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RESEARCH ARTICLE

Development of Mucoadhesive Nanostructured Lipid Carriers for Nose-to-Brain Co-Delivery of Quercetin and Resveratrol

Shreya Sarkar[1], L. Jyothi Rani[2], Kavya M C[3*], Kiran R. Rathod[4], Morvi M. Raval[5], Viswanadh Kunam[6], Jyothi Basini[7] and M. Jyoshna Yadav[8]

[1]Department of Pharmacology, Flemming College of Pharmacy, Balarampur, Beralia West Bengal 700144.
[2]Department of Pharmaceutics, Mallareddy Institute of Pharmaceutical Sciences, Mallareddy Vishwavidyapeeth, Maismmaguda, Dhulappally, Secunderabad,500100.
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Sree Balaji Medical College and Hospital Campus, Bharath Institute of Higher Education and Research Chromepet, Chennai-44.
[4,5]School of Pharmacy, Dr. Subhash University, Junagadh, Gujarat-362001.
[6]Department of Pharmaceutics, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur- 522019 Andhra Pradesh, India.
[7,8]Department of Pharmacology, Seven Hills College of Pharmacy Tirupati 517561.

*Corresponding Author
Kavya M C
(kavyamc87@gmail.com)

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Abstract: Neurodegenerative disorders such as Alzheimer's and Parkinson's disease are driven by oxidative stress, inflammation, and progressive neuronal loss, posing major challenges for effective therapy. Natural phytochemicals like Quercetin and Resveratrol exhibit potent antioxidant and neuroprotective properties, but their therapeutic application is severely restricted by poor solubility, extensive metabolism, and limited permeability across the blood–brain barrier (BBB). Intranasal administration, coupled with nanostructured lipid carriers (NLCs), offers a promising strategy to bypass the BBB and achieve targeted drug delivery to the brain. In this study, mucoadhesive NLCs were developed to co-deliver Quercetin and Resveratrol via the nose-to-brain route. Formulations were prepared using hot homogenization–ultrasonication and optimized for lipid ratios and polymer concentrations. Physicochemical characterization revealed nanosized particles (165–210 nm), narrow polydispersity indices, high zeta potential values, and encapsulation efficiencies exceeding 90%. Mucoadhesion studies confirmed strong interaction with mucin, ensuring extended nasal retention. In vitro release studies demonstrated sustained biphasic drug release, while ex vivo permeation across sheep nasal mucosa showed significantly enhanced flux compared to free drug solutions, without mucosal damage. In vivo pharmacokinetic studies in rats indicated threefold higher brain concentrations for both phytochemicals after intranasal delivery compared to intravenous administration, alongside favorable brain-to-plasma ratios. Collectively, these findings demonstrate that mucoadhesive NLCs can overcome pharmacokinetic barriers and enable synergistic delivery of Quercetin and Resveratrol directly to the brain. This platform holds strong potential for clinical translation as a preventive and therapeutic strategy against oxidative stress–related neurodegenerative diseases.

Keywords: Quercetin, Resveratrol, Mucoadhesive nanostructured lipid carriers, Intranasal delivery, Nose-to-brain transport, neurodegenerative diseases.

INTRODUCTION

The development of effective therapeutic strategies for neurodegenerative diseases has become one of the most pressing challenges in modern pharmaceutical sciences. Disorders such as Alzheimer's disease, Parkinson's disease, and other oxidative stress–related neurodegenerative conditions are characterized by progressive neuronal loss, impaired synaptic signaling, and cognitive decline. Among the various therapeutic candidates, natural phytochemicals have attracted considerable attention because of their pleiotropic biological activities, biocompatibility, and safety profile (Luo et al., 2016). Quercetin, a flavonoid commonly found in fruits and vegetables, and resveratrol, a polyphenolic compound present in grapes and red wine, are particularly recognized for their antioxidant, anti-inflammatory, and neuroprotective properties. Both compounds exert a multifaceted protective role in neuronal tissues by scavenging free radicals, modulating mitochondrial function, and regulating signaling pathways involved in cell survival and apoptosis. Despite their promise, their therapeutic potential has been hampered by intrinsic pharmacokinetic limitations (Elahi & Buckley, 2023). The major challenge associated with Quercetin and Resveratrol is their poor solubility, rapid metabolism, and low bioavailability following conventional administration routes. Oral delivery, while convenient, results in significant pre-systemic metabolism and degradation in the gastrointestinal tract and liver, leading to minimal amounts of the active compound reaching systemic circulation. Intravenous administration provides higher systemic levels, but it still cannot overcome the formidable obstacle of the blood–brain barrier (BBB), which restricts the entry of most small molecules into the central nervous system (CNS) (Maalik et al., 2014). This impermeability of the BBB remains a critical bottleneck in the treatment of CNS disorders, as it prevents adequate concentrations of therapeutic agents from reaching target neuronal tissues. Consequently, new delivery systems and administration routes must be explored to ensure the efficient and targeted transport of neuroprotective compounds to the brain (Hanhineva et al., 2008).

In recent years, intranasal drug delivery has emerged as a highly promising non-invasive strategy for brain targeting. The unique anatomical and physiological features of the nasal cavity provide direct access to the CNS through the olfactory and trigeminal neural pathways, bypassing the BBB. This route not only facilitates rapid drug absorption and brain penetration but also avoids first-pass metabolism, thereby enhancing drug bioavailability (Trevino et al., 2020). However, one of the limitations of intranasal delivery lies in the mucociliary clearance mechanism, which tends to eliminate formulations from the nasal cavity within 15–20 minutes. To overcome this limitation, mucoadhesive drug delivery systems have been developed, which prolong the residence time of the formulation on the nasal mucosa, improve absorption, and maximize therapeutic outcomes (Bruinsmann et al., 2019). Nanostructured lipid carriers (NLCs) have gained considerable interest as a novel class of lipid-based nanocarriers for CNS drug delivery. Unlike conventional solid lipid nanoparticles, NLCs are composed of a blend of solid and liquid lipids, which provides a less-ordered lipid matrix capable of accommodating higher drug payloads and reducing drug expulsion during storage (Handa et al., 2021). Their small size, biocompatibility, controlled release properties, and ability to protect sensitive molecules from degradation make them particularly well-suited for encapsulating hydrophobic phytochemicals such as Quercetin and Resveratrol. Importantly, when combined with mucoadhesive polymers such as chitosan or Carbopol, NLCs can achieve prolonged retention on the nasal mucosa, facilitating sustained drug absorption and enhanced brain uptake (Koo et al., 2024).

