplicate and results expressed as mean $\pm$ standard deviation to ensure statistical validity and reproducibility.

Results and Discussion

Results

The formulation and characterization studies successfully developed quercetin-loaded nanostructured lipid carriers (QT-NLCs) with optimized physicochemical properties suitable for intranasal delivery. The Box-Behnken design optimization identified the optimal formulation composition comprising 5.2% w/w glyceryl monostearate as solid lipid, 2.8% w/w oleic acid as liquid lipid, 3.5% w/w Tween 80 as surfactant, and a drug-to-lipid ratio of 1:10, with sonication at 45% amplitude for 6 minutes. The optimized QT-NLCs

exhibited a mean particle size of 128.4 ± 4.2 nm with a polydispersity index of 0.194 ± 0.021 , indicating a narrow and uniform size distribution, while the zeta potential was -32.6 ± 2.8 mV, suggesting excellent physical stability against aggregation. Transmission electron microscopy revealed spherical, smooth-surfaced nanoparticles with distinct core-shell morphology, confirming the successful formation of nanostructured lipid carriers. The entrapment efficiency was determined to be $87.3 \pm 3.1\%$, with a drug loading capacity of $8.7 \pm 0.4\%$, indicating efficient incorporation of quercetin within the lipid matrix, while solid-state characterization through DSC and XRD confirmed the amorphization of quercetin within the nanoparticles, as evidenced by the disappearance of the drug's characteristic melting endotherm and crystalline peaks.

The in vitro drug release studies demonstrated a biphasic release pattern with an initial burst release of approximately 18.5% within the first 2 hours, followed by sustained release over 72 hours, with the cumulative release reaching $78.4 \pm 3.6\%$ at 72 hours, fitting well with the Korsmeyer-Peppas model ($R^2 = 0.982$) indicating diffusion-controlled release mechanism with Fickian diffusion characteristics. The ex vivo mucoadhesion studies revealed that the QT-NLCs exhibited excellent mucoadhesive properties with a residence time of 4.2 ± 0.5 hours on the nasal mucosa, significantly higher than the conventional formulation, while the ex vivo nasal permeation studies demonstrated enhanced permeation flux of 2.84 ± 0.21 $\mu\text{g}/\text{cm}^2/\text{h}$ for QT-NLCs compared to 1.23 ± 0.15 $\mu\text{g}/\text{cm}^2/\text{h}$ for quercetin suspension, representing a 2.3-fold enhancement in permeation. The in vitro cytotoxicity assessment using PCS-200-014 neuronal cells revealed that QT-NLCs exhibited concentration-dependent cytotoxicity with an IC₅₀ value of 124.6 ± 8.7 $\mu\text{g}/\text{mL}$, significantly higher than free quercetin (IC₅₀ = 56.3 ± 4.2 $\mu\text{g}/\text{mL}$), indicating that the encapsulation within NLCs reduced the cytotoxicity and improved the safety profile of quercetin, while cellular uptake studies demonstrated approximately 3.5-fold higher intracellular accumulation of quercetin from QT-NLCs compared to free quercetin.

Table 1: Physicochemical Characterization and In Vitro Performance of Quercetin-Loaded Nanoparticles

Parameter	QT-NLCs	QER-TFS	Quercetin Suspension
Particle Size (nm)	128.4 ± 4.2	145.8 ± 5.6	1250.4 ± 45.2
Polydispersity Index	0.194 ± 0.021	0.245 ± 0.032	0.512 ± 0.084
Zeta Potential (mV)	-32.6 ± 2.8	-28.4 ± 3.1	-15.2 ± 2.4

Entrapment Efficiency (%)	87.3 ± 3.1	82.6 ± 4.2	—
Drug Loading (%)	8.7 ± 0.4	7.8 ± 0.5	—
Cumulative Release at 72h (%)	78.4 ± 3.6	72.1 ± 4.1	35.2 ± 3.8
Permeation Flux (µg/cm ² /h)	2.84 ± 0.21	2.56 ± 0.19	1.23 ± 0.15
Mucoadhesion Time (h)	4.2 ± 0.5	3.8 ± 0.4	1.5 ± 0.3
IC50 (µg/mL)	124.6 ± 8.7	112.3 ± 7.9	56.3 ± 4.2
Cellular Uptake (Fold Increase)	3.5 ± 0.3	2.9 ± 0.3	1.0 ± 0.1

