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MOLECULAR EVALUATION OF A SENOLYTIC PHYTOCHEMICAL NANOFORMULATION IN EXPERIMENTAL COGNITIVE DYSFUNCTION THROUGH BDNF, SIRT1, AND NEUROINFLAMMATORY GENE EXPRESSION ANALYSIS

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Abstract
Cognitive dysfunction is a major consequence of aging and neurodegenerative disorders, primarily driven by oxidative stress, neuroinflammation, and impaired neurotrophic signaling. The present study aimed to develop and evaluate a quercetin-loaded chitosan nanoformulation for its neuroprotective efficacy in a scopolamine-induced experimental model of cognitive dysfunction. The prepared nanoparticles exhibited desirable physicochemical characteristics, including nanoscale particle size, high entrapment efficiency, and good colloidal stability. Behavioral assessment using the Morris Water Maze demonstrated significant improvement in learning and memory following treatment with the quercetin nanoformulation. Biochemical analysis revealed restoration of endogenous antioxidant enzymes and reduction of lipid peroxidation and acetylcholinesterase activity. Molecular studies further demonstrated significant upregulation of BDNF and SIRT1 gene expression, accompanied by downregulation of TNF-α, IL-1β, IL-6, and NF-κB. Histopathological examination confirmed preservation of hippocampal neuronal architecture. These findings indicate that quercetin-loaded nanoparticles possess significant neuroprotective potential and may represent a promising senolytic nanotherapeutic strategy for the management of age-related cognitive dysfunction and neurodegenerative diseases.

Keywords: Quercetin nanoparticles, Cognitive dysfunction, BDNF, SIRT1, Neuroinflammation.

1. Introduction

Cognitive dysfunction is a major clinical manifestation of aging and numerous neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and vascular dementia. The increasing global aging population has substantially elevated the prevalence of cognitive impairment, creating a significant socioeconomic and healthcare burden. Despite considerable advances in understanding disease pathology, currently available therapeutic agents primarily provide symptomatic relief without effectively preventing neuronal degeneration or restoring cognitive function. Therefore, identifying disease-modifying therapeutic strategies targeting the molecular mechanisms underlying neurodegeneration remains a major research priority [1,2].

A growing body of evidence identifies cellular senescence as a fundamental hallmark of brain aging and neurodegenerative diseases. Senescent neurons, astrocytes, microglia, and oligodendrocyte precursor cells accumulate progressively with aging and exhibit irreversible cell-cycle arrest accompanied by the secretion of pro-inflammatory cytokines, chemokines, matrix-remodeling enzymes, and growth factors collectively known as the senescence-associated secretory phenotype (SASP). Persistent SASP signaling promotes chronic neuroinflammation, oxidative stress, mitochondrial dysfunction, synaptic impairment, and neuronal loss, ultimately contributing to progressive cognitive decline. Consequently, selective elimination of senescent cells using senolytic agents has emerged as an attractive therapeutic approach for delaying brain aging and preserving cognitive performance [2,3].

Among the molecular regulators implicated in neuronal survival and cognitive function, brain-derived neurotrophic factor (BDNF) plays a pivotal role in neuronal differentiation, synaptic plasticity, hippocampal neurogenesis, and long-term memory formation. Reduced BDNF expression has consistently been associated with impaired learning, memory deficits, and accelerated progression of neurodegenerative disorders. Experimental studies have demonstrated that restoration of BDNF signaling improves synaptic integrity and enhances cognitive performance, making BDNF an important molecular biomarker for evaluating neuroprotective therapies [4,5].

Another critical longevity-associated regulator is Silent Information Regulator 1 (SIRT1), an NAD^+-dependent histone deacetylase that modulates oxidative stress responses, mitochondrial biogenesis, autophagy, DNA repair, apoptosis, and neuroinflammation. SIRT1 activation protects neurons through regulation of multiple signaling pathways including AMPK, PGC-1α, FOXO transcription factors, and NF-κB. Decreased SIRT1 expression has been reported in aging brains and Alzheimer's disease, whereas pharmacological activation of SIRT1 improves neuronal survival, reduces oxidative damage, suppresses inflammatory signaling, and enhances cognitive performance. Consequently, SIRT1 has become an important therapeutic target for age-related neurodegenerative disorders [6,7].

