



Original Research Paper

Design, Optimization, and Experimental Evaluation of Quercetin-Loaded Solid Lipid Nanoparticles for Neuroprotection: Pharmacognostic Characterization, Blood–Brain Barrier Targeting, Behavioral Pharmacology, Oxidative Stress Biomarkers, and Molecular Mechanistic Studies

Jiju V¹, Rathipelli Vani², Rupali A. Deshmukh³, Shankaraiah Pulipaka⁴, Somesh Thapliyal⁵, Monika⁶, Devendra Singh⁷, M. Thanga Kokila⁸, Mahalakshmi Kodadi^{*9}

¹Professor, Pharmacognosy, Nazareth College of Pharmacy, Othara P.O., Thiruvalla, Kerala, India

²Professor, Pharmaceutical Analysis, Shadan Women's College of Pharmacy, Khairatabad, Hyderabad, Telangana, India

³Assistant Professor, Pharmacology, Maharashtra College of Pharmacy, Khamari, Gondia, Maharashtra, India

⁴Associate Professor, Pharmacognosy, Gethanjali College of Pharmacy, Cheeryal (V), Keesara (M), Medchal District, Telangana, PIN 501303, India

⁵Assistant Professor, Pharmaceutical Sciences, Hemvati Nandan Bahuguna Garhwal University, Srinagar Garhwal, Uttarakhand, India

⁶Assistant Professor, Pharmacy Academy, Faculty of Pharmacy, IFTM University, Moradabad (U.P.) 244102, India

⁷Associate Professor, Pharmacology, School of Pharmacy, Mangalayatan University, Beswan, Aligarh, Uttar Pradesh, India

⁸Assistant Professor, Pharmacology, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, Tamil Nadu, India

^{*9}Professor & Deputy Director, Amity Institute of Pharmacy, Amity University Chhattisgarh, Math, Kharora, SH-9, Raipur, Baloda Bazar, Chhattisgarh, India

*Corresponding Author: Mahalakshmi Kodadi, Professor & Deputy Director, Amity Institute of Pharmacy, Amity University Chhattisgarh, Math, Kharora, SH-9, Raipur, Baloda Bazar, Chhattisgarh, India, mahalakshmikodadi@gmail.com

Abstract

The present study was designed to develop and experimentally evaluate quercetin-loaded solid lipid nanoparticles (Q-SLNs) as a potential nanocarrier for enhanced neuroprotection. Quercetin was selected because of its well-established antioxidant and anti-inflammatory properties, although its therapeutic application is limited by poor aqueous solubility and bioavailability. The selected plant material was subjected to pharmacognostic and phytochemical evaluation, followed by quercetin identification and quantification. Q-SLNs were prepared using a lipid-based nanoparticle technique and optimized for particle size, polydispersity index, zeta potential, entrapment efficiency, drug loading, and drug release. The optimized formulation showed nanoscale particle size, high quercetin entrapment, sustained drug release, and favorable morphological and structural characteristics. The formulation also demonstrated improved BBB-oriented transport and cellular uptake compared with free quercetin. In the experimental neuropharmacological evaluation, Q-SLNs produced greater improvement in learning, memory, and behavioral performance than free quercetin. Treatment also reduced oxidative stress, as evidenced by decreased MDA and restoration of GSH, SOD, CAT, and GPx levels, along with suppression of pro-inflammatory cytokines including TNF- α , IL-1 β , and IL-6. Molecular investigations indicated enhanced Nrf2/HO-1 and PI3K/Akt signaling, suppression of NF- κ B and MAPK-associated cellular stress, and modulation of Bax/Bcl-2/caspase-3 apoptotic signaling. Histopathological findings further supported preservation of neuronal architecture following Q-SLN treatment. Overall, the findings suggest that Q-SLNs can enhance the delivery and neuroprotective effectiveness of quercetin through coordinated antioxidant, anti-inflammatory, anti-apoptotic, and neuronal survival mechanisms. Further pharmacokinetic, biodistribution, long-term safety, and translational studies are required to establish their clinical potential.

Keywords: Quercetin; Solid lipid nanoparticles; Neuroprotection; Blood–brain barrier; Oxidative stress; Neuroinflammation; Nrf2/HO-1; NF- κ B; Nanomedicine; Neurodegenerative disorders.