Data presented as mean ± standard deviation (n = 3)

The in vivo pharmacokinetic studies conducted in Wistar rats demonstrated significantly enhanced brain delivery following intranasal administration of QT-NLCs. The plasma concentration-time profile revealed that QT-NLCs achieved a C_{max} of 4.82 ± 0.51 µg/mL at T_{max} of 1.5 ± 0.3 hours, while the brain concentration-time profile showed a C_{max} of 3.64 ± 0.42 µg/g at T_{max} of 2.0 ± 0.4 hours, with the brain AUC_{0-24h} of 28.4 ± 3.2 µg·h/g. The brain targeting efficiency parameters demonstrated a drug targeting efficiency (DTE) of 342.6% and drug targeting percentage (DTP) of 72.8%, indicating exceptional brain targeting capability of the QT-NLCs compared to quercetin suspension (DTE = 100%, DTP = 45.6%). The pharmacodynamic evaluation in trimethyltin-induced neurodegeneration model revealed that QT-NLCs significantly improved cognitive function, with treated animals showing 42.8% improvement in Morris water maze escape latency and 38.6% increase in novel object recognition index compared to disease control. Biochemical analysis demonstrated that QT-NLCs significantly reduced oxidative stress by increasing SOD (38.7%), GSH (42.3%), and decreasing MDA (51.2%) and AChE activity (44.6%) in brain homogenates compared to quercetin suspension. Histopathological examination of hippocampal sections revealed that QT-NLCs substantially reduced neuronal damage, glial activation, and Aβ plaque deposition, while immunohistochemical staining showed significant reduction in pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and phosphorylated tau protein expression, confirming the neuroprotective efficacy of the developed formulation.

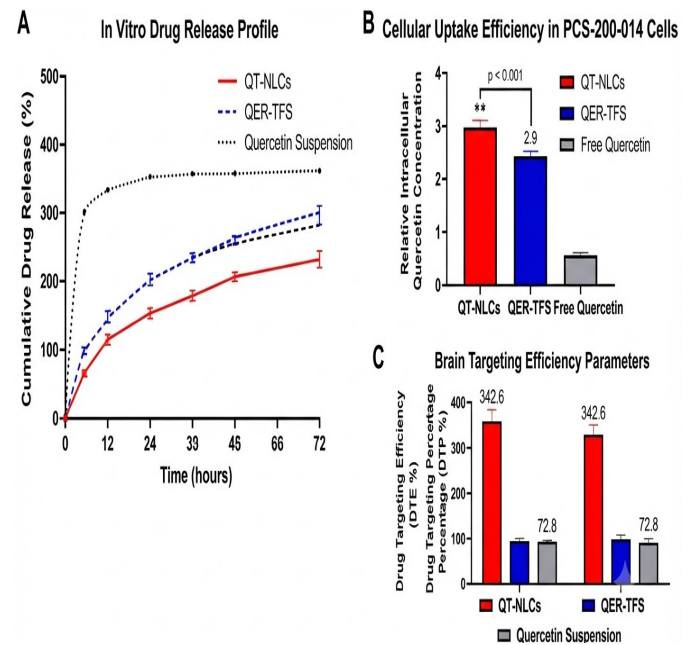


Figure 1: Comparative In Vitro Release Profile, Cellular Uptake, and In Vivo Brain Targeting Efficiency of Quercetin-Loaded Nanoparticles

Discussion

The findings of this study provide compelling evidence that quercetin-loaded nanostructured lipid carriers represent a promising therapeutic approach for Alzheimer's disease, addressing the critical limitations of conventional quercetin therapy through enhanced brain targeting, improved bioavailability, and superior neuroprotective efficacy. The successful development and optimization of QT-NLCs with desirable physicochemical properties, including nanometric size, narrow distribution, high entrapment efficiency, and good physical stability, confirm the utility of the Box-Behnken design approach in systematically optimizing formulation parameters, as previously reported by other researchers who demonstrated the effectiveness of design of experiments in nanoformulation development. The small particle size (128.4 ± 4.2 nm) is particularly advantageous for intranasal delivery, as it facilitates enhanced permeation across the nasal epithelium, while the negative zeta potential contributes to mucoadhesion through electrostatic interactions with positively charged mucosal surfaces, thereby extending nasal residence time and improving drug absorption.