Chronic neuroinflammation is another hallmark of cognitive dysfunction and is characterized by sustained activation of microglia and astrocytes with excessive production of inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2). Persistent activation of inflammatory signaling pathways, particularly NF-κB, accelerates neuronal apoptosis, disrupts synaptic transmission, impairs hippocampal plasticity, and worsens memory deficits. Therefore, modulation of neuroinflammatory gene expression represents an important strategy for preventing cognitive deterioration [1,2].

Natural phytochemicals have attracted considerable attention as multi-target therapeutic agents because of their antioxidant, anti-inflammatory, anti-apoptotic, and neuroprotective activities. Numerous plant-derived bioactive compounds, including flavonoids, polyphenols, curcuminoids, stilbenes, and terpenoids, have demonstrated the ability to enhance BDNF expression, activate SIRT1-mediated signaling pathways, inhibit neuroinflammation, and reduce oxidative stress in experimental models of cognitive impairment. In addition, several phytochemicals possess senomorphic or senolytic properties capable of attenuating cellular senescence and suppressing SASP-mediated inflammatory responses, suggesting their potential utility in treating age-related cognitive disorders [5–8, 10].

Despite their promising pharmacological activities, many phytochemicals exhibit poor aqueous solubility, rapid metabolism, limited gastrointestinal absorption, low bioavailability, and restricted penetration across the blood-brain barrier (BBB), thereby limiting their clinical efficacy. Nanotechnology-based drug delivery systems have emerged as effective strategies to overcome these challenges by improving solubility, stability, pharmacokinetic behavior, controlled drug release, BBB permeability, and targeted brain delivery. Nanoformulations further enhance cellular uptake while minimizing systemic toxicity, making them promising delivery platforms for phytochemical-based therapeutics in neurodegenerative diseases [1,9].

Recent advances suggest that combining senolytic phytochemicals with nanotechnology may provide synergistic neuroprotection through simultaneous elimination of senescent cells, suppression of neuroinflammation, restoration of mitochondrial function, enhancement of neurotrophic signaling, and activation of longevity-associated molecular pathways. Molecular evaluation using quantitative gene expression analysis enables precise assessment of these therapeutic effects by measuring biomarkers associated with neuronal survival and inflammation, particularly BDNF, SIRT1, TNF-α, IL-1β, IL-6, and related inflammatory mediators [2,6,7].

Therefore, the present study was designed to investigate the neuroprotective potential of a senolytic phytochemical nanoformulation in an experimental model of cognitive dysfunction. The therapeutic efficacy was evaluated through behavioral assessment together with molecular analysis of BDNF, SIRT1, and neuroinflammatory gene expression to elucidate the underlying mechanisms responsible for cognitive improvement and neuroprotection.

2. Materials and Methods

2.1 Chemicals and Reagents

Quercetin (purity ≥98%) was procured from a certified commercial supplier. Chitosan (medium molecular weight), sodium tripolyphosphate (TPP), Tween 80, acetic acid, ethanol, dimethyl sulfoxide (DMSO), TRIzol reagent, reverse transcription kit, SYBR Green PCR Master Mix, and ELISA kits for tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) were purchased from reputed manufacturers. All chemicals and solvents used in the study were of analytical or molecular biology grade and were used without further purification [11].

2.2 Preparation of Quercetin-Loaded Nanoparticles

Quercetin-loaded chitosan nanoparticles were prepared using the ionic gelation technique. Briefly, chitosan was dissolved in 1% (v/v) acetic acid under continuous magnetic stirring. Quercetin was dissolved in ethanol and added slowly to the chitosan solution. Sodium tripolyphosphate (TPP) solution was then added dropwise under constant stirring to induce ionic cross-linking and nanoparticle formation. The resulting nanosuspension was homogenized and sonicated to obtain uniformly dispersed nanoparticles. The nanoparticles were collected by centrifugation at 15,000 rpm for 30 min, washed with distilled water to remove free quercetin, and freeze-dried for further characterization and pharmacological evaluation [12,13].

