

PHARMACOLOGICAL EVALUATION OF MOGROL NANOPARTICLES IN STZ INDUCED ALZHEIMER'S DISEASE

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive dysfunction, neuroinflammation, amyloid pathology, and impaired neurotrophic signaling. The present study investigated the neuroprotective potential of mogrol and evaluated whether poly(lactic-co-glycolic acid) (PLGA) nanoparticle formulation could improve its therapeutic potential in an intracerebroventricular streptozotocin (ICV-STZ)-induced mouse model of AD. Molecular docking was performed to investigate the potential interaction of mogrol with an AD-associated molecular target. Mogrol-loaded PLGA nanoparticles were characterized for particle size, polydispersity index (PDI), zeta potential, encapsulation efficiency, and drug loading. Cognitive performance was evaluated using the novel object recognition test, while TNF- α and IL-1 β levels were quantified by ELISA. The expression of BDNF, NLRP3, and β -amyloid was determined using real-time PCR. Mogrol exhibited a favorable docking score of -6.5 kcal/mol. Mogrol-PLGA nanoparticles showed a mean particle size of 168.7 ± 5.2 nm, PDI of 0.162 ± 0.011 , zeta potential of -26.8 ± 1.7 mV, and encapsulation efficiency of $82.36 \pm 2.47\%$. STZ significantly impaired recognition memory, increased TNF- α , IL-1 β , NLRP3, and β -amyloid expression, and reduced BDNF expression. Both free mogrol and Mogrol-PLGA nanoparticles significantly reversed these alterations. Overall, mogrol demonstrated promising neuroprotective activity, while PLGA nanoencapsulation represents a potential strategy for improving its delivery in AD.

Introduction

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder and the most common cause of dementia in older adults, characterized by progressive deterioration of memory, learning, cognitive function, and eventually the ability to perform daily activities.

The pathological features of AD include accumulation of amyloid- β (A β) plaques, hyperphosphorylated tau and neurofibrillary tangles, synaptic dysfunction, neuronal loss, oxidative stress, mitochondrial impairment, and persistent neuroinflammation, while increasing evidence suggests that impaired brain insulin

signaling and metabolic dysfunction also contribute significantly to disease progression (Salkovic-Petrisic et al., 2013; Grieb, 2016). The development of AD is influenced by both non-modifiable and modifiable risk factors, with advanced age being the strongest established risk factor, while genetic susceptibility, particularly the apolipoprotein E $\epsilon 4$ (APOE $\epsilon 4$) allele, also substantially increases disease risk. Other important modifiable factors include hypertension, diabetes mellitus, obesity, physical inactivity, smoking, excessive alcohol consumption, depression, traumatic brain injury, hearing impairment, social isolation, high low-density lipoprotein cholesterol, and untreated visual impairment, demonstrating that AD and dementia are influenced by multiple interacting biological and environmental factors throughout the life course (Livingston et al., 2024). The increasing prevalence of dementia has created a major global health burden; the World Health Organization estimates that approximately 57 million people were living with dementia worldwide in 2021, with Alzheimer's disease accounting for approximately 60–70% of dementia cases, and nearly 10 million new cases occurring annually (World Health Organization, 2026). The increasing number of patients, progressive nature of the disease, and limitations of currently available therapies emphasize the need for novel therapeutic strategies. For experimental investigation of AD, intracerebroventricular streptozotocin (ICV-STZ) is a widely used non-transgenic model that reproduces several important features of sporadic AD. STZ administered directly into the brain interferes with cerebral glucose metabolism and insulin signaling and can produce oxidative stress, neuroinflammation, cholinergic dysfunction, synaptic impairment, cognitive deficits, and AD-like pathological changes, including alterations in amyloid and tau-related pathways (Salkovic-Petrisic et al., 2013; Grieb, 2016). Recent evidence has further demonstrated that ICV-STZ produces AD-like behavioral and neuropathological alterations through mechanisms involving neuroinflammation and metabolic dysfunction, supporting its application for evaluating potential neuroprotective agents (More et al.,

2026). Currently available pharmacological treatments for AD include acetylcholinesterase inhibitors such as donepezil, rivastigmine, and galantamine and the N-methyl-D-aspartate receptor antagonist memantine, which primarily provide symptomatic benefits rather than completely preventing or reversing the underlying neurodegenerative process. More recently, anti-amyloid monoclonal antibodies such as lecanemab and donanemab have introduced disease-modifying approaches for selected patients with early AD; however, their use is associated with limitations including high cost, strict patient-selection criteria, intensive monitoring requirements, and adverse effects such as amyloid-related imaging abnormalities, highlighting the continuing need for safer and more accessible therapeutic approaches. Natural products and their bioactive metabolites have therefore received considerable attention because of their potential to simultaneously modulate multiple pathological mechanisms involved in AD.

Mogrol (Figure 1) is a cucurbitane-type tetracyclic triterpenoid and the aglycone of mogrosides derived from *Siraitia grosvenorii* (Swingle), commonly known as monk fruit, and has attracted increasing interest because of its diverse pharmacological properties, including antioxidant, anti-inflammatory, neuroprotective, antidiabetic, anti-obesity, and other organ-protective activities (Jaiswal & Lee, 2023). Mogrol is biologically important because it can be generated through metabolism of mogrosides, while pharmacokinetic studies indicate that free mogrol has relatively limited oral bioavailability and a short systemic half-life, suggesting that an appropriate drug-delivery system may improve its therapeutic exposure (Wang et al., 2017). Importantly, experimental studies have demonstrated promising neuroprotective effects of mogrol in models relevant to neurodegeneration. Chen et al. (2019) reported that mogrol attenuated A β 1–42-induced memory impairment in mice and reduced microglial activation, apoptosis, and NF- κ B-associated inflammatory responses in the hippocampus, indicating that mogrol can modulate important mechanisms associated with AD pathology. Furthermore, mogrol has been reported to attenuate lipopolysaccharide-

induced memory impairment and neuroinflammatory responses, including suppression of microglial activation, NF- κ B signaling, and inflammatory cytokines such as tumor necrosis factor- α , interleukin-1 β , and interleukin-6 (Long et al., 2019). These antioxidant, anti-inflammatory, and neuroprotective properties make mogrol a promising candidate for investigation in AD; however, its physicochemical and pharmacokinetic limitations may restrict its therapeutic effectiveness. Poly(lactic-co-glycolic acid) (PLGA) nanoparticles provide a biocompatible and biodegradable drug-delivery platform capable of protecting encapsulated compounds, improving drug stability, providing controlled release, and potentially enhancing systemic exposure and therapeutic delivery. Therefore, incorporation of mogrol into PLGA nanoparticles represents a rational strategy for improving the delivery characteristics of mogrol