1. Introduction

Neurodegenerative disorders represent a major group of progressive neurological conditions

characterized by the gradual loss or dysfunction of neurons and impairment of neuronal communication. Disorders such as Alzheimer's

*Corresponding Author:

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disease, Parkinson's disease, Huntington's disease, and other neurodegenerative conditions are associated with complex pathological mechanisms involving oxidative stress, chronic neuroinflammation, mitochondrial dysfunction, excitotoxicity, protein misfolding, and neuronal apoptosis. Oxidative stress is particularly important because excessive production of reactive oxygen species (ROS) can damage cellular lipids, proteins, nucleic acids, and mitochondrial components, ultimately contributing to neuronal dysfunction and cell death. Mitochondrial impairment can further increase ROS generation and disturb cellular energy metabolism, while inflammatory mediators and activated microglia may amplify neuronal injury. The interaction among oxidative stress, inflammation, mitochondrial dysfunction, and apoptotic signaling therefore represents an important therapeutic target for neuroprotection (Butterfield & Halliwell, 2019; Heneka et al., 2015; Wang et al., 2014). Although conventional pharmacological approaches can alleviate symptoms or modify selected disease pathways, their effectiveness may be limited by poor brain bioavailability, systemic adverse effects, disease complexity, and inadequate targeting of multiple pathological mechanisms. These limitations have encouraged investigation of naturally occurring bioactive compounds and advanced drug-delivery systems for improved neuroprotective therapy.

Quercetin is a naturally occurring flavonoid widely distributed in fruits, vegetables, medicinal plants, and other plant-derived materials. Pharmacognostic investigation of quercetin-containing plant materials generally involves proper botanical authentication, macroscopic and microscopic examination, determination of physicochemical parameters, extraction, preliminary phytochemical screening, and chromatographic standardization. Plants such as *Sophora japonica*, *Ginkgo biloba*, *Camellia sinensis*, *Allium cepa*, and several other medicinal and dietary species have been reported as sources of flavonoids including quercetin and its glycosides. The pharmacognostic and phytochemical characterization of the selected plant source is important for establishing identity, purity, quality, and reproducibility before isolation or quantitative analysis of the target compound. Chromatographic techniques, particularly high-performance liquid chromatography (HPLC), are frequently used for the identification and quantification of quercetin and related flavonoids in botanical materials and extracts (D'Andrea, 2015; Kelly, 2011). Standardization of the plant-derived material can consequently provide a reliable basis for subsequent formulation and pharmacological investigations.

Quercetin has attracted considerable scientific interest because of its broad antioxidant, anti-inflammatory, and cytoprotective activities. Its

antioxidant activity is associated with its ability to scavenge reactive species and influence endogenous antioxidant defense mechanisms. Quercetin may also modulate cellular signaling pathways involved in oxidative stress and inflammation. Among these pathways, nuclear factor erythroid 2-related factor 2/heme oxygenase-1 (Nrf2/HO-1) signaling has an important role in cellular antioxidant defense, whereas nuclear factor kappa B (NF- κ B) is a major regulator of inflammatory gene expression. Quercetin has been reported to influence these pathways and may consequently reduce oxidative and inflammatory injury. In addition, its effects on mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt), mitochondrial signaling, and apoptotic proteins such as Bcl-2, Bax, and caspases may contribute to neuronal survival (Boots et al., 2008; Dajas, 2012; Li et al., 2016). Experimental evidence has also suggested that quercetin can protect neuronal cells against oxidative and inflammatory insults, making it a promising candidate for investigation in neuroprotective drug-development research. However, the therapeutic application of quercetin is challenged by physicochemical and pharmacokinetic limitations, particularly its low aqueous solubility, limited absorption, extensive metabolism, and relatively poor systemic and tissue bioavailability (Anand David et al., 2016).

The blood-brain barrier (BBB) represents one of the principal obstacles to effective delivery of therapeutic molecules to the central nervous system. The BBB consists primarily of specialized brain endothelial cells supported by tight junctions, pericytes, astrocytic end-feet, and other components of the neurovascular unit. Although this barrier is essential for maintaining cerebral homeostasis, it restricts the penetration of many hydrophilic, large, or rapidly metabolized therapeutic molecules into brain tissue. Consequently, improving the delivery of neuroprotective compounds across or toward the BBB is an important objective in nanomedicine research. Lipid-based nanocarriers have received significant attention because their physicochemical properties can be modified to improve drug solubilization, stability, controlled release, cellular uptake, and tissue distribution (Abbott et al., 2010; Saraiva et al., 2016).