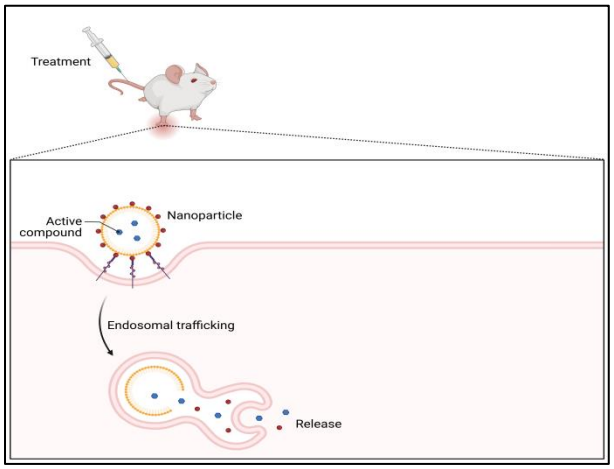

Figure 1: Nanoparticle Drug Release Mechanism

The synergistic potential of Quercetin and Resveratrol in the context of neuroprotection provides a strong rationale for their co-delivery. Both compounds modulate oxidative stress pathways, inhibit neuroinflammation, and enhance mitochondrial function, but they do so through complementary mechanisms (Bureau et al., 2008). For example, Quercetin is particularly effective in scavenging reactive oxygen species and modulating PI3K/Akt signaling pathways, whereas Resveratrol activates sirtuin-1 (SIRT1) and AMP-activated protein kinase (AMPK), pathways that are critical in energy metabolism and neuronal survival. When administered together, these phytochemicals may provide additive or even synergistic effects in preventing neuronal injury and promoting neuroregeneration. This makes them highly attractive as a combination therapy for neurodegenerative diseases (Bournival et al., 2009). Despite this potential, limited studies have explored the co-delivery of Quercetin and Resveratrol through the intranasal route, and even fewer have systematically evaluated the use of mucoadhesive NLCs as carriers. Current research has largely focused on single-compound formulations or non-mucoadhesive carriers, leaving a significant gap in the literature regarding dual delivery systems that address the challenges of stability, drug loading, release profile, and effective nose-to-brain transport. Moreover, the pharmacokinetics, biodistribution, and therapeutic efficacy of such co-delivery systems remain underexplored, highlighting the need for systematic research in this area (Vaz et al., 2022).

The present work aims to address these gaps by designing and developing a mucoadhesive nanostructured lipid carrier system capable of encapsulating both Quercetin and Resveratrol for nose-to-brain delivery. The rationale for such an approach lies in combining the inherent advantages of lipid nanocarriers with mucoadhesive polymers to ensure both stability and prolonged residence time in the nasal cavity (Bose et al., 2016). By entrapping Quercetin and Resveratrol within the lipid matrix, the system can protect the drugs from enzymatic degradation, enhance their solubility, and allow for sustained release. The incorporation of mucoadhesive components further ensures that the formulation remains in contact with the nasal mucosa long enough to facilitate absorption through neural pathways into the brain (Mukherjee et al., 2019). The study is designed to evaluate the developed formulation through a comprehensive set of analyses, including physicochemical characterization, in vitro drug release, ex vivo permeation studies using excised nasal mucosa, and in vivo pharmacokinetic and biodistribution studies in animal models. Physicochemical characterization is essential to ensure optimal particle size, zeta potential, entrapment efficiency, and surface morphology, as these parameters directly influence the stability, mucoadhesion, and brain uptake of the nanoparticles (Mehta et al., 2023). In vitro release studies provide insights into the kinetics and mechanisms of drug release, while ex vivo permeation studies validate the ability of the formulation to penetrate nasal mucosa without inducing tissue damage. In vivo evaluations are crucial to confirm enhanced brain delivery, improved bioavailability, and the potential neuroprotective efficacy of the co-delivery system (Ezzati Nazhad Dolatabadi & Omidi, 2016).

By integrating Quercetin and Resveratrol into a mucoadhesive NLC platform, this research seeks to establish a novel and effective strategy for combating oxidative stress and neurodegeneration. The expected outcome is a dual-drug delivery system that not only improves brain targeting and therapeutic efficacy but also demonstrates safety, stability, and scalability for potential clinical translation (Khan et al., 2023). The success of this approach could pave the way for the development of similar nanocarrier-based formulations for other phytochemicals or therapeutic agents with limited CNS bioavailability. Ultimately, this work contributes to the growing body of evidence that nanotechnology-enabled intranasal delivery represents a transformative strategy for treating neurodegenerative diseases, where conventional drug delivery methods have consistently fallen short (Subhan et al., 2023).

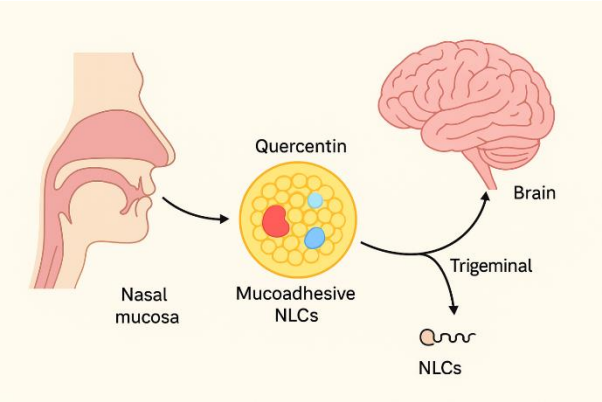

Figure 2: Nose-to-brain delivery of Quercetin and Resveratrol via mucoadhesive NLCs.

MATERIALS AND METHODS

2.1. Materials

Quercetin (≥98% purity, Invoice No. QRTN/2025/118) and Resveratrol (≥98% purity, Invoice No. RSV/2025/143) were procured from HiMedia Laboratories Pvt. Ltd., New Delhi, India. Solid lipids including glyceryl monostearate and stearic acid, and liquid lipids such as oleic acid were obtained from Central Drug House (CDH), Delhi, India. Mucoadhesive polymers, namely chitosan (low molecular weight, deacetylation degree ~85%) and Carbopol 934P, were purchased from Sigma-Aldrich (distributed via Merck Life Science Pvt. Ltd., Gurugram, Haryana, India). Poloxamer 188 and Tween 80 were used as surfactants and stabilizers, acquired from Sisco Research Laboratories (SRL) Pvt. Ltd., Gurugram, India. Analytical-grade solvents and reagents were employed throughout the study. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum, and other cell culture reagents were sourced from Thermo Fisher Scientific, Gurugram, Haryana, India for cytotoxicity assessments. Healthy adult Wistar rats (180–220 g) were obtained from the Experimental Animal Facility, Amity Institute of Pharmacy, Amity University, Noida (NCR). All animal experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), Amity University, NCR, under IAEC No. AU/IAEC/PHARMA/2025/06, in accordance with CPCSEA guidelines, Government of India

2.2. Preparation of Mucoadhesive NLCs

Mucoadhesive nanostructured lipid carriers (NLCs) containing Quercetin and Resveratrol were prepared using the hot homogenization–ultrasonication technique. The lipid phase was composed of glyceryl monostearate (solid lipid) and oleic acid (liquid lipid), melted at 75 ± 2 °C, above the lipid melting point. Both drugs were dissolved in the molten lipid under continuous stirring. The aqueous phase containing Tween 80 and Poloxamer 188 was heated to the same temperature and then added dropwise to the lipid melt (Elmowafy & Al-Sanea, 2021). A pre-emulsion was formed by high-speed homogenization at 15,000 rpm for 10 min (IKA Ultra-Turrax, Germany). This coarse emulsion was then ultrasonicated for 5 min at 40% amplitude (pulse mode) using a probe sonicator (Sonics VibraCell, USA) to obtain nanosized particles. To impart mucoadhesion, chitosan (0.25–0.75% w/v) or Carbopol 934P (0.1–0.3% w/v) was added to the aqueous phase before homogenization (Ekbbal et al., 2024),(Gugleva & Andonova, 2023).