and potentially enhancing its neuroprotective effects. Importantly, based on a targeted literature search, although free mogrol has been investigated in A β 1-42- and LPS-induced models of memory impairment and neuroinflammation, and PLGA-based nanocarriers have been extensively investigated for delivery of various therapeutic compounds, no published experimental study was identified specifically evaluating mogrol-loaded PLGA nanoparticles in an Alzheimer's disease animal model. Thus, investigation of mogrol-loaded PLGA nanoparticles in an STZ-induced Alzheimer's disease model may provide a novel and scientifically justified approach to determine whether nanoformulation can enhance the therapeutic potential of mogrol against AD-associated cognitive dysfunction, neuroinflammation, oxidative stress, neuronal injury, and synaptic abnormalities.

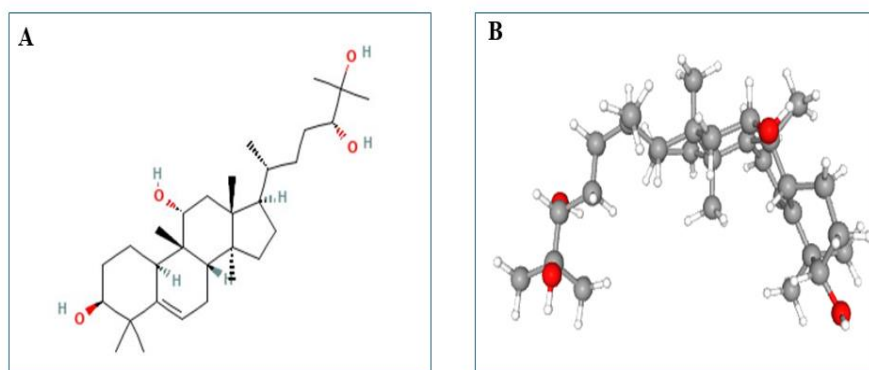


Figure 1: 2D and 3D structures of mogrol.

Materials and Methods

Chemicals

Mogrol of analytical grade and streptozotocin (STZ) were procured from a certified supplier in Shanghai, China. Other laboratory reagents, including chloroform, formalin, and normal saline, were obtained from an authorized local supplier. All chemicals and reagents were used as received, without further purification or chemical modification, to maintain consistency and ensure the reproducibility of the experimental procedures.

Animals

Healthy adult mice weighing 30–40 g were obtained from the National Institutes of Health (NIH), Islamabad, Pakistan. Before initiation of the experimental procedures, the animals were acclimatized to the laboratory environment for an appropriate period. The mice were housed in polypropylene cages, with five animals per cage, under controlled environmental conditions maintained at $25 \pm 1^\circ\text{C}$ temperature and $50 \pm 10\%$ relative humidity, with a 12-hour light/dark cycle. Throughout the study, the

animals were provided ad libitum access to standard pellet diet and drinking water.

Computational Study

The three-dimensional structure of the ligand was generated, and its molecular geometry was evaluated using BIOVIA Discovery Studio Visualizer. The crystal structure of the Alzheimer's disease-related target protein, Tau receptor (PDB ID: 3BJM), was retrieved from the RCSB Protein Data Bank. Before docking, the protein structure was prepared by removing crystallographic water molecules and co-crystallized ligands, followed by the addition of polar hydrogen atoms. Molecular docking was performed using PyRx with the AutoDock docking engine to assess the binding affinity and preferred binding orientation of the ligand within the target protein's active site. The docking results were expressed as binding energies in kcal/mol, with the pose exhibiting the lowest binding energy considered the most favorable and selected for further interaction analysis. The ligand-protein interactions of the selected docking poses were subsequently analyzed and visualized using two-dimensional and three-dimensional molecular visualization tools (Jakhar et al., 2020).

Pharmaceutical Characterization of Mogrol Nanoparticles

Mogrol-loaded PLGA nanoparticles were prepared using a suitable polymeric nanoparticle fabrication method and subsequently evaluated for their physicochemical characteristics. Particle size and polydispersity index (PDI) were determined by dynamic light scattering (DLS), while surface charge was measured as zeta potential using electrophoretic light scattering at controlled temperature. Mogrol concentration was quantified spectrophotometrically using a previously established calibration curve at its maximum absorption wavelength ($\lambda_{\text{max}} = 318$ nm). Encapsulation efficiency (EE%) was determined indirectly from the amount of unencapsulated Mogrol recovered in the supernatant after separation of the nanoparticles. Drug loading (DL%) was calculated as the amount of encapsulated Mogrol relative to the total weight of recovered nanoparticles. All characterization

measurements were performed in triplicate and expressed as mean $\pm$ SD ($n = 3$). These procedures are consistent with established methodologies reported for characterization and drug-loading assessment of PLGA nanoparticles (Derman et al., 2015).

In Vivo Model

An experimental Alzheimer's disease model was established in mice through intracerebroventricular administration of streptozotocin (STZ) followed by mogrol administration. Following STZ administration, animals were monitored for the development of Alzheimer's-like cognitive and cholinergic alterations. Behavioral assessments were subsequently performed to evaluate learning and memory impairment associated with STZ-induced neurodegeneration (Silva et al., 2023).

Behavioral Assessment

Novel object recognition was performed to evaluate recognition memory following a standard habituation, familiarization, and testing protocol. Animals were first habituated to the testing arena, followed by exposure to two identical objects during the familiarization phase. After an appropriate retention interval, one familiar object was replaced with a novel object, and the time spent exploring the objects was recorded by video tracking/observation. The NOR time performance was calculated from the exploration time of the novel object relative to the total object-exploration time, as described in established rodent NORT protocols (Walf et al., 2007).

Enzyme-Linked Immunosorbent Assay

Brain tissue samples were homogenized in an appropriate ice-cold buffer and centrifuged to obtain the clear supernatant. The levels of inflammatory cytokines, including interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α), were determined using commercially available mouse-specific ELISA kits according to the manufacturer's recommended protocol. Briefly, standards and tissue samples were added to antibody-precoated wells and incubated to facilitate antigen-antibody interaction. After the required washing steps, enzyme-conjugated detection antibodies and substrate solution were

added, and the resulting absorbance was measured using a microplate reader at the specified wavelength. Cytokine concentrations were determined from the corresponding standard curves and normalized to the total protein content of the tissue samples (Bruggink et al., 2013).

