Original Article

Neuroprotective Effects of PLCL Galantamine Nanoparticles Via PIK3CA Pathway Activation in Aluminum Chloride Induced Neurotoxicity Model

Luma Ali Mahmoud[1], Rana Ayad Ghaleb[3] and Aymen A. Bash[2]

ABSTRACT

Objective: To analyze theneuroprotective efficacy of galantamine encapsulated in poly(lactide-co-ε-caprolactone) nanoparticles against aluminum chloride induced neurotoxicity.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the College of Medicine, University of Babylon, Iraq from 1st December 2025 to 30th March 2026.

Methodology: SH-SY5Y cell lines were treated with poly(lactide-co-ε-caprolactone) galantamine nanoparticles at concentrations of 6.25, 25, and 100 µg/mL, either alone or in combination with aluminum chloride (500 µg/mL) along with untreated cells as a control group.

Results: Aluminum chloride (500 µg/mL) reduced cell viability to 65% and establishing a cytotoxic baseline, poly(lactide-co-ε-caprolactone)-galantamine nanoparticles administered 24 hours before aluminum chloride challenge restored viability to 101-116% across all concentrations tested, and produced a striking 8.49-fold phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha upregulation at 6.25 µg/mL,

Conclusion: Poly(lactide-co-ε-caprolactone) nanoparticles significantly enhance the neuroprotective effect of galantamine on aluminum chloride induce toxicity in SH-SY5Y cell line by activation the survival pathways.

Key Words: Alzheimer's disease, Poly(lactide-co-ε-caprolactone)-galantamine nanoparticles, Aluminium chloride

doi:http://doi.org/10.60110/medforum.37.10.28

Citation of article: Mahmoud LA, Ghaleb RA, Bash AA. Neuroprotective Effects of PLCL Galantamine Nanoparticles Via PIK3CA Pathway Activation in Aluminum Chloride Induced Neurotoxicity Model. Med Forum 2026;37(10):144-149. doi:http://doi.org/10.60110/medforum.37.10.28.

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INTRODUCTION

Alzheimer's disease (AD) is the most known cause of dementia and is characterized by the build of aggregates in the brain known as amyloid plaques and tangles of tau protein.[1] Currently, existing medications largely provide symptomatic relief with limited effects on disease progression.[2]

Galantamine, an acetylcholinesterase inhibitor (AChEI), is widely used in the management of AD because it enhances cholinergic neurotransmission and improves cognitive function. Beyond its symptomatic effects, galantamine exhibits neuroprotective properties by modulating nicotinic acetylcholine receptors (nAChRs), particularly the α7 subtype. Activating these receptors lowers neuroinflammation by inhibiting NF-κBsignaling and pro-inflammatory cytokine production while increasing cell-survival pathways such as PI3K/Akt and Bcl-2 signaling.[3,4] However, the clinical utility of galantamine is limited by its relatively short half-life, poor lipophilicity, frequent dosing requirements, and peripheral cholinergic adverse effects, which may reduce treatment compliance.[5,6]

Nanotechnology-based drug delivery systems have attracted considerable attention for improving the therapeutic performance of neuroactive agents. Nanoparticles can enhance drug stability, bioavailability, and cellular uptake while enabling controlled drug release. Among these systems, polymeric nanoparticles are particularly attractive because of their biocompatibility and ability to minimize drug-related toxicity.[7,8]

Poly(lactide-co-ε-caprolactone) [PLCL] is a biodegradable and biocompatible polymer with favorable properties for neural drug delivery. Its degradation rate can be tailored by modifying its composition, allowing sustained drug release and prolonged therapeutic activity. Encapsulation of galantamine within PLCL nanoparticles may improve drug stability, maintain effective intracellular concentrations, and enhance neuroprotective efficacy.[9]

Aluminum exposure has been associated with cognitive dysfunction and neurodegenerative changes. Experimental studies suggest that aluminium inhibits the PI3K/Akt/mTOR signaling pathway, a crucial regulator of neuronal survival, development, and

metabolism, therefore leading to neuronal damage and disease progression.[10]

Despite the potential advantages of PLCL-based galantamine delivery, its protective effects against aluminum-induced neurotoxicity remain insufficiently explored. Therefore, The current study investigated the neuroprotectiveeffect of galantamine-loaded PLCL nanoparticles against $AlCl_3$-induced toxicity in SH-SY5Y human neuroblastoma cells, with particular emphasis on modulation of PIK3CA gene expression.