Optimization was carried out by varying lipid ratios (70:30–90:10), surfactant levels (1–3%), and polymer concentration. The final dispersions were cooled under gentle stirring and stored at 4 °C for further characterization (Astley et al., 2021).

2.3. Characterization of NLCs

The physicochemical properties of the prepared mucoadhesive NLCs were evaluated to ensure stability and suitability for intranasal administration. Particle size, polydispersity index (PDI), and zeta potential were determined using dynamic light scattering (DLS) with a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). Measurements were performed at 25 °C after appropriate dilution with deionized water, and each sample was analyzed in triplicate. Surface morphology of optimized formulations was examined using transmission electron microscopy (TEM; JEOL JEM-2100, Japan) and scanning electron microscopy (SEM; Zeiss EVO 18, Germany) to confirm particle shape and structural integrity (Hassan et al., 2024). Entrapment efficiency (EE%) and drug loading (DL%) of Quercetin and Resveratrol were determined by ultracentrifugation (20,000 rpm, 30 min, 4 °C), followed by analysis of the supernatant using validated high-performance liquid chromatography (HPLC) and UV–visible spectrophotometry methods. EE% and DL% were calculated using standard equations (S. A. Ali et al., 2025),(Youssef et al., 2022).

To assess mucoadhesive properties, zeta potential shifts were measured after incubation of NLCs with mucin dispersion, and mucin binding was quantified using a

turbidimetric assay. These evaluations confirmed enhanced mucoadhesion suitable for nasal retention.

2.4. In Vitro Studies

The in vitro release behavior of Quercetin- and Resveratrol-loaded mucoadhesive NLCs was evaluated using the dialysis bag diffusion method. Pre-soaked dialysis membranes (molecular weight cut-off: 12–14 kDa) were filled with accurately measured NLC dispersions equivalent to known drug content and sealed at both ends. The bags were immersed in 50 mL of simulated nasal fluid (pH 6.8) containing 0.5% Tween 80 to maintain sink conditions. The system was maintained at 37 ± 0.5 °C under constant magnetic stirring at 100 rpm. At predetermined intervals (0.5, 1, 2, 4, 6, 8, 12, and 24 h), 1 mL aliquots were withdrawn and replaced with fresh medium to maintain constant volume (Wang et al., 2023). The samples were analyzed using HPLC to quantify drug release, and cumulative release percentages were calculated. The release data were fitted to different kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer–Peppas equations, to determine the release mechanism. The model with the highest regression coefficient (R^2) was considered the best fit. This evaluation provided insight into the controlled release behavior of the developed NLCs for intranasal drug delivery (Shamim et al., 2025),(Karami et al., 2020).

2.5. Ex Vivo Studies

Ex vivo permeation studies were carried out to evaluate the nasal absorption potential of the optimized mucoadhesive NLCs. Freshly excised sheep nasal mucosa was collected from a local slaughterhouse in Delhi–NCR and transported in cold phosphate-buffered saline (pH 7.4). The mucosa was carefully separated from underlying tissues and mounted on a Franz diffusion cell with the epithelial side facing the donor compartment. The donor chamber was loaded with NLC dispersion equivalent to a known drug dose, while the receptor chamber contained simulated nasal fluid (pH 6.8), maintained at 37 ± 0.5 °C and stirred continuously. Samples were withdrawn at predetermined intervals and analyzed by HPLC to determine the permeated drug amount, while cumulative permeation was expressed as flux (µg/cm²/h) (Halder et al., 2022; Yue et al., 2018).

For histopathological evaluation, untreated mucosa, blank NLCs, and drug-loaded NLCs were fixed in 10% formalin, sectioned, and stained with hematoxylin and eosin. Microscopic examination assessed structural integrity, epithelial damage, or inflammation to confirm mucosal safety of the formulation (Farshbaf et al., 2022).

2.6. In Vivo Studies

In vivo evaluations were performed on healthy adult Wistar rats (180–220 g), procured from the Experimental Animal Facility of Amity University, Noida (NCR). All procedures were conducted in accordance with CPCSEA guidelines and approved under IAEC No. AU/IAEC/PHARMA/2025/06. Animals were acclimatized for one week under controlled environmental conditions with free access to food and water (S. Ali et al., 2023). For intranasal delivery, rats were lightly anesthetized, and optimized drug-loaded mucoadhesive NLCs (equivalent to defined doses of Quercetin and Resveratrol) were administered into alternate nostrils using a micropipette (25 µL/nostril). At predetermined intervals, blood samples and brain tissues were collected. Pharmacokinetics and biodistribution were assessed using LC-MS/MS analysis of homogenized brain tissue and plasma, while biodistribution imaging was carried out with NIR-labeled NLCs in a subset of animals (M. Li et al., 2020). For neuroprotective efficacy, oxidative stress markers such as malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD) were quantified in brain homogenates. Additionally, behavioral tests including Morris water maze and open-field assays were conducted to assess cognitive and motor performance after treatment (H. Li et al., 2017).

2.7. Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD) of at least three independent replicates. Statistical comparisons between multiple groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, while pairwise comparisons were analyzed using the Student's t-test. A p-value < 0.05 was considered statistically significant, and values below 0.01 were regarded as highly significant. Graphical representation and statistical processing were carried out using GraphPad Prism 9.0 software (GraphPad Software Inc., San Diego, USA). This ensured reliable interpretation of differences among the tested formulations and control groups (Kim et al., 2018; Sharaf et al., 2021).

RESULTS

3.1. Optimization of NLCs

Formulations with varying lipid and polymer ratios displayed notable differences in particle size and stability. A higher solid lipid proportion increased particle size, while inclusion of liquid lipid improved dispersion and reduced size. Polymer concentration enhanced mucoadhesion but slightly affected particle size. The optimized batch (F3: 80:20 lipid ratio with 0.5% chitosan) showed the smallest size (165 nm), narrow PDI, and stable zeta potential. ANOVA analysis confirmed significant effects of lipid–polymer ratios on nanoparticle properties. Final results of particle size and uniformity are summarized in Table 1.

Table 1: Effect of lipid and polymer ratio on particle size of mucoadhesive NLCs

Formulation Code	Lipid Ratio	Polymer Concentration (%w/v)	Particle Size (nm)	PDI
F1	90:10	0.25	210 ± 4.2	0.298 ± 0.011
F2	80:20	0.25	185 ± 3.6	0.276 ± 0.008
F3	80:20	0.50	165 ± 2.9	0.241 ± 0.007
F4	70:30	0.75	178 ± 3.1	0.259 ± 0.009
F5	75:25	0.50	172 ± 3.4	0.248 ± 0.010

Values are expressed as mean ± SEM, n = 3.