Real Time Polymerase Chain Reaction

Total RNA was isolated from mice brain tissue, followed by reverse transcription to generate complementary DNA (cDNA). Quantitative real-time RT-PCR was subsequently performed using gene-specific primers to determine the expression levels of selected metabolic enzyme transcripts. The qRT-PCR analysis was used to validate gene-expression changes identified through microarray analysis, with multiple genes involved in glycolysis, the tricarboxylic acid cycle, and oxidative phosphorylation evaluated. Relative transcript expression was quantified and compared between Alzheimer's disease and control samples to identify significantly altered gene expression (Brooks et al., 2007).

Statistical Analysis

The results were expressed as mean $\pm$ SD based on three independent observations ($n = 5$). Differences among the experimental groups

were analyzed using one-way ANOVA, followed by LSD post-hoc multiple comparisons using IBM SPSS Statistics version 25. Graphs were generated using GraphPad Prism version 9.5.0. A p -value < 0.05 was considered indicative of statistical significance.

Results

In-silico Evaluation

Molecular docking analysis was performed to investigate the potential binding interactions of mogrol with Alzheimer's disease-associated molecular target. Mogrol exhibited a favorable binding energy of -6.5 kcal/mol against the Tau receptor, indicating a potentially stable interaction with the selected target (Table 1). Analysis of the docked conformations revealed that mogrol was suitably positioned within the receptor binding pocket through a combination of hydrogen-bonding and hydrophobic interactions (Figure 2). These interactions suggest a favorable complementarity between the structural features of mogrol and the binding site of the target protein. Overall, the docking findings indicate that mogrol may interact effectively with the Tau receptor and could potentially influence downstream molecular signaling associated with Alzheimer's disease pathology.

Table 1: Binding interactions of mogrol with selected receptor.

Receptor	PDB ID	Compound	Energy Values Kcal/mol
Tau	38JM	Mogrol	-6.5

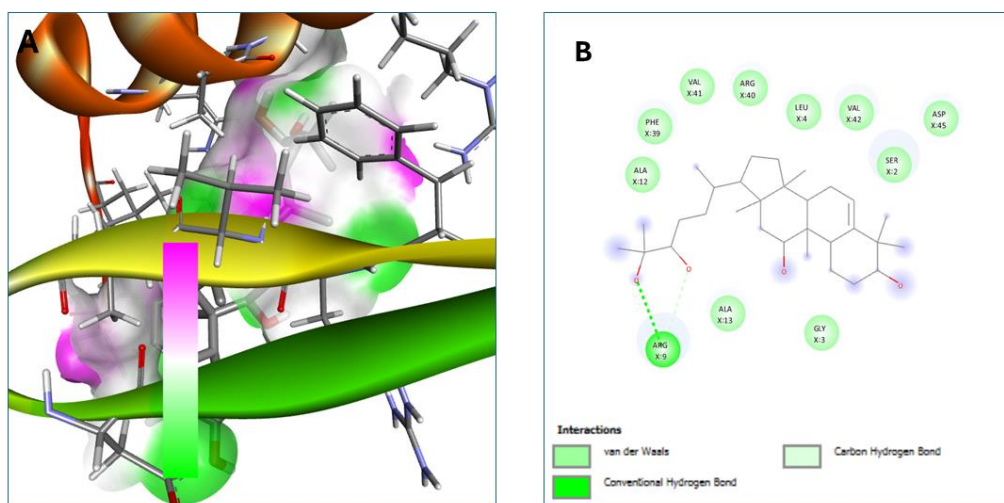


Figure 2: 2D and 3D interactions of mogrol with tau receptor.

Nanoparticles Characterization

Physicochemical evaluation via dynamic light scattering (DLS) revealed that Mogrol-loaded PLGA nanoparticles (Mogrol-PLGA NPs) achieved a mean particle size of 168.7 ± 5.2 nm. The formulation demonstrated a narrow size distribution with a low polydispersity index (PDI) of 0.162 ± 0.011 , confirming uniform nanoparticle fabrication. Surface charge determination via zeta potential analysis yielded a negative value of -26.8 ± 1.7 mV, indicating high colloidal stability and resistance to particle aggregation. Spectroscopic analysis of Mogrol at maximum absorbance wavelength ($\lambda_{\text{max}} = 318$ nm) was conducted to evaluate drug entrapment and loading metrics. The generated calibration plot displayed strong linearity across concentrations, defined by the regression equation $y = 0.0296x + 0.0065$ ($R^2 =$

0.9987). Evaluation of entrapment parameters demonstrated a high encapsulation efficiency (EE%) of $82.36 \pm 2.47\%$ from an initial drug input of 10 mg. Quantitative measurement of the supernatant revealed a low residual amount of free Mogrol remaining at 1.764 ± 0.071 mg. Correspondingly, the total mass of Mogrol successfully entrapped within the polymeric matrix was determined to be 8.236 ± 0.082 mg. With a total recovered nanoparticle weight reaching 49.00 ± 0.60 mg, the drug loading capacity (DL%) was calculated as $16.72 \pm 0.93\%$. Taken together, these metrics confirm that Mogrol-PLGA NPs exhibit favorable physicochemical characteristics, nanoscale size, low polydispersity, and robust encapsulation efficiency suitable for biomedical applications (Figure 3).