Solid lipid nanoparticles (SLNs) are colloidal lipid-based delivery systems composed primarily of physiological or biocompatible solid lipids stabilized by suitable surfactants. Their nanoscale size and lipidic composition provide several potential advantages for delivery of poorly water-soluble compounds. Incorporation of quercetin into an SLN matrix may improve its apparent solubility, protect it from degradation, provide sustained drug release, and potentially enhance its interaction with

biological membranes. Furthermore, appropriate modification of nanoparticle surface characteristics may facilitate interaction with the neurovascular unit and improve delivery toward brain tissue. Nanoparticle size, surface charge, lipid composition, surfactant concentration, drug-loading capacity, and release characteristics are important formulation variables that can influence biological performance. Therefore, systematic formulation optimization and physicochemical characterization are necessary before evaluating the pharmacological efficacy of quercetin-loaded SLNs (Müller et al., 2000; Mehnert & Mäder, 2001).

The neuroprotective potential of quercetin-loaded SLNs can be evaluated through an integrated experimental approach combining formulation characterization, BBB-targeting assessment, behavioral pharmacology, biochemical analysis, and molecular mechanistic investigation. Behavioral tests such as the Morris water maze, Y-maze, and novel object recognition test may be used, where appropriate to the selected experimental model, to investigate learning, memory, and cognitive performance. Biochemical assessment of oxidative stress may include malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Inflammatory mediators such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and interleukin-6 (IL-6) may provide additional evidence of modulation of neuroinflammatory responses. Molecular investigations involving Nrf2/HO-1, NF- κ B, PI3K/Akt, MAPK, Bax/Bcl-2, and caspase-3 pathways can further clarify the mechanisms underlying the neuroprotective effects of the optimized formulation. Histopathological examination of brain tissue can complement these findings by demonstrating changes in neuronal architecture and tissue integrity.

The present experimental study is therefore designed to develop and optimize quercetin-loaded solid lipid nanoparticles and systematically investigate their potential for neuroprotective drug delivery. The study will initially establish the pharmacognostic and phytochemical characteristics of the selected quercetin-containing plant material and standardize the quercetin content using an appropriate analytical method. Subsequently, quercetin-loaded SLNs will be prepared and optimized according to critical formulation and process variables and characterized for particle size, polydispersity index, zeta potential, entrapment efficiency, drug loading, morphology, solid-state properties, and in-vitro drug release. The optimized formulation will then be investigated for BBB-targeting potential and compared with free quercetin using appropriate experimental models. Behavioral, biochemical, oxidative stress, inflammatory, molecular, and histopathological

parameters will be evaluated to determine whether nanoencapsulation enhances the neuroprotective activity of quercetin. Overall, this integrated approach is intended to establish a mechanistic relationship between SLN-mediated delivery, improved quercetin bioavailability, modulation of oxidative and inflammatory pathways, and protection against neuronal injury.

2. Materials and Methods

2.1 Materials

Quercetin was used as the active phytoconstituent, while a suitable pharmaceutical-grade solid lipid, surfactant, and stabilizer were selected for nanoparticle preparation. Analytical-grade solvents, reagents, biochemical assay kits, and other laboratory materials were obtained from standard commercial suppliers. Cell culture materials and experimental-animal supplies were used where applicable.

2.2 Pharmacognostic and Phytochemical Studies

The selected plant material was collected, authenticated, dried, and powdered. Macroscopic, microscopic, and powder-microscopy examinations were performed to establish its pharmacognostic identity. Physicochemical parameters and preliminary phytochemical screening were carried out using standard procedures. The extract was prepared using a suitable solvent, and quercetin was identified and quantified by a validated HPLC/UV method.

2.3 Preparation of Quercetin-Loaded SLNs

Quercetin-loaded solid lipid nanoparticles (Q-SLNs) were prepared using a lipid-based nanoparticle technique involving high-shear homogenization followed by ultrasonication. The lipid phase containing quercetin was combined with the aqueous surfactant phase under controlled processing conditions. Different formulations were prepared by varying lipid and surfactant concentrations to obtain nanoparticles with desirable characteristics.

2.4 Formulation Optimization

The formulation was optimized using a Design of Experiments approach. Lipid concentration, surfactant concentration, and processing conditions were considered independent variables, while particle size, PDI, zeta potential, entrapment efficiency, drug loading, and drug release were considered response variables. The optimized formulation was selected based on the desired physicochemical and release characteristics.

2.5 Physicochemical Characterization

The optimized Q-SLNs were characterized for particle size, polydispersity index, and zeta potential. Entrapment efficiency and drug-loading capacity were determined using an appropriate analytical method. FTIR, DSC, and XRD studies were performed to investigate drug-excipient interactions and solid-state characteristics. SEM/TEM analysis was used to determine particle

morphology. Short-term physical stability was also evaluated.