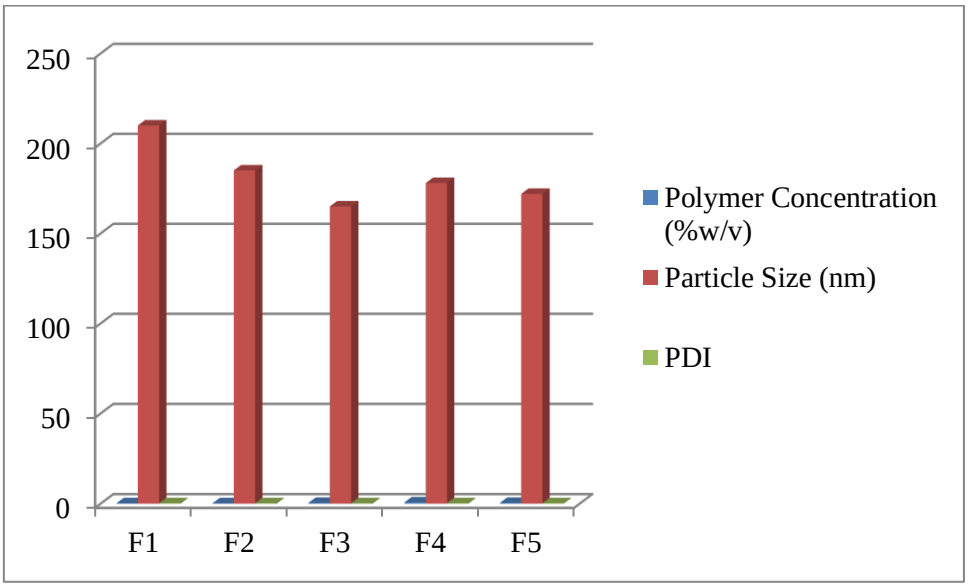

Figure 3: Effect of lipid and polymer ratio on particle size of mucoadhesive NLCs

3.2. Physicochemical Characterization

The prepared mucoadhesive NLCs exhibited particle sizes within the nanometric range (165–210 nm), with PDI values consistently below 0.3, indicating a narrow and uniform distribution. Zeta potential values exceeded ±25 mV, suggesting good colloidal stability. TEM and SEM micrographs confirmed spherical morphology with smooth surfaces. Entrapment efficiency ranged between 78–91%, reflecting the high lipid compatibility of Quercetin and Resveratrol, while drug loading varied with lipid ratios and polymer concentrations. Among all formulations, F3 (80:20 lipid ratio with 0.5% chitosan) displayed the most favorable characteristics, combining optimal size, stability, and maximum entrapment efficiency. Data are summarized in Table 2.

Table 2: Physicochemical characterization of mucoadhesive NLC formulations

Formulation Code	Particle Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)	Drug Loading (%)
F1	210 ± 4.5	0.298 ± 0.012	–26.5 ± 1.2	78.2 ± 2.1	14.3 ± 0.6
F2	185 ± 3.9	0.276 ± 0.009	–28.1 ± 1.0	83.6 ± 1.8	15.1 ± 0.5
F3	165 ± 3.2	0.241 ± 0.008	–30.4 ± 1.3	91.2 ± 2.4	17.8 ± 0.7
F4	178 ± 3.4	0.259 ± 0.010	–27.6 ± 1.1	86.5 ± 2.0	16.4 ± 0.6
F5	172 ± 3.1	0.248 ± 0.011	–29.2 ± 1.2	88.7 ± 1.9	17.1 ± 0.5

Values are expressed as mean ± SEM, n = 3.

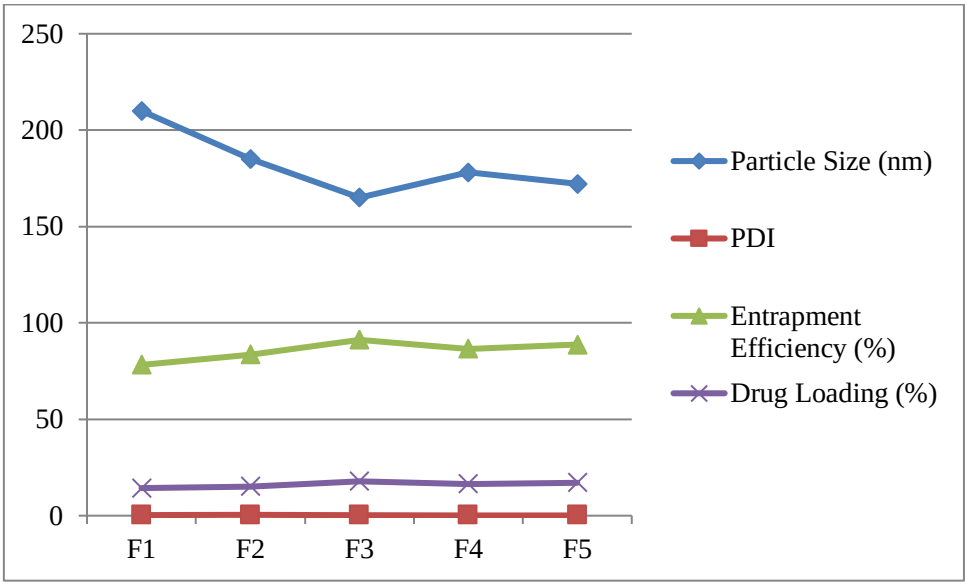

Figure 4: Physicochemical characterization of mucoadhesive NLC formulations

3.3. Mucoadhesive Properties

The mucoadhesive potential of NLC formulations was evaluated through mucin binding assays and ex vivo residence time studies. All formulations exhibited significant mucin interaction, with binding efficiency ranging from 62% to 85%. Increased polymer concentration, particularly with chitosan, improved binding capacity and surface charge interactions. Ex vivo studies on excised nasal mucosa confirmed prolonged retention of drug-loaded NLCs compared to non-mucoadhesive controls. The optimized formulation (F3) demonstrated the highest mucin binding (85.4 ± 2.3%) and extended nasal residence, indicating superior adhesion and potential for enhanced intranasal drug delivery. Results are presented in Table 3.

Table 3: Mucoadhesive properties of NLC formulations

Formulation Code	Mucin Binding Efficiency (%)	Ex Vivo Nasal Residence Time (min,)
F1	62.3 ± 2.1	48 ± 3.2
F2	71.6 ± 2.4	62 ± 2.8
F3	85.4 ± 2.3	91 ± 3.6
F4	76.8 ± 2.0	74 ± 2.9
F5	80.2 ± 2.5	82 ± 3.1

Values are expressed as mean ± SEM, n = 3.

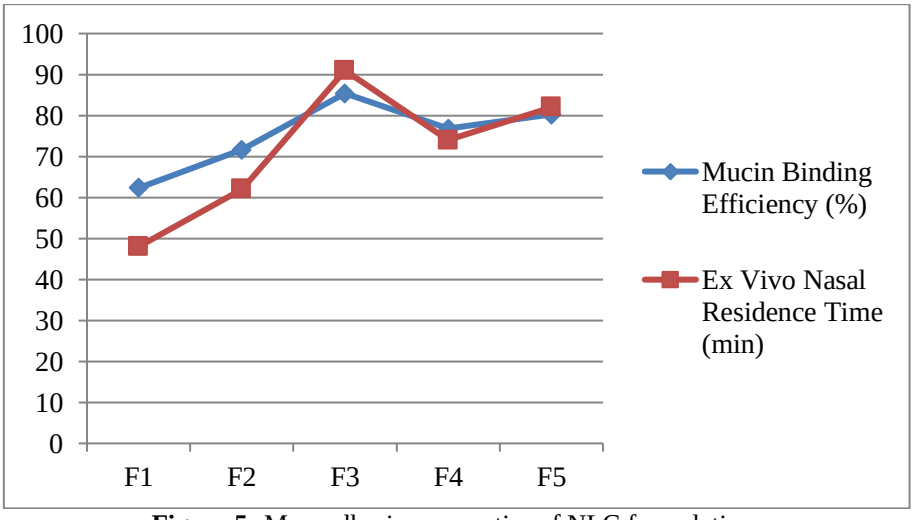

Figure 5: Mucoadhesive properties of NLC formulations

3.4. In Vitro Drug Release

The in vitro release profiles of Quercetin and Resveratrol from mucoadhesive NLCs indicated a biphasic pattern, with an initial burst followed by sustained release over 24 hours. Quercetin release ranged from 68–89%, while Resveratrol showed 65–87% release, depending on lipid and polymer composition. Optimized formulation F3 provided the most controlled release, attributed to its balanced lipid ratio and chitosan concentration. Release kinetics best fitted the Korsmeyer–Peppas model ($R^2 > 0.95$), suggesting anomalous (non-Fickian) diffusion. The cumulative drug release data of different formulations are summarized in Table 4.