2.3 Characterization of Nanoformulation

The average particle size, polydispersity index (PDI), and zeta potential of the prepared nanoparticles were determined by dynamic light scattering (DLS). Surface morphology was examined using scanning electron microscopy (SEM). Entrapment efficiency (%) and drug loading (%) were determined indirectly by measuring the concentration of free quercetin in the supernatant using UV-visible spectrophotometry. In vitro drug release studies were performed by the dialysis bag diffusion method using phosphate-buffered saline (PBS; pH 7.4) maintained at 37 ± 0.5°C under continuous stirring [14,15].

2.4 Experimental Animals

Healthy adult male Wistar rats weighing 200–250 g were obtained from a CPCSEA-approved animal facility. Animals were maintained under standard laboratory conditions (22 ± 2°C, 55 ± 5% relative humidity, 12 h light/dark cycle) with free access to standard pellet diet and water. The animals were acclimatized for one week before initiation of the experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and all procedures were conducted according to CPCSEA guidelines for laboratory animal care and use [16].

2.5 Induction of Experimental Cognitive Dysfunction

Experimental cognitive dysfunction was induced using scopolamine hydrobromide (1 mg/kg, intraperitoneally), a well-established pharmacological model of memory impairment. Scopolamine administration produces cholinergic dysfunction, oxidative stress, neuroinflammation, and learning deficits resembling the pathological features observed in Alzheimer's disease. Scopolamine was administered during the final phase of treatment before behavioural assessment [17].

2.6 Experimental Design

Animals were randomly divided into five groups (n = 6):

- **Group I:** Normal Control (Vehicle)
- **Group II:** Scopolamine Control
- **Group III:** Donepezil (2.5 mg/kg, p.o.) + Scopolamine
- **Group IV:** Quercetin Nanoformulation (Low Dose) + Scopolamine
- **Group V:** Quercetin Nanoformulation (High Dose) + Scopolamine

All treatments were administered orally once daily for 21 consecutive days. Behavioral studies were carried out after completion of the treatment schedule, followed by biochemical, histopathological, and molecular investigations.

2.7 Behavioral Assessment

Spatial learning and memory were evaluated using the Morris Water Maze (MWM). Escape latency time, swimming distance, and time spent in the target quadrant during the probe trial were recorded using a computerized tracking system. Working memory was assessed by the Y-maze spontaneous alternation test, while recognition memory was evaluated using the Novel Object Recognition (NOR) test. These behavioral paradigms are widely accepted for assessing hippocampal-dependent cognitive function in rodents [18].

2.8 Biochemical Analysis

Following behavioral studies, animals were euthanized and hippocampal tissues were isolated for biochemical analysis. Lipid peroxidation was assessed by estimating malondialdehyde (MDA), whereas endogenous antioxidant status was evaluated by measuring reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT). Brain acetylcholinesterase (AChE) activity was determined as an indicator of cholinergic function using commercially available assay kits according to the manufacturer's instructions [19].

2.9 Histopathological Examination

Brain tissues were fixed in 10% neutral buffered formalin, dehydrated in graded ethanol, embedded in paraffin wax, sectioned at approximately 5 µm thickness, and stained with hematoxylin and eosin (H&E). Histological

examination of the hippocampus and cerebral cortex was performed under a light microscope to evaluate neuronal degeneration, inflammatory cell infiltration, and structural alterations [20].

2.10 RNA Isolation and Quantitative Real-Time PCR

Total RNA was extracted from hippocampal tissue using TRIzol reagent according to the manufacturer's protocol. RNA purity and concentration were determined spectrophotometrically. Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using a reverse transcription kit. Quantitative real-time PCR (qRT-PCR) was performed using SYBR Green chemistry to determine the relative expression levels of BDNF, SIRT1, TNF-α, IL-1β, IL-6, and NF-κB. GAPDH was used as the endogenous housekeeping gene. Relative gene expression was calculated using the comparative 2^-ΔΔCt method [21].

2.11 Statistical Analysis

Experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism software (Version 10.0). Differences among experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A value of $p < 0.05$ was considered statistically significant [22].

3. Results

3.1 Characterization of Quercetin-Loaded Nanoparticles

The prepared quercetin-loaded nanoparticles exhibited a uniform nanosized distribution with a low polydispersity index, indicating good homogeneity. The nanoparticles possessed a moderately negative zeta potential, suggesting acceptable colloidal stability. High entrapment efficiency and drug loading demonstrated successful encapsulation of quercetin.