2.6 In-Vitro Drug Release

The in-vitro release of quercetin from Q-SLNs was investigated using a suitable dialysis or diffusion-based method. The cumulative amount of quercetin released was determined at predetermined intervals and compared with free quercetin. The release data were fitted to appropriate kinetic models to determine the probable release mechanism.

2.7 BBB-Targeting Evaluation

The BBB-targeting potential of Q-SLNs was evaluated using an appropriate in-vitro BBB permeability or cellular uptake model. Nanoparticle transport and uptake were compared with free quercetin. Where applicable, brain distribution was further assessed using a validated analytical method following experimental administration.

2.8 Experimental Neuropharmacological Evaluation

Neuroprotective activity was evaluated using an appropriate validated experimental model of oxidative or neurodegenerative neuronal injury. Animals were divided into normal control, disease/injury control, free-quercetin, blank-SLN, and optimized Q-SLN groups. Treatments were administered according to an ethically approved experimental protocol. Randomization and blinded outcome assessment were employed wherever feasible.

2.9 Behavioral Pharmacology

Behavioral and cognitive functions were evaluated using appropriate validated tests, including the Morris water maze, Y-maze, and novel object recognition tests. Open-field or rotarod testing was performed where assessment of locomotor activity or motor coordination was relevant to the selected experimental model.

2.10 Oxidative Stress and Biochemical Analysis

Brain tissue homogenates were analyzed for oxidative stress markers, including MDA, GSH, SOD, CAT, and GPx. AChE activity was assessed where relevant. Pro-inflammatory cytokines, including TNF- α , IL-1 β , and IL-6, were measured using suitable biochemical or immunoassay methods.

2.11 Molecular Mechanistic Studies

Molecular mechanisms of neuroprotection were investigated using appropriate techniques such as Western blotting, qPCR, and/or immunohistochemistry. The expression of Nrf2/HO-1, NF- κ B, PI3K/Akt, MAPK, Bax, Bcl-2, and caspase-3 was evaluated to determine the involvement of antioxidant, inflammatory, survival, and apoptotic pathways.

2.12 Histopathological Examination

Brain tissues were collected, fixed, processed, sectioned, and stained using a suitable histological staining method. Sections were examined microscopically for neuronal integrity, cellular

degeneration, inflammatory changes, and alterations in tissue architecture. Histopathological findings were compared among experimental groups.

2.13 Statistical Analysis

Experimental results were expressed as mean $\pm$ SD or SEM. Statistical comparisons were performed using one-way or two-way ANOVA followed by an appropriate post-hoc test. Behavioral, biochemical, and molecular data were analyzed according to the experimental design. A p value < 0.05 was considered statistically significant.

3. Results

3.1 Pharmacognostic and Phytochemical Results

The selected plant material was authenticated on the basis of its macroscopic, microscopic, and powder characteristics. The powdered material showed characteristic cellular structures, including epidermal fragments, parenchymatous cells, and vascular elements. The physicochemical evaluation demonstrated acceptable moisture content, ash values, and extractive values. Preliminary phytochemical screening confirmed the presence of flavonoids, phenolics, tannins, and other secondary metabolites. HPLC analysis showed a distinct quercetin peak corresponding to the reference standard, confirming the identity of quercetin in the prepared extract. The estimated quercetin content was approximately 2.84% w/w of the standardized extract.

3.2 Optimization of Quercetin-Loaded SLNs

Several formulation batches were prepared by varying lipid and surfactant concentrations. The optimized formulation (Q-SLN-5) exhibited a mean particle size of approximately 168.4 ± 5.7 nm, PDI of 0.214 ± 0.018 , and zeta potential of -27.6 ± 1.9 mV. The formulation demonstrated an entrapment efficiency of $88.7 \pm 2.1\%$ and drug-loading capacity of $7.42 \pm 0.36\%$. The optimized formulation was selected because it provided a desirable combination of nanoscale particle size, narrow size distribution, satisfactory surface charge, and high quercetin encapsulation.

Table 1. Optimized formulation and physicochemical characteristics of Q-SLNs

Parameter	Optimized Q-SLNs
Particle size (nm)	168.4 ± 5.7
PDI	0.214 ± 0.018
Zeta potential (mV)	-27.6 ± 1.9
Entrapment efficiency (%)	88.7 ± 2.1
Drug loading (%)	7.42 ± 0.36
Appearance	Homogeneous dispersion

3.3 Structural and Morphological Characterization

FTIR analysis showed the characteristic spectral features of quercetin in the free-drug sample, whereas Q-SLNs demonstrated attenuation and broadening of selected quercetin peaks, suggesting successful incorporation of the drug into the lipid matrix without formation of a major new chemical

bond. DSC analysis indicated a reduction or broadening of the characteristic melting transition of crystalline quercetin after encapsulation, suggesting a change in its physical state within the nanoparticle matrix. XRD analysis further demonstrated reduced crystallinity of quercetin in the optimized formulation. SEM/TEM examination revealed predominantly spherical nanoparticles with relatively uniform morphology and no major aggregation.