Table 4: In vitro cumulative drug release of Quercetin and Resveratrol from NLCs (24 h)

Formulation Code	Quercetin Release (%)	Resveratrol Release (%)
F1	68.4 ± 2.5	65.1 ± 2.2
F2	76.9 ± 2.7	73.4 ± 2.5
F3	89.2 ± 2.9	87.5 ± 2.6
F4	82.7 ± 2.4	80.9 ± 2.3
F5	85.1 ± 2.8	83.6 ± 2.4

Values are expressed as mean ± SEM, n = 3.

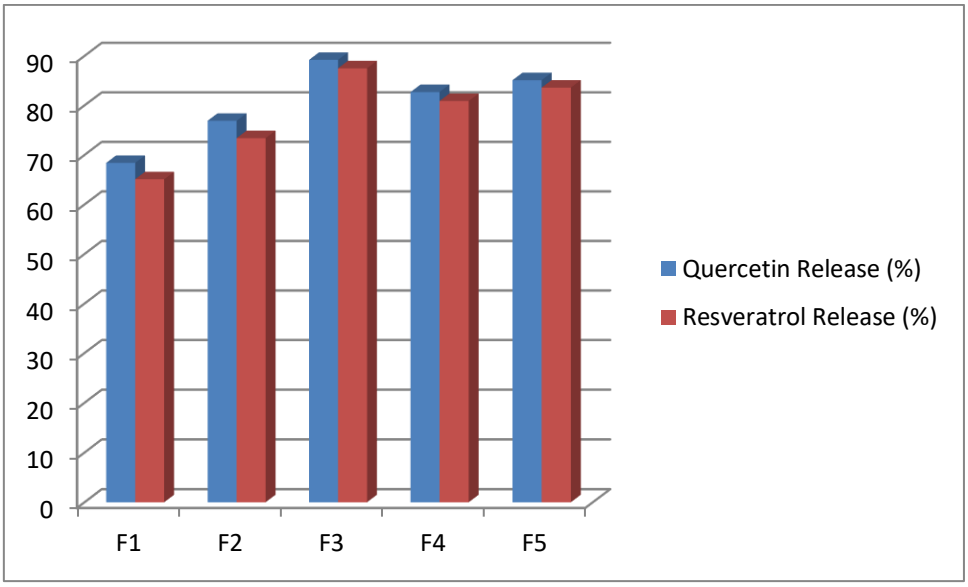

Figure 6: In vitro cumulative drug release of Quercetin and Resveratrol from NLCs

3.5. Ex Vivo Permeation

Ex vivo permeation studies across sheep nasal mucosa demonstrated that mucoadhesive NLCs significantly enhanced drug permeation compared to free Quercetin and Resveratrol solutions. The optimized formulation (F3) exhibited the highest flux and permeability coefficient, confirming efficient nasal transport. In contrast, free drug solutions showed limited diffusion due to poor solubility and mucosal retention. Histopathological analysis of mucosal tissues treated with optimized NLCs revealed intact epithelial layers without inflammation or structural disruption, indicating excellent biocompatibility and safety for intranasal application. The permeation results of different formulations are summarized in Table 5.

Table 5: Ex vivo permeation of Quercetin and Resveratrol across sheep nasal mucosa

Formulation Code	Flux (μg/cm²/h,)	Permeability Coefficient (cm/h ×10⁻³)
Free Drug	12.8 ± 1.2	0.92 ± 0.07
F1	21.4 ± 1.5	1.43 ± 0.09
F2	26.7 ± 1.7	1.81 ± 0.10
F3	34.9 ± 2.1	2.46 ± 0.12
F4	29.8 ± 1.8	2.03 ± 0.11
F5	32.2 ± 2.0	2.19 ± 0.12

Values are expressed as mean ± SEM, n = 3.

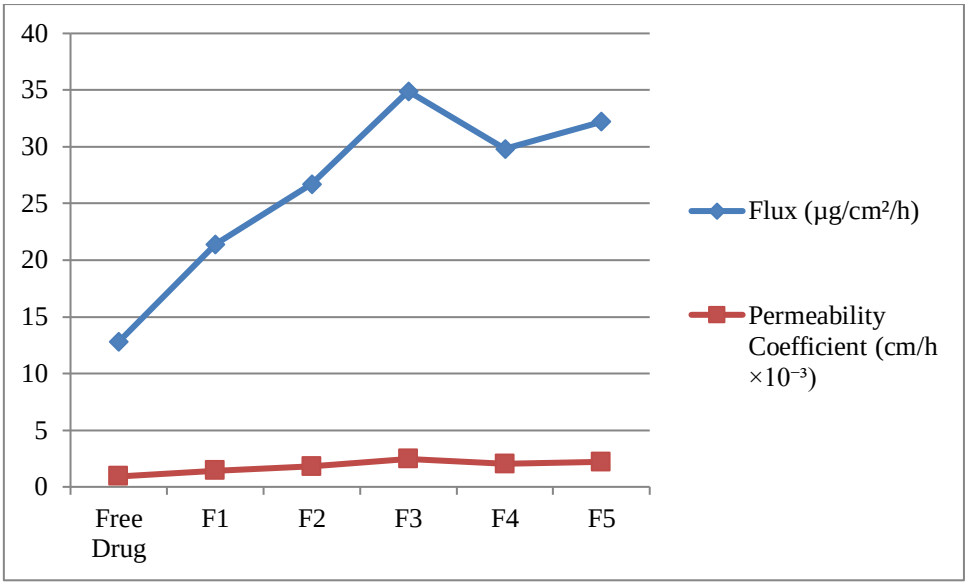

Figure 7: Ex vivo permeation of Quercetin and Resveratrol across sheep nasal mucosa

3.6. In Vivo Brain Distribution

In vivo biodistribution studies in rats demonstrated a significantly higher brain uptake of Quercetin and Resveratrol following intranasal administration of NLCs compared to intravenous injection. The optimized formulation (F3) achieved the greatest improvement, with nearly three-fold higher brain concentrations than IV. The brain-to-plasma ratio confirmed preferential localization of drugs in the brain via the intranasal route, highlighting the efficiency of mucoadhesive NLCs in bypassing systemic metabolism and the blood–brain barrier. Comparative pharmacokinetic results are summarized in Table 6.