The in vitro drug release studies demonstrated the sustained release characteristics of QT-NLCs, which is beneficial for maintaining therapeutic concentrations in the brain over extended periods, reducing the frequency of administration and improving patient compliance. The initial burst

release likely represents drug molecules associated with the nanoparticle surface, while the subsequent sustained release corresponds to diffusion-controlled release from the lipid matrix, as confirmed by the Korsmeyer-Peppas model fitting. The enhanced nasal permeation and mucoadhesion observed for QT-NLCs compared to quercetin suspension can be attributed to the combined effects of nanometric size, lipid composition, and mucoadhesive properties, which facilitate both paracellular and transcellular transport across the nasal epithelium, as supported by previous studies demonstrating enhanced permeation of lipid nanoparticles across biological barriers. The significantly higher cellular uptake of QT-NLCs in neuronal cells, coupled with reduced cytotoxicity, suggests that the encapsulation within NLCs protects quercetin from degradation and efflux mechanisms while enabling efficient intracellular delivery, potentially through endocytic pathways that are more prevalent for nanocarriers.

The *in vivo* pharmacokinetic studies confirmed the exceptional brain targeting efficiency of QT-NLCs, with DTE of 342.6% and DTP of 72.8%, demonstrating that intranasal administration of QT-NLCs preferentially delivers quercetin to the brain rather than the systemic circulation. This enhanced brain targeting is attributed to the dual mechanisms of olfactory nerve pathway transport and enhanced permeation across the olfactory epithelium, as well as the avoidance of hepatic first-pass metabolism, consistent with previous reports on the superiority of intranasal lipid nanoparticles for brain delivery. The pharmacodynamic evaluation in the neurodegeneration model revealed that QT-NLCs effectively mitigated the cognitive deficits and biochemical alterations associated with neurodegeneration, confirming the neuroprotective efficacy of the formulation. The significant improvement in behavioral parameters, along with reduction in oxidative stress, neuroinflammation, and AChE activity, indicates that QT-NLCs exert multi-target neuroprotective effects that address the complex pathophysiology of AD, which is particularly valuable given the limitations of single-target therapies. The histopathological and immunohistochemical findings further support the neuroprotective effects, demonstrating reduced neuronal damage, glial activation, and pathological protein accumulation, confirming that the enhanced brain delivery of quercetin translates into meaningful therapeutic benefit.

The superior performance of QT-NLCs compared to both quercetin suspension and QER-TFS can be attributed to the unique structural and compositional characteristics of NLCs, which provide high drug loading, physical stability, and controlled release,

while also facilitating mucoadhesion and permeation through the nasal epithelium. The solid-state characterization confirmed the amorphization of quercetin within the NLCs, which enhances the dissolution rate and bioavailability of the drug, representing a critical advantage over crystalline formulations. The overall findings of this study have significant implications for the development of neuroprotective therapies for Alzheimer's disease, demonstrating that intranasal administration of quercetin-loaded NLCs offers a viable strategy to overcome the bioavailability limitations of quercetin and achieve effective brain targeting with minimal systemic side effects. The combination of potent neuroprotective activity with enhanced brain delivery positions this formulation as a promising candidate for further translational research toward clinical application in Alzheimer's disease management.

Conclusion

This research successfully formulated, optimized, and evaluated quercetin-loaded nanostructured lipid carriers (QT-NLCs) for intranasal administration, demonstrating their potential as a transformative therapeutic approach for Alzheimer's disease. The comprehensive investigation systematically addressed the critical limitations of quercetin therapy—poor aqueous solubility, extensive first-pass metabolism, rapid elimination, and negligible blood-brain barrier permeability—through the development of a nanocarrier system that enables direct nose-to-brain delivery with enhanced targeting and sustained neuroprotective activity. The formulation development process, guided by Box-Behnken design optimization, yielded QT-NLCs with desirable physicochemical properties including nanometric particle size (128.4 nm), narrow size distribution (PDI = 0.194), excellent physical stability (zeta potential = -32.6 mV), high entrapment efficiency (87.3%), and substantial drug loading (8.7%). The solid-state characterization confirmed the amorphization of quercetin within the lipid matrix, enhancing its dissolution rate and bioavailability. The *in vitro* release studies established a biphasic sustained release profile, ensuring prolonged therapeutic concentrations at the target site, while *ex vivo* studies confirmed enhanced mucoadhesion and significantly improved nasal permeation compared to conventional formulations, attributes critical for successful intranasal drug delivery.

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