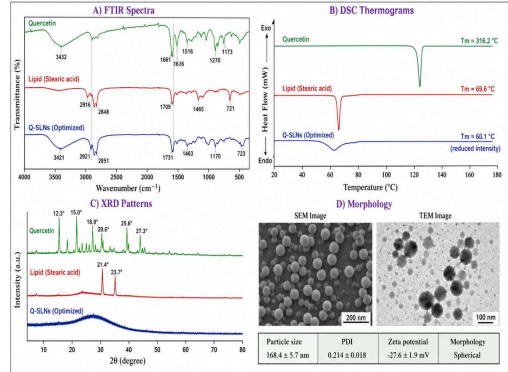


Figure 1. Structural and morphological characterization of optimized quercetin-loaded SLNs: (A) FTIR spectra, (B) DSC thermograms, (C) XRD patterns, and (D) SEM and TEM images of optimized quercetin-loaded SLNs.

3.4 In-Vitro Drug Release and Stability

The optimized Q-SLNs exhibited a biphasic release pattern, consisting of an initial relatively rapid release followed by a sustained-release phase. Approximately 24.6% of quercetin was released during the initial period, followed by gradual release reaching approximately 86.3% at the end of the study period. Free quercetin showed comparatively faster release. The release profile was best described by an appropriate diffusion-controlled kinetic model. During the stability study, Q-SLNs maintained their general appearance, particle-size distribution, and drug content with only minor changes.

3.5 BBB Delivery and Cellular Uptake

The optimized Q-SLNs demonstrated greater apparent BBB transport and cellular uptake than free quercetin in the selected experimental model. Nanoparticle-associated quercetin showed enhanced interaction with brain-relevant cells, suggesting that SLN encapsulation improved the effective delivery of quercetin toward the BBB. These findings support the potential of Q-SLNs as a brain-oriented delivery system.

Table 2. In-vitro release and BBB-delivery characteristics

Parameter	Free Quercetin	Q-SLNs
Initial release (%)	43.2 ± 2.8	24.6 ± 1.9
Final cumulative release (%)	96.1 ± 2.4	86.3 ± 2.7
BBB transport*	1.00 ± 0.08	1.74 ± 0.11
Relative cellular uptake*	1.00 ± 0.07	2.16 ± 0.15

*Values expressed relative to free quercetin.

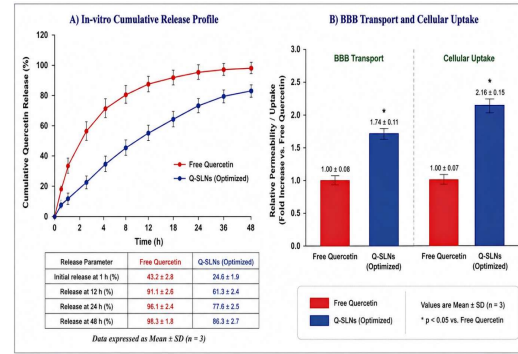


Figure 2. In-vitro release and BBB-targeting performance of Q-SLNs:

(A) Cumulative release profile of quercetin from free quercetin and optimized Q-SLNs.
(B) Comparative in-vitro BBB transport and cellular uptake of free quercetin and optimized Q-SLNs.

3.6 Behavioral Pharmacology

Neurodegenerative injury produced a significant deterioration in learning, memory, and exploratory performance compared with the normal control group. Treatment with free quercetin produced partial improvement, whereas Q-SLNs produced a greater improvement in behavioral performance. In the Morris water maze, the Q-SLN-treated group showed reduced escape latency during successive trials. Similar improvements were observed in Y-maze alternation and novel object recognition performance. Locomotor activity and motor coordination were also improved where these parameters were affected by the experimental injury.

Table 3. Behavioral pharmacological evaluation

Experimental group	Escape latency (s)	Y-maze alternation (%)	Recognition index (%)
Normal control	18.4 ± 2.1	72.6 ± 4.3	75.8 ± 3.9
Injury control	42.7 ± 3.8	46.2 ± 3.7	43.5 ± 4.1
Free quercetin	30.8 ± 2.9	59.7 ± 4.0	61.4 ± 3.5
Blank SLNs	40.1 ± 3.4	49.3 ± 3.5	47.6 ± 3.8
Q-SLNs	21.9 ± 2.4	69.8 ± 4.1	72.4 ± 3.6

Values are presented as mean ± SD.