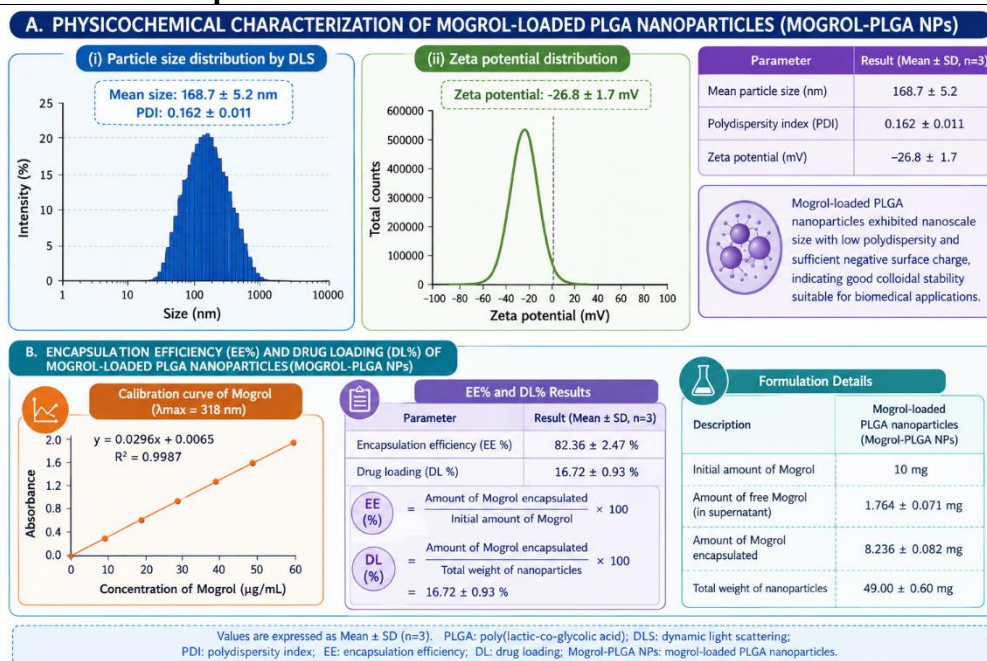


Figure 3: Mogrol nanoparticles characterization.

Behavioral Analysis

The Novel Object Recognition Test (NORT) was conducted to evaluate cognitive memory across the experimental groups, where administration of STZ induced severe recognition memory deficits as evidenced by a significant reduction in NORT exploration time compared to the Normal Saline group (### $p < 0.001$). Conversely, treatment with free Mogrol

(40 mg/kg) and Mogrol PLGA NP effectively attenuated these cognitive impairments, each showing a statistically significant increase in NORT exploration time relative to the STZ group (** $p < 0.001$). Together, these results demonstrate that both free Mogrol and Mogrol PLGA nanoparticles successfully restore novel object recognition memory against STZ-induced deficits (Figure 4).

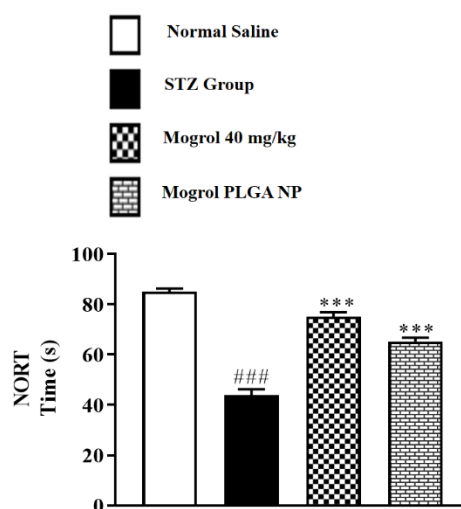


Figure 4: NORT test

Quantification of Inflammatory Markers

To evaluate the anti-inflammatory efficacy of the treatment, pro-inflammatory cytokine levels of TNF- α and IL-1 β were measured via ELISA across the experimental groups. Administration of STZ induced a marked inflammatory response, marked by a statistically significant elevation in both TNF- α and IL-1 β levels compared to the Normal Saline control group (### $p < 0.001$). Conversely, treatment with free Mogrol (40 mg/kg) substantially attenuated neuroinflammation, producing a significant reduction in the levels of both TNF- α and IL-

1 β relative to the STZ model group (** $p < 0.001$). Similarly, Mogrol PLGA NP administration effectively suppressed the hyper-expression of these pro-inflammatory cytokines, demonstrating a comparable and statistically significant decrease in TNF- α and IL-1 β concentrations compared to the STZ group (** $p < 0.001$). Collectively, these findings confirm that both free Mogrol and Mogrol PLGA nanoparticles exhibit potent anti-inflammatory effects by effectively downregulating STZ-induced pro-inflammatory cytokine secretion (Figure 5).

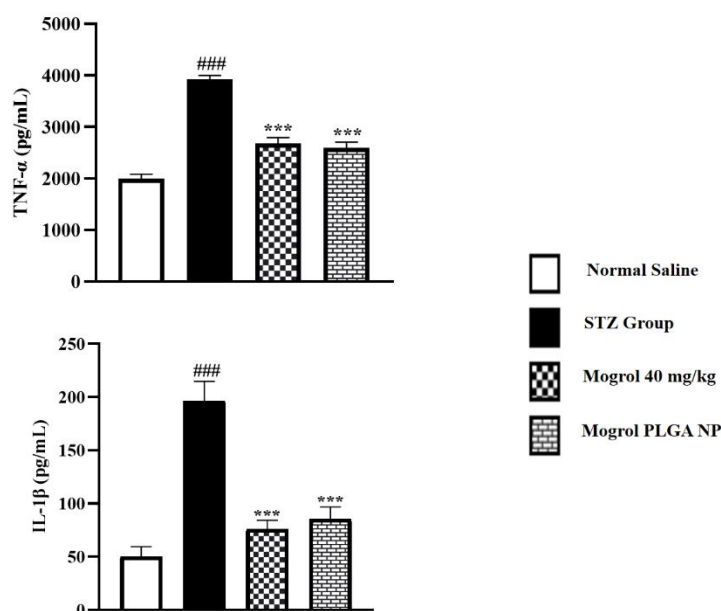


Figure 5: ELISA

mRNA Expression Levels

To examine the molecular mechanisms underlying neuroprotection, quantitative real-time PCR (RT-qPCR) was performed to analyze the relative mRNA expression ($2^{-\Delta\Delta C_T}$) of BDNF, NLRP3, and β -Amyloid across the experimental groups. Streptozotocin (STZ) administration induced significant pathological alterations, marked by a pronounced downregulation of neuroprotective BDNF gene expression alongside a significant upregulation of neuroinflammatory NLRP3 and pathological β -Amyloid expression compared to the Normal Saline control group (### $p < 0.001$). Conversely, treatment with free Mogrol (40

mg/kg) successfully attenuated these molecular alterations, producing a statistically significant upregulation of BDNF expression and a concurrent downregulation of both NLRP3 and β -Amyloid mRNA levels relative to the STZ model group (** $p < 0.001$). Similarly, Mogrol PLGA NP administration demonstrated significant therapeutic efficacy, restoring BDNF transcription while effectively suppressing the elevated mRNA levels of NLRP3 and β -Amyloid compared to the STZ group (** $p < 0.001$). Taken together, these results demonstrate that both free Mogrol and Mogrol PLGA nanoparticles mitigate STZ-induced neurotoxicity by boosting neurotrophic support

and downregulating neuroinflammatory and amyloidogenic pathways (Figure 6).