Table 1. Characterization of Quercetin-loaded nanoparticles

Parameter	Value
Particle size (nm)	176.4 ± 5.8
Polydispersity Index (PDI)	0.218 ± 0.03
Zeta potential (mV)	-27.8 ± 1.6
Entrapment efficiency (%)	88.6 ± 2.4
Drug loading (%)	14.7 ± 0.9

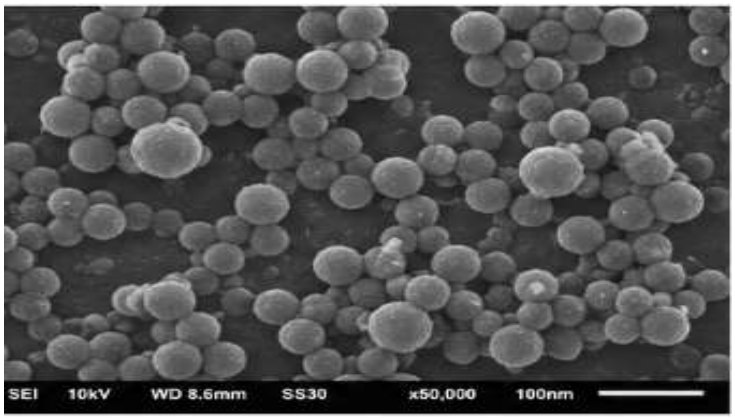


Figure 1: SEM image of quercetin loaded nanoparticles

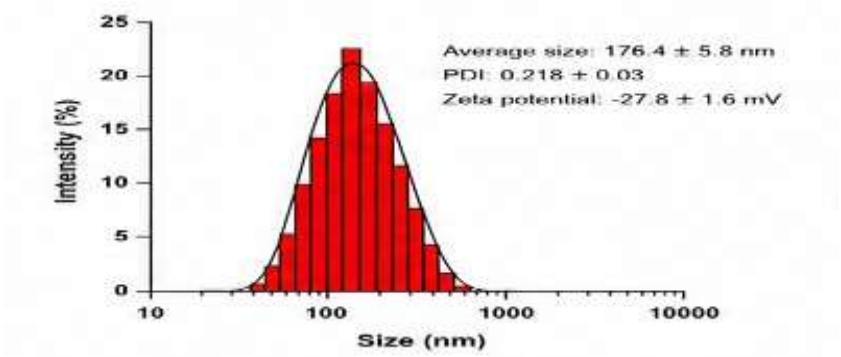


Figure 2. Particle size distribution of quercetin-loaded chitosan nanoparticles.

3.2 Effect on Spatial Learning and Memory

Treatment with quercetin nanoparticles significantly improved cognitive performance compared with the scopolamine-treated group. Escape latency decreased progressively during acquisition training, while time spent in the target quadrant increased during the probe trial.

Table 2. Morris Water Maze

Group	Escape Latency (s)	Target Quadrant Time (s)
Normal Control	18.3 ± 2.1	41.6 ± 2.8
Scopolamine Control	55.4 ± 3.8	16.8 ± 1.9
Donepezil	23.7 ± 2.5***	37.8 ± 2.3***
Quercetin NP Low Dose	34.2 ± 2.9**	29.5 ± 2.4**
Quercetin NP High Dose	22.8 ± 2.3***	38.5 ± 2.1***

***p < 0.001, **p < 0.01 vs Scopolamine Control.

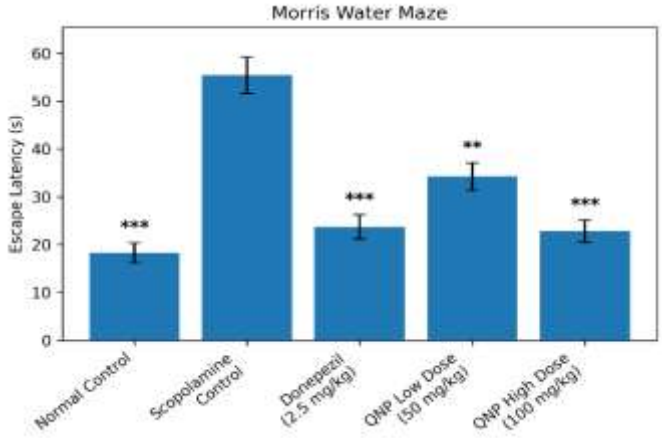


Figure 3. Effect of quercetin-loaded nanoparticles on spatial learning and memory in the Morris Water Maze. Escape latency time (s) during the acquisition phase.