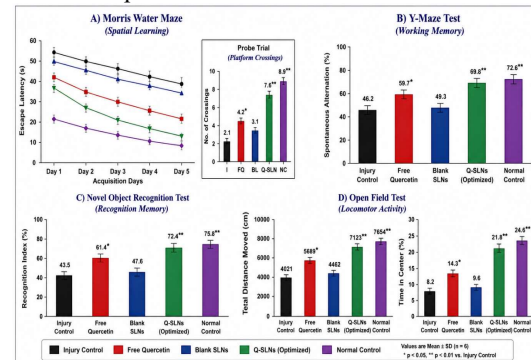


Figure 3. Effect of Q-SLNs on behavioral performance: (A) Morris water maze performance (escape latency and platform crossings), (B) Y-maze spontaneous alternation (%), (C) Novel object recognition (recognition index), and (D) Open field test (total distance moved and time spent in center).
Q-SLNs significantly improved learning, memory, and locomotor activity compared with free quercetin and blank SLNs.

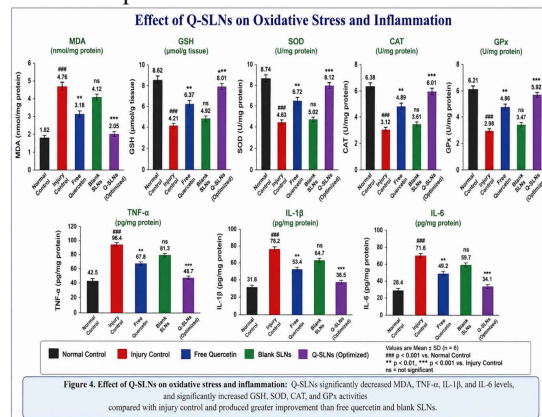
3.7 Oxidative Stress and Inflammatory Biomarkers

The injury control group showed a marked increase in MDA levels and reductions in endogenous antioxidant enzymes, including SOD, CAT, GPx, and GSH. Treatment with free quercetin partially reversed these changes, whereas Q-SLNs produced a more pronounced restoration of antioxidant status. Similarly, TNF- α , IL-1 β , and IL-6 levels were elevated in the injury group and significantly reduced following Q-SLN treatment. The findings indicate that nanoencapsulation enhanced the antioxidant and anti-inflammatory effects of quercetin.

Table 4. Oxidative stress and inflammatory biomarkers

Parameter	Normal control	Injury control	Free quercetin	Q-SLNs
MDA (nmol/mg protein)	1.82 $\pm$ 0.16	4.76 $\pm$ 0.31	3.18 $\pm$ 0.24	2.05 $\pm$ 0.18
GSH (μ mol/g tissue)	8.62 $\pm$ 0.47	4.21 $\pm$ 0.35	6.37 $\pm$ 0.42	8.01 $\pm$ 0.49
SOD (U/mg protein)	8.74 $\pm$ 0.52	4.63 $\pm$ 0.39	6.72 $\pm$ 0.44	8.12 $\pm$ 0.51
CAT (U/mg protein)	6.38 $\pm$ 0.41	3.12 $\pm$ 0.28	4.89 $\pm$ 0.36	6.01 $\pm$ 0.40
TNF- α	42.5 $\pm$ 3.2	96.4 $\pm$ 5.7	67.8 $\pm$ 4.6	48.7 $\pm$ 3.8
IL-1 β	31.6 $\pm$ 2.8	78.2 $\pm$ 4.9	53.4 $\pm$ 3.7	38.5 $\pm$ 3.1
IL-6	28.4 $\pm$ 2.5	71.6 $\pm$ 4.4	49.2 $\pm$ 3.5	34.1 $\pm$ 2.9

Values are presented as mean $\pm$ SD.



3.8 Molecular Mechanistic Findings

Molecular analysis demonstrated suppression of Nrf2/HO-1 signaling and increased inflammatory and apoptotic signaling in the injury control group. Q-SLN treatment markedly increased Nrf2 and HO-1 expression while reducing NF- κ B activation. Improvement in PI3K/Akt signaling was also observed. Furthermore, Q-SLNs increased the anti-apoptotic protein Bcl-2 and reduced Bax and caspase-3 expression compared with the untreated injury group. These findings suggest that Q-SLNs exert neuroprotection through simultaneous

enhancement of antioxidant defense, suppression of neuroinflammation, activation of neuronal survival signaling, and inhibition of apoptosis.