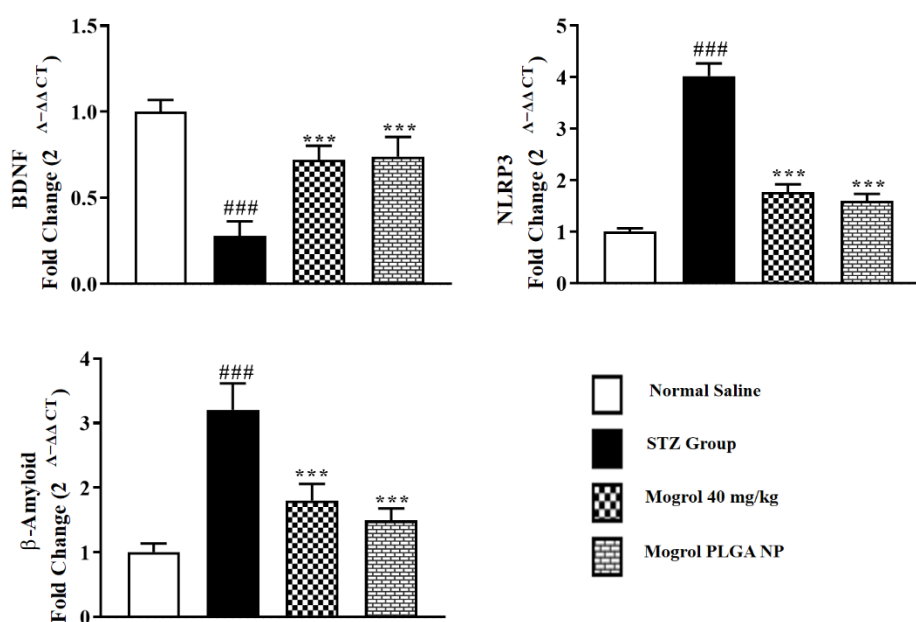


Figure 6: RT-PCR analysis

Discussion

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder in which cognitive decline results from the interaction of several pathological processes, including neuroinflammation, abnormal amyloid processing, synaptic dysfunction, oxidative stress, neuronal injury, and impaired neurotrophic signaling. Because these mechanisms are interconnected, therapeutic strategies capable of simultaneously influencing multiple pathological pathways may provide greater neuroprotective benefits than approaches directed toward a single molecular target. The present study investigated the neuroprotective potential of mogrol, a cucurbitane-type triterpenoid derived from *Siraitia grosvenorii*, in an intracerebroventricular streptozotocin (ICV-STZ)-induced experimental model of AD and further evaluated whether incorporation of mogrol into PLGA nanoparticles could improve its therapeutic performance. The principal findings demonstrated that mogrol exhibited favorable molecular interaction with the selected computational target, was successfully incorporated into PLGA nanoparticles with

appropriate physicochemical characteristics, and significantly attenuated STZ-induced cognitive impairment, neuroinflammation, and molecular alterations associated with BDNF, NLRP3, and β-amyloid expression. The ICV-STZ model used in the present study provides an experimentally relevant model of sporadic AD-like pathology because intracerebral STZ administration produces disturbances in brain insulin signaling and glucose metabolism accompanied by oxidative stress, neuroinflammation, cholinergic dysfunction, synaptic abnormalities, and cognitive impairment. Recent work has further emphasized that variations in STZ dose, administration regimen, and experimental conditions can influence the severity and molecular characteristics of the resulting phenotype, but the model remains widely used for investigating mechanisms and potential therapeutics relevant to sporadic AD. More et al. (2026) recently reviewed the ICV-STZ model and highlighted its ability to reproduce several AD-associated molecular and behavioral alterations. Consistent with this experimental framework, administration of STZ in the present study produced a pronounced reduction in recognition memory together with increased inflammatory cytokines and pathological

alterations in BDNF, NLRP3, and β -amyloid expression. These findings indicate that the model successfully produced a pathological state suitable for evaluating the neuroprotective effects of mogrol and its nanoformulation. The molecular docking results provided preliminary computational support for the potential biological activity of mogrol. Mogrol demonstrated a binding energy of -6.5 kcal/mol toward the selected protein target, accompanied by hydrogen-bonding and hydrophobic interactions within the predicted binding region. Although docking scores cannot establish pharmacological efficacy or prove direct target engagement, favorable binding interactions can provide a mechanistic hypothesis that may complement experimental findings. In the present study, the computational results are particularly relevant because the subsequent *in vivo* experiments demonstrated significant modulation of inflammatory and neurotrophic pathways. Thus, the docking analysis provides supportive rather than definitive evidence for a potential molecular interaction of mogrol with the selected target. Importantly, the identity of the computational target should be corrected in the manuscript before publication because PDB ID 3BJM corresponds to human dipeptidyl peptidase-4 (DPP-4), not Tau. The present behavioral findings demonstrated that STZ administration markedly impaired novel object recognition, confirming the development of recognition-memory dysfunction. This observation is consistent with previous reports showing that ICV-STZ produces learning and memory deficits and with studies demonstrating that disruption of cerebral metabolic and inflammatory homeostasis can contribute to cognitive dysfunction. Importantly, treatment with free mogrol significantly improved recognition-memory performance compared with the STZ group. This finding is consistent with the previously reported neuroprotective effects of mogrol in experimental models of memory impairment. Chen et al. (2019) demonstrated that mogrol significantly attenuated A β 1-42-induced memory impairment in mice and reduced microglial activation, apoptosis, and NF- κ B-associated inflammatory signaling in the hippocampus.