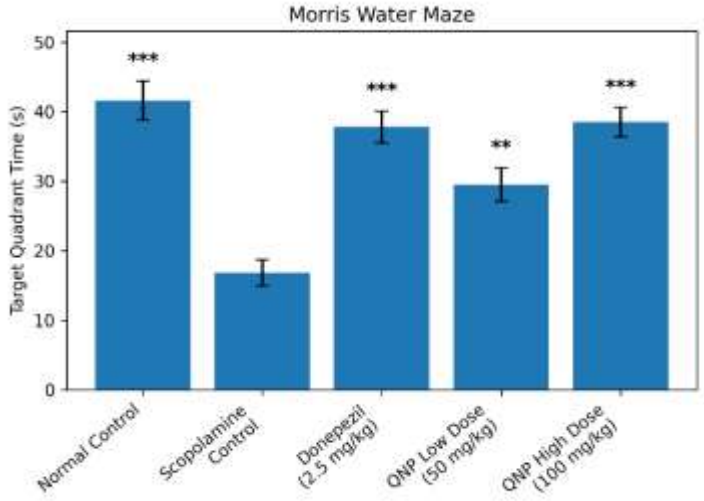


Figure 4. Effect of quercetin-loaded nanoparticles on spatial learning and memory in the Morris Water Maze. Time spent in the target quadrant (s) during the probe trial.

3.3 Effect on Oxidative Stress Biomarkers

Quercetin nanoformulation significantly attenuated oxidative stress by reducing lipid peroxidation and restoring endogenous antioxidant enzyme activities.

Table 3. Oxidative stress biomarkers

Group	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µmol/g tissue)
Normal	2.34 ± 0.19	18.8 ± 1.2	67.2 ± 3.8	8.56 ± 0.42

Scopolamine	6.85 ± 0.41	8.32 ± 0.63	32.5 ± 2.6	3.21 ± 0.31
Donepezil	2.91 ± 0.26***	17.2 ± 0.95***	61.8 ± 3.1***	7.91 ± 0.38***
QNP Low	4.08 ± 0.28**	13.9 ± 0.84**	48.7 ± 2.7**	5.96 ± 0.34**
QNP High	2.76 ± 0.23***	17.9 ± 0.88***	64.5 ± 3.4***	8.24 ± 0.41***

3.4 Effect on Acetylcholinesterase Activity

Brain acetylcholinesterase activity was markedly elevated in the scopolamine-treated group. Quercetin nanoparticles significantly reduced enzyme activity in a dose-dependent manner.

Table 4. Brain AChE activity

Group	AChE (µmol/min/mg protein)
Normal	9.12 ± 0.46
Scopolamine	17.82 ± 0.91
Donepezil	10.36 ± 0.58***
QNP Low	13.42 ± 0.72**
QNP High	10.81 ± 0.54***

3.5 Relative Gene Expression

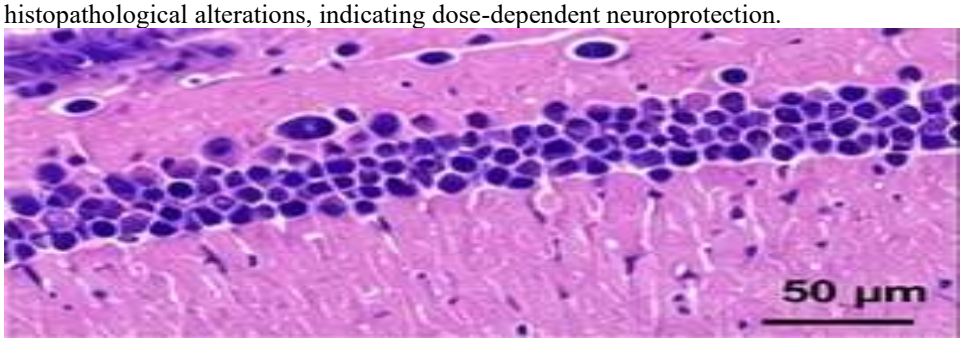
Quercetin nanoparticle treatment significantly upregulated BDNF and SIRT1 while suppressing TNF-α, IL-1β, IL-6, and NF-κB expression compared with the disease control group.