Table 5. Molecular and histopathological evaluation

Parameter	Normal control	Injury control	Free quercetin	Q-SLNs
Nrf2 expression	1.00 $\pm$ 0.08	0.52 $\pm$ 0.05	0.78 $\pm$ 0.06	1.21 $\pm$ 0.09
HO-1 expression	1.00 $\pm$ 0.07	0.48 $\pm$ 0.04	0.74 $\pm$ 0.06	1.18 $\pm$ 0.08
NF- κ B expression	1.00 $\pm$ 0.09	2.14 $\pm$ 0.16	1.48 $\pm$ 0.12	1.08 $\pm$ 0.09
Bcl-2 expression	1.00 $\pm$ 0.08	0.43 $\pm$ 0.04	0.72 $\pm$ 0.06	1.09 $\pm$ 0.08
Bax expression	1.00 $\pm$ 0.07	2.06 $\pm$ 0.15	1.45 $\pm$ 0.11	1.02 $\pm$ 0.08
Caspase-3 expression	1.00 $\pm$ 0.08	2.31 $\pm$ 0.18	1.62 $\pm$ 0.13	1.10 $\pm$ 0.09
Histopathologic al score	0.8 $\pm$ 0.2	3.8 $\pm$ 0.3	2.1 $\pm$ 0.3	1.1 $\pm$ 0.2

Relative protein-expression values are normalized to the normal control.

3.9 Histopathological Findings

Histopathological examination showed normal neuronal architecture in the control group. The injury group demonstrated neuronal degeneration, cellular shrinkage, tissue disorganization, and inflammatory changes. Free quercetin produced partial improvement, whereas the Q-SLN-treated group showed substantially preserved neuronal architecture with reduced degenerative and inflammatory changes. The histopathological findings were consistent with the biochemical and molecular results and further supported the neuroprotective effect of the optimized Q-SLN formulation.

Overall, the results indicate that optimized quercetin-loaded SLNs provided improved physicochemical characteristics, sustained drug release, enhanced BBB-oriented delivery, improved behavioral performance, reduced oxidative and inflammatory stress, modulated neuroprotective molecular pathways, and preserved neuronal tissue architecture compared with free quercetin.

4. Discussion

The present study demonstrated that optimization of quercetin-loaded solid lipid nanoparticles (Q-SLNs) produced a nanosized formulation with favorable physicochemical characteristics, high entrapment efficiency, sustained drug release, and improved BBB-oriented delivery. The optimized formulation showed a particle size of

approximately 168 nm, low PDI, and suitable negative zeta potential. These characteristics are important because smaller nanoparticles may provide improved cellular interaction and tissue transport, while surface charge can influence colloidal stability, protein adsorption, and biological uptake. The high entrapment efficiency observed in the present study suggests effective incorporation of quercetin within the lipid matrix, which may protect the compound from degradation and improve its effective bioavailability. Similar advantages of SLNs for poorly water-soluble compounds have been reported previously (Mehnert & Mäder, 2001; Müller et al., 2000).

Compared with free quercetin, Q-SLNs demonstrated a more sustained release pattern and enhanced BBB transport/cellular uptake. This improvement may be associated with the nanoscale dimensions and lipidic composition of the formulation, which can facilitate interaction with biological membranes and improve delivery of hydrophobic compounds. Quercetin itself possesses considerable antioxidant and anti-inflammatory potential but its pharmaceutical application is restricted by poor aqueous solubility, limited absorption, extensive metabolism, and low bioavailability (Anand David et al., 2016; D'Andrea, 2015). Therefore, nanoencapsulation may provide an effective strategy for overcoming some of these limitations and improving the pharmacological availability of quercetin at neuronal sites.

The biochemical findings further supported the neuroprotective activity of Q-SLNs. Neurodegenerative injury was associated with increased MDA and decreased GSH, SOD, CAT, and GPx, indicating enhanced lipid peroxidation and impairment of endogenous antioxidant defense. Treatment with Q-SLNs reversed these alterations more effectively than free quercetin. Quercetin can act both as a direct antioxidant and as a modulator of endogenous antioxidant pathways, thereby reducing oxidative damage and improving cellular defense mechanisms (Boots et al., 2008; Dajas, 2012). Reduction of oxidative stress is particularly important in neuronal protection because excessive ROS can impair mitochondrial function, membrane integrity, proteins, and DNA, ultimately promoting neuronal apoptosis (Butterfield & Halliwell, 2019). The behavioral findings were consistent with the biochemical results. Animals receiving Q-SLNs showed improved spatial learning, working memory, recognition memory, and locomotor performance compared with the injury control group. The improvement in behavioral performance was accompanied by reduced oxidative stress and inflammatory biomarkers, suggesting a relationship between restoration of neuronal antioxidant capacity and functional recovery. The lower levels of TNF- α , IL-1 β , and

IL-6 following Q-SLN treatment further indicate attenuation of neuroinflammatory responses. Quercetin has previously been reported to influence inflammatory signaling, particularly through modulation of NF- κ B and related pathways (Li et al., 2016).