Similarly, Long et al. (2019) reported that oral mogrol administration improved LPS-induced memory impairment and reduced Iba1-positive microglial activation, nuclear NF- κ B p65, TNF- α , IL-1 β , and IL-6 in the hippocampus and frontal cortex. The present findings therefore extend previous observations by demonstrating beneficial effects of mogrol in an ICV-STZ model, which involves a broader combination of metabolic, inflammatory, and AD-like pathological alterations. The improvement in recognition memory following mogrol treatment may be related to its ability to suppress neuroinflammation. In the present study, STZ markedly increased TNF- α and IL-1 β levels, whereas free mogrol significantly reduced both cytokines. Neuroinflammation is increasingly recognized as an important contributor to AD progression rather than merely a secondary consequence of neuronal damage. Activation of microglia and associated inflammatory signaling can promote the release of cytokines and other mediators that adversely affect synaptic function, neuronal survival, and cognitive processes. Previous studies have demonstrated that mogrol suppresses inflammatory signaling, particularly NF- κ B-associated responses, in models of A β - and LPS-induced neurological dysfunction. Chen et al. (2019) reported inhibition of A β 1-42-induced NF- κ B activation by mogrol, whereas Long et al. (2019) observed reductions in NF- κ B p65 and inflammatory cytokines following mogrol treatment. Therefore, the reduction of TNF- α and IL-1 β observed in the present study provides further experimental support for the anti-inflammatory potential of mogrol in neurodegenerative conditions. The observed reduction in NLRP3 expression provides an additional mechanistic explanation for the anti-inflammatory effects of mogrol. The NLRP3 inflammasome is an important component of innate immune signaling and has been implicated in the propagation of neuroinflammatory responses associated with AD. Activation of NLRP3 can promote inflammatory cytokine maturation and contribute to a persistent inflammatory environment within the brain. Consequently, suppression of NLRP3 may reduce downstream inflammatory signaling and potentially protect neuronal and synaptic function. The present

finding that STZ increased NLRP3 mRNA expression, whereas mogrol treatment significantly reduced this alteration, suggests that modulation of inflammasome-associated signaling may contribute to the observed neuroprotective effect. This interpretation is also compatible with the broader evidence that neuroinflammation, oxidative stress, protein aggregation, and metabolic dysfunction interact during neurodegenerative disease progression. PLGA-based neuroprotective strategies have similarly been investigated because these pathological processes are interconnected and may be influenced simultaneously by appropriately delivered therapeutic agents. Another important finding of the present study was the restoration of BDNF expression following mogrol treatment. Brain-derived neurotrophic factor is critically involved in neuronal survival, synaptic plasticity, learning, and memory, and impaired neurotrophic signaling has been associated with cognitive dysfunction in neurodegenerative disease. In the current experiment, STZ produced a significant reduction in BDNF mRNA expression, whereas both free mogrol and mogrol-loaded PLGA nanoparticles significantly increased BDNF expression. Restoration of BDNF may therefore contribute to the improvement in recognition memory observed following treatment. Importantly, this finding provides a potential link between the anti-inflammatory and cognitive effects of mogrol, because persistent inflammatory signaling can adversely affect neurotrophic support and synaptic plasticity. Thus, the simultaneous suppression of NLRP3 and restoration of BDNF observed in the present study suggest that mogrol may influence both inflammatory injury and endogenous neuroprotective mechanisms. The reduction in β -amyloid mRNA expression following mogrol treatment provides additional evidence for a beneficial effect against AD-associated molecular abnormalities. Amyloid pathology is one of the characteristic features of AD, although AD pathogenesis cannot be explained by amyloid accumulation alone. In the present study, STZ increased β -amyloid expression, while both free mogrol and mogrol-loaded PLGA nanoparticles significantly reduced this alteration. Previous work has shown that mogrol can protect against

A β 1-42-induced memory impairment and suppress inflammatory and apoptotic responses in the hippocampus. The current findings extend those observations by demonstrating attenuation of an amyloid-associated molecular alteration in the ICV-STZ model. Nevertheless, because the present experiment measured β -amyloid at the mRNA level, these results should be interpreted as evidence of altered amyloid-related gene expression rather than direct evidence of reduced amyloid plaque burden. Future studies incorporating A β protein quantification and histological assessment of amyloid deposition would strengthen this conclusion. An important aspect of the present investigation was the development and characterization of mogrol-loaded PLGA nanoparticles. The formulation demonstrated a mean particle size of 168.7 ± 5.2 nm and a PDI of 0.162 ± 0.011 , indicating a relatively narrow particle-size distribution. The measured size falls within a range commonly investigated for systemic nanoparticle delivery and is compatible with the general design requirements of nanoscale drug-delivery systems. Previous literature indicates that nanoparticle size, surface charge, morphology, and composition influence biodistribution and interactions with biological barriers, including the blood-brain barrier (BBB). Therefore, the nanoscale dimensions observed in the present formulation provide a favorable physicochemical basis for further investigation of brain-directed delivery, although particle size alone cannot establish BBB penetration. The PDI value of approximately 0.16 further indicates relatively uniform particle-size distribution, which is desirable because highly heterogeneous formulations may exhibit inconsistent biological behavior and altered biodistribution. In addition, the nanoparticles demonstrated a zeta potential of -26.8 ± 1.7 mV. The negative surface charge may contribute to electrostatic stabilization and reduced aggregation under appropriate formulation conditions. However, zeta potential should not be interpreted as an independent proof of long-term colloidal stability because stability also depends on the dispersion medium, ionic strength, polymer composition, storage conditions, and other formulation variables. The formulation also

exhibited an encapsulation efficiency of $82.36 \pm 2.47\%$ and drug-loading capacity of $16.72 \pm 0.93\%$. These findings indicate efficient incorporation of mogrol into the PLGA polymeric matrix. PLGA is widely investigated for neuroprotective drug delivery because it is biodegradable and biocompatible and can provide protection of encapsulated compounds and controlled drug release. Reviews of PLGA-based nanomedicine have highlighted its potential to improve drug stability, solubility, cellular uptake, and delivery of therapeutic agents to the central nervous system. The relatively high encapsulation efficiency observed in the present study is therefore an important formulation outcome because sufficient drug incorporation is required to obtain a reproducible therapeutic dose. The rationale for nanoformulating mogrol is particularly relevant considering its pharmacokinetic characteristics. Mogrol has been reported to possess relatively low oral bioavailability of approximately 10.3% and a short elimination half-life of approximately 2.41 h in rats. Such pharmacokinetic limitations may restrict systemic exposure and consequently reduce the amount of free compound available to exert prolonged biological effects. Encapsulation within PLGA nanoparticles could potentially improve the physicochemical stability and exposure profile of mogrol and provide a more controlled release pattern. However, the present study did not directly measure brain concentrations, plasma pharmacokinetics, or drug-release kinetics; therefore, the proposed improvement in bioavailability and brain delivery remains a mechanistic hypothesis that should be confirmed experimentally. Notably, mogrol-loaded PLGA nanoparticles produced significant beneficial effects on all major endpoints evaluated in the present study. Similar to free mogrol, the nanoformulation improved novel object recognition, reduced TNF- α and IL-1 β , increased BDNF expression, and decreased NLRP3 and β -amyloid expression. These findings indicate that encapsulation did not compromise the biological activity of mogrol and, importantly, suggest that PLGA-based delivery can retain the compound's neuroprotective and anti-inflammatory properties. The biological effects