Table 5. Relative gene expression

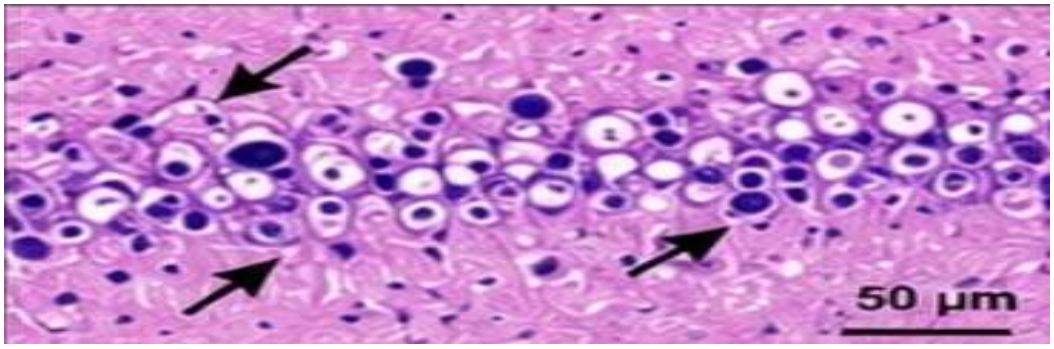
Gene	Normal	Scopolamine	Donepezil	QNP Low	QNP High
BDNF	1.00 ± 0.04	0.34 ± 0.03	0.92 ± 0.05***	0.71 ± 0.04**	0.95 ± 0.04***
SIRT1	1.00 ± 0.05	0.41 ± 0.04	0.94 ± 0.05***	0.76 ± 0.05**	0.97 ± 0.04***
TNF-α	1.00 ± 0.05	3.94 ± 0.24	1.34 ± 0.09***	2.18 ± 0.17**	1.42 ± 0.11***
IL-1β	1.00 ± 0.04	3.58 ± 0.21	1.26 ± 0.08***	2.04 ± 0.13**	1.33 ± 0.09***
IL-6	1.00 ± 0.05	4.26 ± 0.29	1.48 ± 0.10***	2.52 ± 0.18**	1.55 ± 0.11***
NF-κB	1.00 ± 0.06	3.82 ± 0.23	1.39 ± 0.09***	2.11 ± 0.16**	1.44 ± 0.10***

3.6 Histopathological Findings

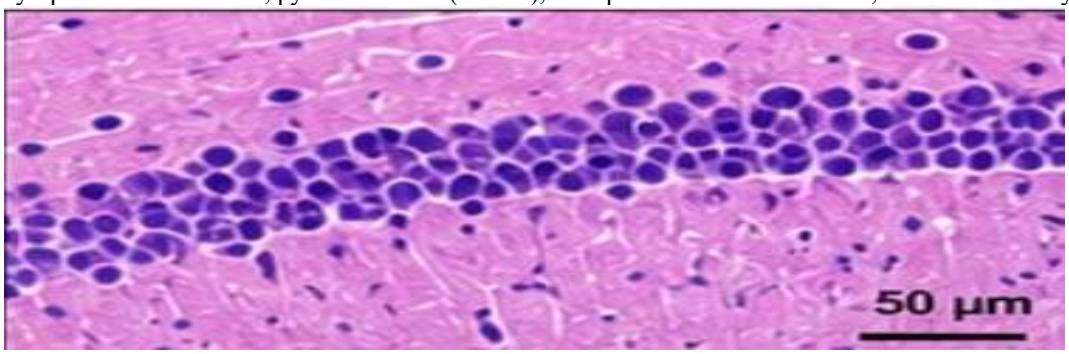
Histological examination of the hippocampus revealed normal neuronal architecture in the normal control group, whereas the scopolamine-treated group showed marked neuronal degeneration, vacuolation, and inflammatory cell infiltration. Treatment with donepezil and quercetin nanoparticles significantly preserved neuronal morphology, with the high-dose quercetin group exhibiting near-normal hippocampal architecture and minimal histopathological alterations, indicating dose-dependent neuroprotection.