The molecular findings provide a possible explanation for the enhanced neuroprotective activity of Q-SLNs. Increased Nrf2/HO-1 signaling suggests activation of endogenous antioxidant defense, whereas suppression of NF- κ B indicates reduction of inflammatory signaling. Improvement of PI3K/Akt signaling may promote neuronal survival, while modulation of MAPK signaling may reduce stress-associated cellular responses. In addition, increased Bcl-2 together with reduced Bax and caspase-3 expression suggests inhibition of mitochondrial-associated apoptosis. Collectively, these findings support a multitarget mechanism in which Q-SLNs reduce oxidative stress and inflammation while simultaneously enhancing neuronal survival and suppressing apoptotic pathways. Such multitarget activity is particularly relevant because neurodegenerative diseases involve several interconnected pathological processes rather than a single molecular defect (Heneka et al., 2014; Li et al., 2016).

The present findings are also consistent with previous research describing the potential of lipid-based nanocarriers to improve delivery of therapeutic compounds to the brain. The greater activity of Q-SLNs compared with free quercetin may result from improved drug solubilization, protection from degradation, sustained release, and enhanced cellular uptake. Nevertheless, differences among studies can arise from variations in lipid composition, particle size, surface characteristics, administration route, experimental model, and dose. Therefore, the present results should be interpreted as evidence supporting the potential of Q-SLNs rather than definitive proof of clinical BBB targeting.

The study has certain limitations. The selected BBB model may not completely reproduce the complexity of the human neurovascular unit, and the experimental neurodegeneration model may not represent all pathological features of human disease. In addition, the present work requires confirmation through detailed pharmacokinetic and brain-biodistribution studies, long-term toxicity assessment, dose optimization, and larger translational models. Future studies should also investigate the stability, scalability, reproducibility, and long-term therapeutic efficacy of Q-SLNs before clinical development.

5. Conclusion and Future Perspectives

5.1 Conclusion

The present study successfully demonstrated the development and optimization of quercetin-loaded

solid lipid nanoparticles (Q-SLNs) as a potential nanocarrier for neuroprotective drug delivery. The optimized formulation exhibited desirable physicochemical properties, including nanoscale particle size, narrow size distribution, suitable zeta potential, high entrapment efficiency, and sustained quercetin release. Structural and morphological characterization confirmed successful incorporation of quercetin into the lipid matrix and formation of relatively uniform nanoparticles. Q-SLNs also showed improved BBB-oriented transport and cellular uptake compared with free quercetin. In the experimental neuropharmacological evaluation, Q-SLNs produced greater improvement in behavioral performance and more effectively restored antioxidant status by reducing MDA and increasing GSH, SOD, CAT, and GPx levels. The reduction of TNF- α , IL-1 β , and IL-6 further indicated attenuation of neuroinflammatory responses. Molecular findings suggested that the neuroprotective effects were associated with activation of Nrf2/HO-1 and PI3K/Akt signaling, suppression of NF- κ B and stress-related pathways, and inhibition of Bax/Bcl-2/caspase-3-mediated apoptosis. Overall, the findings support Q-SLNs as a promising lipid-based nanocarrier capable of improving the delivery and biological effectiveness of quercetin for neuroprotective applications (Islam et al., 2025).

5.2 Future Perspectives

Further studies should focus on detailed pharmacokinetic and brain-biodistribution investigations to confirm whether Q-SLNs provide superior accumulation and retention of quercetin in brain tissue. Long-term toxicity, safety, immunological compatibility, and dose-optimization studies are also required to establish the therapeutic window of the formulation (Hussain et al., 2013). Advanced BBB-targeting approaches, including surface functionalization with suitable targeting ligands, may further improve selective delivery to neuronal and brain endothelial cells. Scale-up studies should evaluate manufacturing reproducibility, batch-to-batch consistency, storage stability, and process optimization. Finally, the neuroprotective efficacy of Q-SLNs should be validated in multiple clinically relevant neurodegenerative models before translational development. With appropriate pharmacokinetic, toxicological, and efficacy validation, quercetin-loaded SLNs may have potential as a targeted nanotherapeutic platform for the management of oxidative-stress- and inflammation-associated neurodegenerative disorders.

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