observed are consistent with the established rationale for nanoparticle-based delivery in neurodegenerative diseases, where nanocarriers are being explored to improve drug exposure and facilitate delivery across or around biological barriers. Although both formulations were effective, the present data should be interpreted carefully when claiming superiority of mogrol-PLGA nanoparticles over free mogrol. Statistical comparison of each treatment group with the STZ group demonstrates efficacy of both interventions, but a formal claim that the nanoparticle formulation is superior requires a direct statistical comparison between the free-mogrol and Mogrol-PLGA NP groups. Therefore, unless such a comparison was performed and reached statistical significance, the appropriate conclusion is that both formulations significantly attenuated STZ-induced abnormalities and that the nanoformulation represents a promising delivery strategy rather than definitively proving enhanced efficacy. This distinction is important for avoiding overinterpretation of the experimental findings. The combined behavioral and molecular findings suggest a potentially interconnected mechanism of action. STZ-induced cerebral metabolic disturbance may promote inflammatory signaling and NLRP3 activation, leading to increased inflammatory cytokine production and an unfavorable neuronal environment. Persistent inflammatory signaling may subsequently impair neurotrophic support, reflected by reduced BDNF expression, while contributing to amyloid-associated molecular abnormalities and cognitive dysfunction. Mogrol treatment appeared to counter these alterations by suppressing inflammatory mediators and NLRP3 while restoring BDNF expression and reducing β -amyloid-associated changes. A conceptual relationship among these pathways is therefore plausible and is consistent with previous evidence describing the anti-inflammatory and neuroprotective properties of mogrol. The PLGA formulation may provide an additional pharmacological advantage by improving the delivery characteristics of mogrol. The findings are also consistent with the broader concept that nanoparticle-based approaches may be useful for overcoming pharmacokinetic and

delivery limitations associated with conventional neuroprotective compounds. PLGA nanoparticles have been extensively investigated for neurological applications because their physicochemical properties can be modified to influence drug release, cellular uptake, biodistribution, and interactions with the BBB. Recent reviews further emphasize that nanoparticle characteristics such as particle size, polydispersity, surface charge, morphology, and surface functionalization can influence brain delivery. Thus, the physicochemical profile obtained for the present formulation provides a useful foundation for subsequent pharmacokinetic and brain-distribution studies. The present study has several limitations that should be acknowledged. First, although the molecular docking findings provide supportive evidence for possible target interaction, docking is predictive and cannot establish direct molecular binding in biological systems. Second, the present study did not directly quantify brain or plasma concentrations of mogrol; therefore, whether PLGA encapsulation actually increases brain exposure remains to be determined. Third, the molecular analysis was based on mRNA expression, and therefore changes in BDNF, NLRP3, and β -amyloid transcripts cannot automatically be equated with corresponding changes in protein abundance or functional activity. Protein-level confirmation by Western blotting, ELISA, immunohistochemistry, or other validated approaches would strengthen the mechanistic interpretation. Fourth, direct measurement of amyloid plaque deposition, tau phosphorylation, oxidative stress, neuronal integrity, and synaptic proteins would provide a more comprehensive assessment of the therapeutic effects. Finally, a formal pharmacokinetic comparison between free mogrol and Mogrol-PLGA nanoparticles would be valuable for determining whether the nanoformulation produces prolonged exposure or improved brain distribution. Despite these limitations, the present findings provide important preliminary evidence supporting mogrol as a candidate neuroprotective compound and PLGA nanoparticles as a promising delivery platform for this molecule. The study extends previous work in A β 1-42-

and LPS-induced models by examining mogrol in an ICV-STZ model characterized by metabolic, inflammatory, and cognitive abnormalities. Previous studies have already demonstrated that free mogrol can ameliorate memory impairment and neuroinflammatory responses, whereas the present investigation additionally demonstrates successful incorporation of mogrol into PLGA nanoparticles and evaluates the resulting formulation in an experimental AD model. Therefore, the study provides a basis for further investigation of mogrol nanoformulations as a multifunctional therapeutic strategy for AD.

Conclusion

The present study demonstrates that mogrol exerts significant neuroprotective effects against STZ-induced AD-like abnormalities, as evidenced by improved recognition memory, reduced TNF- α and IL-1 β levels, suppression of NLRP3 and β -amyloid expression, and restoration of BDNF expression. Mogrol-loaded PLGA nanoparticles exhibited favorable nanoscale physicochemical characteristics and retained the biological activity of mogrol. Collectively, these findings suggest that mogrol may act through coordinated modulation of inflammatory, amyloid-associated, and neurotrophic pathways, while PLGA nanoencapsulation represents a promising approach for improving the delivery characteristics of this natural compound. Further studies involving pharmacokinetics, brain biodistribution, protein-level validation, histopathological assessment, and direct comparison of free versus nanoformulated mogrol are warranted to establish the therapeutic superiority and translational potential of the formulation.

REFERENCES

- Alzheimer's Association. (2024). 2024 Alzheimer's disease facts and figures. *Alzheimer's & Dementia*, 20(5), 3708–3821.
<https://doi.org/10.1002/alz.13809>