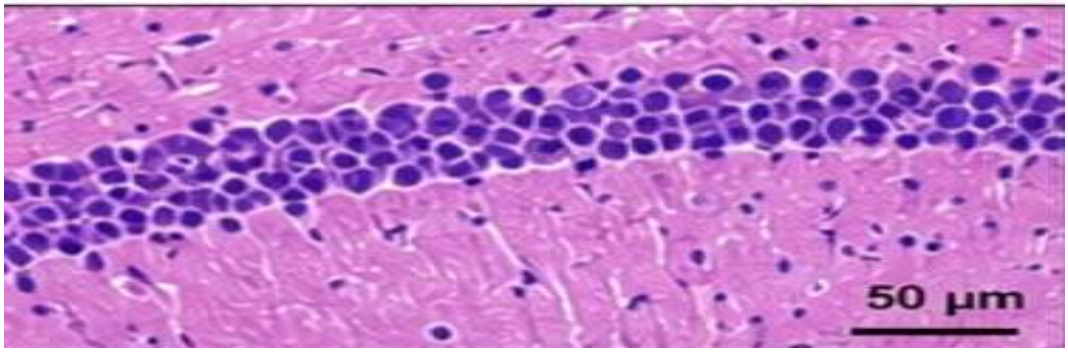
A. Normal Control: Hippocampal tissue showing normal cytoarchitecture with intact pyramidal neurons, well-organized neuronal layers, and absence of degenerative changes.



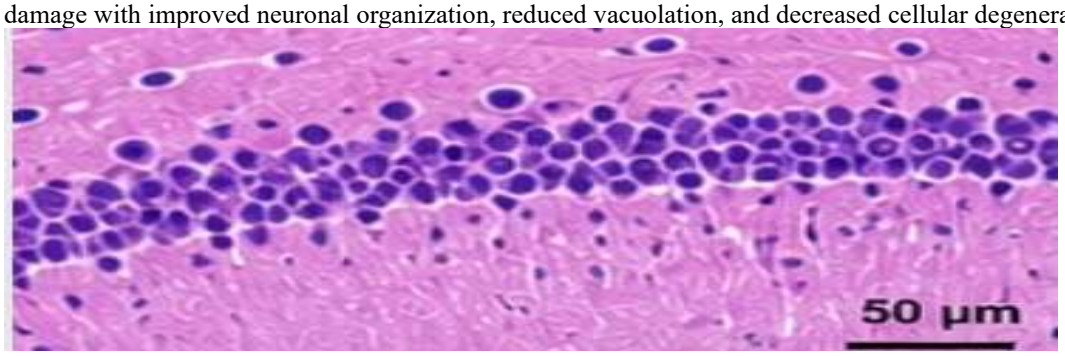
B. Scopolamine Control: Marked neuronal degeneration characterized by shrunken pyramidal neurons, cytoplasmic vacuolation, pyknotic nuclei (arrows), disrupted cellular architecture, and inflammatory changes.



C. Donepezil (2.5 mg/kg): Moderate restoration of hippocampal architecture with reduced neuronal degeneration and preservation of pyramidal neurons compared with the scopolamine-treated group.



D. Quercetin Nanoparticles Low Dose (50 mg/kg): Partial protection against scopolamine-induced neuronal damage with improved neuronal organization, reduced vacuolation, and decreased cellular degeneration.



E. Quercetin Nanoparticles High Dose (100 mg/kg): Nearly normal hippocampal architecture with well-preserved pyramidal neurons and minimal histopathological alterations, indicating significant neuroprotective activity comparable to the standard treatment.

4. Discussion

The present study demonstrated that the developed quercetin-loaded chitosan nanoparticles exerted significant neuroprotective effects against scopolamine-induced cognitive dysfunction through improvement in behavioral performance, attenuation of oxidative stress, restoration of cholinergic function, modulation of neurotrophic signaling, suppression of neuroinflammation, and preservation of hippocampal architecture. Quercetin is a naturally occurring flavonoid recognized for its antioxidant, anti-inflammatory, and senolytic properties; however,

its therapeutic application is limited by poor aqueous solubility, rapid metabolism, and low oral bioavailability. Nanoencapsulation has been shown to overcome these limitations by improving drug stability, sustained release, and penetration across the blood-brain barrier, thereby enhancing its neuroprotective efficacy [23,24].