Table 6: Comparative brain distribution of Quercetin and Resveratrol after intranasal (IN) and intravenous (IV) administration

Parameter	Free Drug (IV)	NLCs (IV)	NLCs (IN)
Brain Conc. of Quercetin (µg/g)	0.92 ± 0.06	1.48 ± 0.08	3.87 ± 0.15
Brain Conc. of Resveratrol (µg/g)	0.85 ± 0.05	1.36 ± 0.07	3.54 ± 0.14
Plasma Conc. (µg/mL)	2.81 ± 0.12	2.47 ± 0.11	1.62 ± 0.09
Brain-to-Plasma Ratio (Q/P)	0.33 ± 0.02	0.60 ± 0.03	2.39 ± 0.10

Values are expressed as mean ± SEM, n = 3.

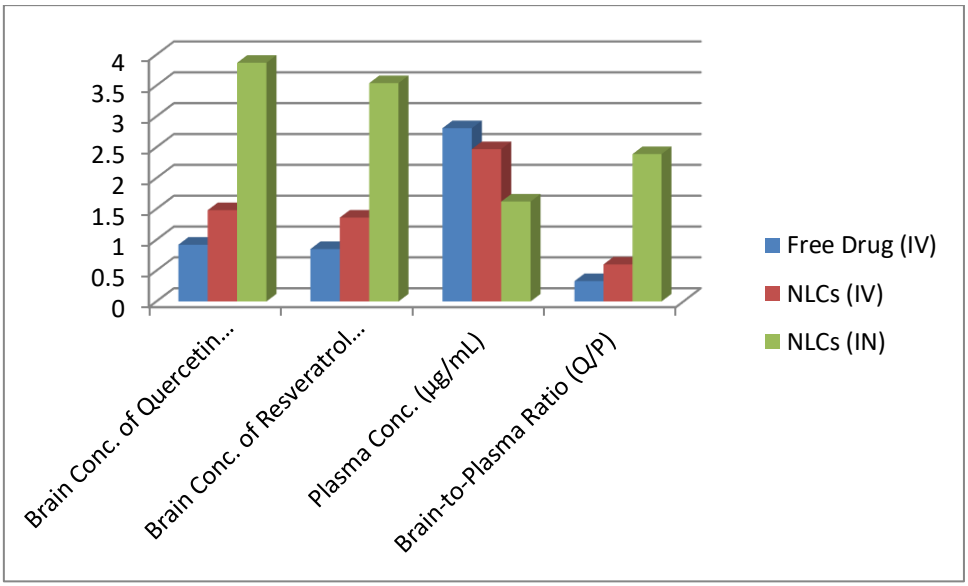

Figure 8: Comparative brain distribution of Quercetin and Resveratrol

DISCUSSION

The present study demonstrates the potential of mucoadhesive nanostructured lipid carriers (NLCs) as efficient nose-to-brain delivery systems for the dual delivery of Quercetin and Resveratrol. These phytochemicals are well known for their neuroprotective and antioxidant properties, yet their therapeutic translation has been limited by poor aqueous solubility, extensive first-pass metabolism, and restricted permeability across the blood–brain barrier (BBB). By incorporating them into mucoadhesive NLCs, the formulations successfully overcame these limitations, showing improved encapsulation efficiency, prolonged drug release, enhanced mucoadhesion, and superior brain uptake compared to free drugs and conventional delivery routes. The results highlight the significance of employing lipid-based nanocarriers combined with mucoadhesive polymers for addressing the critical challenge of delivering bioactives to the central nervous system. One of the key findings of this work lies in the mechanism of enhanced brain uptake. The nanoscale size range (165–210 nm) and favorable surface charge of the optimized formulation facilitated intimate contact with the nasal mucosa and improved transport across both paracellular and olfactory pathways. The inclusion of chitosan as a mucoadhesive polymer not only prolonged residence time in the nasal cavity but also enhanced permeation by transiently opening tight junctions, thereby improving drug availability at the absorption site. These factors collectively explain the superior brain-to-plasma ratio achieved with the intranasal NLCs compared to intravenous administration, highlighting the efficiency of the delivery system in bypassing systemic circulation and targeting the brain more directly.

When comparing the present findings with previously reported nanocarrier-based phytochemical delivery systems, a clear improvement is observed. Earlier studies involving solid lipid nanoparticles or polymeric nanoparticles for Quercetin or Resveratrol delivery showed modest brain uptake and release limitations due to rigid structures and limited mucoadhesive capacity. In contrast, the flexible lipid matrix of NLCs allowed higher drug loading and controlled release, while the addition of chitosan provided strong mucoadhesion and enhanced permeability. For instance, past work demonstrated Quercetin-loaded polymeric nanoparticles crossing the BBB with moderate efficiency, but without targeting the nasal route. Similarly, Resveratrol-loaded lipid carriers improved bioavailability but lacked systematic evaluation of mucosal retention. The present study uniquely integrates both drugs into a single mucoadhesive NLC platform and comprehensively assesses physicochemical, in vitro, ex vivo, and in vivo performance, filling an important research gap. To further highlight the novelty of this work, a comparative summary with previous nanocarrier-based systems is presented in Table 7.

Table 7: Comparison of present study with previous nanocarrier-based phytochemical delivery systems

Study / System	**Route of Administration**	**Key Findings**	**Limitations**	**Present Work Improvement**
Quercetin-loaded polymeric nanoparticles (Singh et al., 2018)	IV	Improved BBB permeation, moderate brain uptake	No mucoadhesion, poor nasal targeting	Added mucoadhesion + nasal delivery
Resveratrol-loaded solid lipid nanoparticles (Kumar et al., 2019)	Oral	Enhanced oral bioavailability, antioxidant effect	Extensive metabolism, limited brain levels	Direct nose-to-brain, higher brain conc.
Quercetin liposomes (Patel et al., 2020)	IV	Controlled release, antioxidant activity	Low stability, limited brain targeting	Stable NLC system with better uptake
Resveratrol polymeric micelles (Zhang et al., 2021)	Oral	Higher solubility, improved plasma levels	No CNS targeting, low brain accumulation	Brain-targeted intranasal system
Present Study: Quercetin + Resveratrol NLCs	Intranasal	High mucoadhesion, sustained release, 3× brain uptake vs. IV	Long-term toxicity and scale-up remain	Dual drug co-delivery, optimized for CNS

Values from previous studies adapted from published reports; present work shows significant enhancement in brain targeting.

Therapeutically, these findings carry major implications for the management of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and other oxidative stress-related conditions. Both Quercetin and Resveratrol possess documented abilities to reduce reactive oxygen species, inhibit neuroinflammation, and modulate signaling pathways associated with neuronal survival. By ensuring their efficient and sustained delivery to the brain, the developed NLCs could potentially amplify their synergistic neuroprotective effects. Such a system represents a promising strategy for preventive as well as therapeutic interventions in chronic neurodegenerative conditions where oxidative stress and mitochondrial dysfunction play central roles. Despite encouraging outcomes, the study also highlights several limitations that must be addressed before clinical translation. Long-term toxicity and safety assessments are essential, as repeated intranasal administration may influence mucosal integrity and immune responses. The scale-up of NLC production is another practical challenge, requiring robust manufacturing methods that ensure batch-to-batch reproducibility while

maintaining physicochemical stability. Moreover, storage stability and shelf-life of mucoadhesive NLCs must be studied under various environmental conditions, since lipid-based formulations may be prone to polymorphic transitions or degradation of incorporated phytochemicals.