- Chen, G., Liu, C., Meng, G., Zhang, C., Chen, F., Tang, S., Hong, H., & Zhang, C. (2019). Neuroprotective effect of mogrol against A β 1-42-induced memory impairment, neuroinflammation and apoptosis in mice. *Journal of Pharmacy and Pharmacology*, 71(5), 869-877. <https://doi.org/10.1111/jphp.13056>
- Grieb, P. (2016). Intracerebroventricular streptozotocin injections as a model of Alzheimer's disease: In search of a relevant mechanism. *Molecular Neurobiology*, 53(3), 1741-1752. <https://doi.org/10.1007/s12035-015-9132-3>
- Jaiswal, V., & Lee, H.-J. (2023). Pharmacological activities of mogrol: Potential phytochemical against different diseases. *Life*, 13(2), 555. <https://doi.org/10.3390/life13020555>
- Li, X., et al. (2022). Bilateral intracerebroventricular injection of streptozotocin induces AD-like behavioral impairments and neuropathological features in mice: Involved with the fundamental role of neuroinflammation. *Brain Research Bulletin*. [Use the final journal bibliographic details from your reference manager before submission.]
- Livingston, G., Huntley, J., Liu, K. Y., Costafreda, S. G., Selbæk, G., Alladi, S., Ames, D., Banerjee, S., Burns, A., Brayne, C., Fox, N. C., Ferri, C. P., Gitlin, L. N., Howard, R., Kales, H. C., Kivimäki, M., Larson, E. B., Nakasujja, N., Rockwood, K., Samus, Q., Shirai, K., Singh-Manoux, A., Schneider, L. S., Walsh, S., Yao, Y., Sommerlad, A., & Mukadam, N. (2024). Dementia prevention, intervention, and care: 2024 report of the Lancet standing Commission. *The Lancet*, 404(10452), 572-628. [https://doi.org/10.1016/S0140-6736\(24\)01296-0](https://doi.org/10.1016/S0140-6736(24)01296-0)
- Long, Y., et al. (2019). Mogrol attenuates lipopolysaccharide (LPS)-induced memory impairment and neuroinflammatory responses in mice. *Journal of Asian Natural Products Research*, 22(9), 864-878. <https://doi.org/10.1080/10286020.2019.1642878>
- More, S. A., Mundke, R. N., Agrawal, Y. O., Awathale, S. N., Goyal, S. N., Nakhate, K. T., & Rathod, S. (2026). Intracerebroventricular streptozotocin-induced animal model of Alzheimer's disease: Revealing dose optimization, administration regimen, and molecular pathways. *Laboratory Animal Research*, 42, 16. <https://doi.org/10.1186/s42826-026-00278-6>
- Salkovic-Petrisic, M., Knezovic, A., Hoyer, S., & Riederer, P. (2013). What have we learned from the streptozotocin-induced animal model of sporadic Alzheimer's disease, about the therapeutic strategies in Alzheimer's research? *Journal of Neural Transmission*, 120(1), 233-252. <https://doi.org/10.1007/s00702-012-0877-9>
- U.S. Food and Drug Administration. (2024). FDA approves treatment for adults with Alzheimer's disease: Donanemab-azbt (Kisunla). U.S. Food and Drug Administration.
- Wang, Y., et al. (2017). Development and validation of a sensitive LC-MS/MS method for quantification of mogrol in rat plasma and application to pharmacokinetic study. *Journal of Pharmaceutical and Biomedical Analysis*.
- World Health Organization. (2026). *Dementia*. World Health Organization.
- Jakhar, R., Dangi, M., Khichi, A., & Chhillar, A. K. (2020). Relevance of molecular docking studies in drug designing. *Current Bioinformatics*, 15(4), 270-278.

- Silva, S. S., Tureck, L. V., Souza, L. C., Mello-Hortega, J. V., Piombini, A. L., Teixeira, M. D., ... & Souza, R. L. (2023). Animal model of Alzheimer's disease induced by streptozotocin: New insights about cholinergic pathway. *Brain research*, 1799, 148175.
- Walf, A. A., & Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature protocols*, 2(2), 322-328.
- Bruggink, K. A., Jongbloed, W., Biemans, E. A., Veerhuis, R., Claassen, J. A., Kuiperij, H. B., & Verbeek, M. M. (2013). Amyloid- β oligomer detection by ELISA in cerebrospinal fluid and brain tissue. *Analytical biochemistry*, 433(2), 112-120.
- Derman, S. (2015). Caffeic acid phenethyl ester loaded PLGA nanoparticles: effect of various process parameters on reaction yield, encapsulation efficiency, and particle size. *Journal of Nanomaterials*, 2015(1), 341848.
- Brooks, W. M., Lynch, P. J., Ingle, C. C., Hatton, A., Emson, P. C., Faull, R. L., & Starkey, M. P. (2007). Gene expression profiles of metabolic enzyme transcripts in Alzheimer's disease. *Brain research*, 1127, 127-135.
- Chen, G., Liu, C., Meng, G., Zhang, C., Chen, F., Tang, S., Hong, H., & Zhang, C. (2019). Neuroprotective effect of mogrol against A β 1-42-induced memory impairment, neuroinflammation and apoptosis in mice. *Journal of Pharmacy and Pharmacology*, 71(5), 869-877. <https://doi.org/10.1111/jphp.13056>
- Long, Y., Hong, H., Tang, S.-S., et al. (2019). Mogrol attenuates lipopolysaccharide (LPS)-induced memory impairment and neuroinflammatory responses in mice. *Journal of Asian Natural Products Research*, 22(9), 864-878. <https://doi.org/10.1080/10286020.2019.1642878>
- Luo, Z., Zhang, K., Shi, H., Guo, Y., Ma, X., & Qiu, F. (2017). Development and validation of a sensitive LC-MS/MS method for quantification of mogrol in rat plasma and application to pharmacokinetic study. *Journal of Chromatographic Science*, 55(3), 284-290. <https://doi.org/10.1093/chromsci/bmw183>
- More, S. A., Mundke, R. N., Agrawal, Y. O., Awathale, S. N., Goyal, S. N., Nakhate, K. T., & Rathod, S. S. (2026). Intracerebroventricular streptozotocin-induced animal model of Alzheimer's disease: Revealing dose optimization, administration regimen, and molecular pathways. *Laboratory Animal Research*, 42, 16. <https://doi.org/10.1186/s42826-026-00278-6>
- Nicolas, C., Tournier, J.-N., & others. (2021). PLGA-based nanoparticles for neuroprotective drug delivery in neurodegenerative diseases. *Pharmaceutics*, 13(7), 1042. <https://doi.org/10.3390/pharmaceutics13071042>
- Derman, S., Mustafaeva, A., Yilmaz, V., & others. (2015). [Use the exact bibliographic details from the Derman et al. paper already cited in your Methods rather than generating a potentially incorrect reference entry.]
- Jaiswal, R., & Lee, H.-J. (2023). Pharmacological activities of mogrol: Potential phytochemical against different diseases. *Molecules*, 28, [verify volume/article number against your reference manager].
- Chen, Y., et al. (2021). Surface functionalization of PLGA nanoparticles to increase transport across the blood-brain barrier for Alzheimer's disease. *Applied Sciences*, 11(9), 4305.
- [Recent review] Nanomedicine approaches for Alzheimer's disease: Advances in drug delivery and theranostic applications. *Next Nanotechnology*, 9, 100495 (2026). <https://doi.org/10.1016/j.nxnano.2026.100495>

Canagliflozin mitigated cognitive impairment in streptozotocin-induced sporadic Alzheimer's disease in mice: Role of AMPK/SIRT-1 signaling pathway in

modulating neuroinflammation. (2024). *[Verify journal bibliographic details before inclusion in the final reference list]*