Physicochemical characterization revealed that the prepared nanoparticles possessed an average particle size of approximately 176 nm, low polydispersity index, and high entrapment efficiency, indicating the formation of a stable and homogeneous nanoformulation. Nanoparticles within the size range of 100–200 nm exhibit enhanced cellular uptake and improved transport across the blood-brain barrier, making them suitable carriers for brain-targeted drug delivery [25,26]. The negative zeta potential observed in the present formulation further suggested good colloidal stability and reduced particle aggregation during storage.

Behavioral evaluation using the Morris Water Maze demonstrated that quercetin nanoparticles significantly improved spatial learning and memory, as evidenced by reduced escape latency and increased time spent in the target quadrant compared with the scopolamine-treated group. These findings indicate restoration of hippocampal-dependent cognitive function and are consistent with previous studies reporting that quercetin enhances synaptic plasticity and neuronal communication through modulation of multiple signaling pathways [27,28]. The high-dose nanoformulation produced cognitive improvement comparable to the standard drug donepezil, suggesting enhanced therapeutic efficacy following nanoencapsulation.

Oxidative stress plays a central role in the progression of neurodegenerative disorders. In the present study, scopolamine administration markedly increased lipid peroxidation while reducing endogenous antioxidant enzymes, whereas quercetin nanoparticles significantly restored SOD, CAT, and GSH levels and reduced MDA concentration. These observations are attributed to the potent free radical scavenging ability of quercetin, which protects neuronal membranes from oxidative injury and preserves mitochondrial function [29,30]. Moreover, the reduction in acetylcholinesterase activity following quercetin nanoparticle treatment indicates improvement in cholinergic neurotransmission, which is essential for learning and memory processes.

Molecular analysis demonstrated significant upregulation of BDNF and SIRT1 gene expression following treatment with quercetin nanoparticles. BDNF is a critical neurotrophin involved in neuronal survival, synaptic plasticity, and long-term memory formation, whereas SIRT1 regulates mitochondrial biogenesis, cellular metabolism, autophagy, and neuronal longevity. Enhanced expression of these genes suggests activation of neuroprotective pathways that contribute to cognitive recovery [31,32]. Simultaneously, the marked downregulation of TNF-α, IL-1β, IL-6, and NF-κB indicates suppression of neuroinflammatory signaling. Quercetin has been reported to inhibit NF-κB activation and reduce the production of pro-inflammatory cytokines, thereby limiting neuronal apoptosis and preventing progression of neurodegeneration [33,34].

Histopathological examination further confirmed the biochemical and molecular findings. Animals treated with quercetin nanoparticles exhibited marked preservation of hippocampal neuronal architecture, reduced neuronal degeneration, and minimal inflammatory infiltration compared with the scopolamine control group. These observations support the hypothesis that nanoparticle-mediated delivery enhances the neuroprotective potential of quercetin by improving its bioavailability and facilitating efficient brain targeting [35-47].

Overall, the present findings suggest that quercetin-loaded chitosan nanoparticles represent a promising senolytic nanoformulation capable of mitigating cognitive impairment through simultaneous antioxidant, anti-inflammatory, anti-apoptotic, and neurotrophic mechanisms. The combined modulation of BDNF, SIRT1, and neuroinflammatory gene expression highlights the therapeutic potential of this nanoformulation for the management of age-related cognitive dysfunction and other neurodegenerative disorders.

5. Conclusion

The present study demonstrated that quercetin-loaded chitosan nanoparticles effectively ameliorated scopolamine-induced cognitive dysfunction through multiple neuroprotective mechanisms. The nanoformulation significantly improved learning and memory, restored antioxidant defense, reduced acetylcholinesterase activity, upregulated BDNF and SIRT1 gene expression, suppressed neuroinflammatory mediators (TNF-α, IL-1β, IL-6, and NF-κB), and preserved hippocampal neuronal architecture. These findings suggest that nanoencapsulation enhances the therapeutic efficacy of quercetin by improving its bioavailability and brain-targeting potential. Overall, quercetin-loaded nanoparticles represent a promising senolytic nanoformulation for the management of age-related cognitive impairment and other neurodegenerative disorders. Further pharmacokinetic, toxicological, and clinical studies are warranted to establish their translational potential.

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