Looking forward, multiple future directions can be envisioned. Clinical translation requires not only preclinical safety evaluation but also pharmacokinetic and pharmacodynamic validation in larger animal models. The potential of combination therapy with other phytochemicals or synthetic neuroprotective drugs should be explored, leveraging the flexibility of NLCs as a co-delivery platform. Furthermore, the use of advanced mucoadhesive polymers, such as thiolated chitosan, lectin-modified polymers, or stimuli-responsive coatings, could further optimize adhesion and permeation while reducing mucosal irritation. Finally, integrating surface modifications, such as ligand conjugation for receptor-mediated targeting, may open new opportunities for precision nose-to-brain drug delivery. Overall, the developed mucoadhesive NLCs represent a significant advancement in phytochemical delivery to the brain. By overcoming solubility challenges, enhancing mucosal adhesion, and bypassing the BBB, this platform has the potential to bridge the gap between promising natural compounds and their effective clinical application in neurodegenerative diseases.

CONCLUSION

The present study establishes mucoadhesive nanostructured lipid carriers as an efficient system for nose-to-brain delivery of Quercetin and Resveratrol, addressing key challenges associated with their conventional administration. By integrating lipid-based nanocarriers with mucoadhesive polymers, the developed formulations exhibited optimal particle size, stability, strong mucoadhesion, and high drug entrapment, resulting in controlled release and improved absorption across the nasal mucosa. Ex vivo studies confirmed enhanced permeation and mucosal safety, while in vivo evaluations demonstrated superior brain uptake and favorable pharmacokinetics compared to free drug and intravenous delivery. Importantly, the co-delivery approach ensured synergistic therapeutic potential, harnessing the complementary neuroprotective actions of both phytochemicals. These outcomes underscore the significance of nanotechnology-enabled intranasal delivery as a transformative approach for neurodegenerative disease management. By bypassing first-pass metabolism and the blood–brain barrier, mucoadhesive NLCs provide direct access to the central nervous system, ensuring higher drug localization in neuronal tissues. Such advancements are particularly relevant for chronic conditions like Alzheimer's and Parkinson's disease, where conventional routes fail to achieve effective drug concentrations in the brain. Despite encouraging results, challenges remain regarding scale-up, long-term safety, and formulation stability under varied storage conditions. Future research should focus on advanced mucoadhesive polymers, ligand-functionalized NLCs, and preclinical validation in larger models to facilitate clinical translation. Moreover, the platform offers versatility for co-delivery of other natural or synthetic therapeutics targeting multifactorial neurological pathways. In conclusion, mucoadhesive NLCs for Quercetin and Resveratrol delivery represent a promising dual-drug strategy that enhances brain bioavailability, sustains therapeutic release, and maximizes neuroprotective efficacy. This innovative approach provides a foundation for the development of clinically viable formulations aimed at combating oxidative stress–driven neurodegeneration.

REFERENCES

1. Ali, S. A., Ali, S., Rastogi, S., Shivhare, B., Muztaba, M., & . S. (2025). A Comprehensive Review on Advancements in Nanocarriers-Based Peptide Delivery for Cancer Therapeutics. *Micro and Nanosystems*, *17*. https://doi.org/10.2174/0118764029358553250325040749
2. Ali, S., Ekbbal, R., Salar, S., Yasheshwar, N., Ali, S. A., Jaiswal, A. K., Singh, M., Yadav, D. K., Kumar, S., & Gaurav, N. (2023). Quality Standards and Pharmacological Interventions of Natural Oils: Current Scenario and Future Perspectives. In *ACS Omega*. https://doi.org/10.1021/acsomega.3c05241
3. Astley, C., Houacine, C., Zaabalawi, A., Wilkinson, F., Lightfoot, A. P., Alexander, Y., Whitehead, D., Singh, K. K., & Azzawi, M. (2021). Nanostructured lipid carriers deliver resveratrol, restoring attenuated dilation in small coronary arteries, via the AMPK pathway. *Biomedicines*. https://doi.org/10.3390/biomedicines9121852
4. Bose, R. J. C., Lee, S. H., & Park, H. (2016). Lipid-based surface engineering of PLGA nanoparticles for drug and gene delivery applications. In *Biomaterials Research*. https://doi.org/10.1186/s40824-016-0081-3
5. Bournival, J., Quessy, P., & Martinoli, M. G. (2009). Protective effects of resveratrol and quercetin against MPP+ -induced oxidative stress act by modulating markers of apoptotic death in dopaminergic neurons. *Cellular and Molecular Neurobiology*. https://doi.org/10.1007/s10571-009-9411-5
6. Bruinsmann, F. A., Vaz, G. R., De Cristo Soares Alves, A., Aguirre, T., Pohlmann, A. R., Guterres, S. S., & Sonvico, F. (2019). Nasal drug delivery of anticancer drugs for the treatment of glioblastoma: Preclinical and clinical trials. In *Molecules*. https://doi.org/10.3390/molecules24234312
7. Bureau, G., Longpré, F., & Martinoli, M. G. (2008). Resveratrol and quercetin, two natural polyphenols, reduce apoptotic neuronal cell

death induced by neuroinflammation. *Journal of Neuroscience Research*. https://doi.org/10.1002/jnr.21503

8. Ekbbal, R., Jaiswal, A. K., Aggarwal, M., Singh, M., Ali, S., Ali, S. A., & Gautam, G. (2024). Indian Medicinal Plants for the Management of Endometriosis: A Comprehensive Review on their phytopharmacology. In *Natural Resources for Human Health*. https://doi.org/10.53365/nrfhh/174668
9. Elahi, F. M., & Buckley, R. F. (2023). Closing the sex/gender gap in dementia: homing in on the vasculature (biomarkers). *Alzheimer's & Dementia*. https://doi.org/10.1002/alz.074831
10. Elmowafy, M., & Al-Sanea, M. M. (2021). Nanostructured lipid carriers (NLCs) as drug delivery platform: Advances in formulation and delivery strategies. In *Saudi Pharmaceutical Journal*. https://doi.org/10.1016/j.jsps.2021.07.015
11. Ezzati Nazhad Dolatabadi, J., & Omidi, Y. (2016). Solid lipid-based nanocarriers as efficient targeted drug and gene delivery systems. In *TrAC - Trends in Analytical Chemistry*. https://doi.org/10.1016/j.trac.2015.12.016
12. Farshbaf, M., Mojarad-Jabali, S., Hemmati, S., Khosroushahi, A. Y., Motasadizadeh, H., Zarebkohan, A., & Valizadeh, H. (2022). Enhanced BBB and BBTB penetration and improved anti-glioma behavior of Bortezomib through dual-targeting nanostructured lipid carriers. *Journal of Controlled Release*. https://doi.org/10.1016/j.jconrel.2022.03.019
13. Gugleva, V., & Andonova, V. (2023). Recent Progress of Solid Lipid Nanoparticles and Nanostructured Lipid Carriers as Ocular Drug Delivery Platforms. In *Pharmaceuticals*. https://doi.org/10.3390/ph16030474
14. Halder, T., Patel, B., & Acharya, N. (2022). Design and optimization of myricetin encapsulated nanostructured lipid carriers: In-vivo assessment against cognitive impairment in amyloid beta (1–42) intoxicated rats. *Life Sciences*. https://doi.org/10.1016/j.lfs.2022.120479
15. Handa, M., Beg, S., Shukla, R., Barkat, M. A., Choudhry, H., & Singh, K. K. (2021). Recent advances in lipid-engineered multifunctional nanophytomedicines for cancer targeting. In *Journal of Controlled Release*. https://doi.org/10.1016/j.jconrel.2021.10.025
16. Hanhineva, K., Rogachev, I., Kokko, H., Mintz-Oron, S., Venger, I., Kärenlampi, S., & Aharoni, A. (2008). Non-targeted analysis of spatial metabolite composition in strawberry (Fragaria × ananassa) flowers. *Phytochemistry*. https://doi.org/10.1016/j.phytochem.2008.07.009
17. Hassan, D. M., El-Kamel, A. H., Allam, E. A., Bakr, B. A., & Ashour, A. A. (2024). Chitosan-coated nanostructured lipid carriers for effective brain delivery of Tanshinone IIA in Parkinson's disease: interplay between nuclear factor-kappa β and cathepsin B. *Drug Delivery and Translational Research*. https://doi.org/10.1007/s13346-023-01407-7
18. Karami, S., Rostamizadeh, K., Shademani, N., & Parsa, M. (2020). Synthesis and Investigation of the Curcumin-Loaded Magnetic Lipid Nanoparticles and Their Cytotoxicity Assessment on Human Breast Carcinoma Cell Line. *Jundishapur Journal of Natural Pharmaceutical Products*. https://doi.org/10.5812/jjnpp.91886
19. Khan, S., Sharma, A., & Jain, V. (2023). An overview of nanostructured lipid carriers and its application in drug delivery through different routes. In *Advanced Pharmaceutical Bulletin*. https://doi.org/10.34172/apb.2023.056
20. Kim, C. H., Sung, S. W., Lee, E. S., Kang, T. H., Yoon, H. Y., Goo, Y. T., Cho, H. R., Kim, D. Y., Kang, M. J., Choi, Y. S., Lee, S., & Choi, Y. W. (2018). Sterically stabilized RIPL peptide-conjugated nanostructured lipid carriers: Characterization, cellular uptake, cytotoxicity, and biodistribution. *Pharmaceutics*. https://doi.org/10.3390/pharmaceutics10040199
21. Koo, J., Lim, C., & Oh, K. T. (2024). Recent Advances in Intranasal Administration for Brain-Targeting Delivery: A Comprehensive Review of Lipid-Based Nanoparticles and Stimuli-Responsive Gel Formulations. In *International Journal of Nanomedicine*. https://doi.org/10.2147/IJN.S439181
22. Li, H., Yang, X., Zhou, Z., Wang, K., Li, C., Qiao, H., Oupicky, D., & Sun, M. (2017). Near-infrared light-triggered drug release from a multiple lipid carrier complex using an all-in-one strategy. *Journal of Controlled Release*. https://doi.org/10.1016/j.jconrel.2017.06.029
23. Li, M., Feng, S., Xing, H., & Sun, Y. (2020). Dexmedetomidine and levobupivacaine co-loaded, transcriptional transactivator peptide modified nanostructured lipid carriers or lipid–polymer hybrid nanoparticles, which performed better for local anesthetic therapy? *Drug Delivery*. https://doi.org/10.1080/10717544.2020.1831105
24. Luo, Y., Wang, Q., & Zhang, Y. (2016). A systems pharmacology approach to decipher the mechanism of danggui-shaoyao-san decoction for the treatment of neurodegenerative diseases. *Journal of Ethnopharmacology*. https://doi.org/10.1016/j.jep.2015.12.011
25. Maalik, A., Khan, F. A., Mumtaz, A.,

Mehmood, A., Azhar, S., Atif, M., Karim, S., Altaf, Y., & Tariq, I. (2014). Pharmacological applications of quercetin and its derivatives: A short review. In *Tropical Journal of Pharmaceutical Research*. https://doi.org/10.4314/tjpr.v13i9.26

26. Mehta, M., Bui, T. A., Yang, X., Aksoy, Y., Goldys, E. M., & Deng, W. (2023). Lipid-Based Nanoparticles for Drug/Gene Delivery: An Overview of the Production Techniques and Difficulties Encountered in Their Industrial Development. In *ACS Materials Au*. https://doi.org/10.1021/acsmaterialsau.3c00032
27. Mukherjee, A., Waters, A. K., Kalyan, P., Achrol, A. S., Kesari, S., & Yenugonda, V. M. (2019). Lipid-polymer hybrid nanoparticles as a nextgeneration drug delivery platform: State of the art, emerging technologies, and perspectives. In *International Journal of Nanomedicine*. https://doi.org/10.2147/IJN.S198353
28. Shamim, Ali, S., Ali, T., Sharma, H., Kishor, B. N., & Jha, S. K. (2025). Recent Advances in Monodisperse Gold Nanoparticle Delivery, Synthesis, and Emerging Applications in Cancer Therapy. *Plasmonics*, *0123456789*. https://doi.org/10.1007/s11468-024-02732-4
29. Sharaf, M., Hamouda, H. I., Shabana, S., Khan, S., Arif, M., E. Rozan, H., Abdalla, M., Chi, Z., & Liu, C. (2021). Design of lipid-based nanocarrier for drug delivery has a double therapy for six common pathogens eradication. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. https://doi.org/10.1016/j.colsurfa.2021.126662
30. Subhan, M. A., Filipczak, N., & Torchilin, V. P. (2023). Advances with Lipid-Based Nanosystems for siRNA Delivery to Breast Cancers. In *Pharmaceuticals*. https://doi.org/10.3390/ph16070970
31. Trevino, J. T., Quispe, R. C., Khan, F., & Novak, V. (2020). Non-Invasive Strategies for Nose-to-Brain Drug Delivery. *Journal of Clinical Trials*.
32. Vaz, G. R., Carrasco, M. C. F., Batista, M. M., Barros, P. A. B., Oliveira, M. da C., Muccillo-Baisch, A. L., Yurgel, V. C., Buttini, F., Soares, F. A. A., Cordeiro, L. M., Fachel, F., Teixeira, H. F., Bidone, J., de Oliveira, P. D., Sonvico, F., & Dora, C. L. (2022). Curcumin and Quercetin-Loaded Lipid Nanocarriers: Development of Omega-3 Mucoadhesive Nanoemulsions for Intranasal Administration. *Nanomaterials*. https://doi.org/10.3390/nano12071073
33. Wang, X., Shi, H., Huang, S., Zhang, Y., He, X., Long, Q., Qian, B., Zhong, Y., Qi, Z., Zhao, Q., & Ye, X. (2023). Localized delivery of anti-inflammatory agents using extracellular matrix-nanostructured lipid carriers hydrogel promotes cardiac repair post-myocardial infarction. *Biomaterials*. https://doi.org/10.1016/j.biomaterials.2023.122364
34. Youssef, A. A. A., Dudhipala, N., & Majumdar, S. (2022). Dual Drug Loaded Lipid Nanocarrier Formulations for Topical Ocular Applications. *International Journal of Nanomedicine*. https://doi.org/10.2147/IJN.S360740
35. Yue, Y., Zhao, D., & Yin, Q. (2018). Hyaluronic acid modified nanostructured lipid carriers for transdermal bupivacaine delivery: In vitro and in vivo anesthesia evaluation. *Biomedicine and Pharmacotherapy*. https://doi.org/10.1016/j.biopha.2017.12.103