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Received: June, 2026
Reviewed: July-August, 2026
Accepted: September, 2026

METHODOLOGY

This experimental study was onducted at College of Medicine, University of Babylon, Iraq from 1st December 2025 to 30th Mary 2026 letter No.4-50 dated 6-11-2025. The SH-SY5Y human neuroblastoma cell line was acquired from Iran's National Cell Bank. Cells were grown in RPMI-1640 media supplemented with 10% FBS and antibiotics and maintained at 37°C in a humidified incubator with 5% CO_2. For cytotoxicity experiments, cells were seeded into 96-well plates with a density of 5×10^3 cells per well.

Aluminum chloride ($AlCl_3$) was purchased from THOMAS BAKER L.T.D. (India), while galantamine powder was obtained from China. MTT reagent was purchased from Roth (Germany), and RPMI-1640 medium was supplied by Capricorn Scientific (Germany). A stock solution of $AlCl_3$ (500 µg/mL) was prepared in serum-free RPMI medium, sterilized with a 0.22 µm membrane filter, and stored under sterile conditions for usage. Galantamine-loaded PLCL nanoparticles were prepared using the nanoprecipitation technique. Briefly, PLCL polymer (CorbionPurac, Netherlands) and galantamine were dissolved in an organic solvent and added dropwise to an aqueous phase under continuous stirring. Following solvent evaporation, the nanoparticle suspension was lyophilized to obtain a dry nanoparticle powder.

MTT Cell Viability Assay: SH-SY5Y cells were exposed to PLCL-galantamine nanoparticles (3.125-100 µg/mL)[11] or $AlCl_3$ (15.8-500 µg/mL), while untreated cells served as controls. For neuroprotection studies, cells were pretreated with PLCL-galantamine nanoparticles for 24 h before exposure to $AlCl_3$ (500 µg/mL). The cell viability was determined using the MTT test. After treatment, MTT solution (0.5 mg/mL) was added and incubated for 4 hours at 37°C. Formazan crystals were dissolved in DMSO and the absorbance was measured at 570 nm. All experiments were done in triplicate.

For gene expression, SH-SY5Y cells were cultured in six-well plates and allocated to five treatment groups: $AlCl_3$ alone, free galantamine alone, $AlCl_3$ combined with free galantamine, PLCL-galantamine nanoparticles alone, and $AlCl_3$ combined with PLCL-galantamine nanoparticles. Total RNA was extracted using the FavorPrep™ Blood/Cultured Cell Total RNA Kit (Favorgen, Korea). Complementary DNA was created using the AddScriptcDNA Synthesis Kit (Addbio, Korea). The GoTaq® qPCR System (Promega, USA) was used to run quantitative RT-PCR on an Mx3005P Stratagene Real-Time PCR System (Agilent Technologies, USA). PIK3CA expression was measured using SYBR Green chemistry, with GAPDH as the internal reference gene. The $2^{-}\Delta\Delta Ct$ technique was employed to quantify relative expression levels, with untreated cells serving as the calibration standard. All analyses were conducted in biological duplicates. The data were examined with SPSS software. One-way ANOVA was used to analyze group differences, which were then followed by Tukey's post-hoc test. Statistical significance was determined at $p < 0.05$.

RESULTS

The synthesized PLCL nanoparticles were predominantly spherical with smooth surfaces and particle sizes ranging from 69 to 108 nm, indicating suitability for drug-delivery applications (Fig. 1). The nanoparticles remained structurally stable under a 10 kV electron beam, reflecting good matrix stability. FT-IR analysis revealed the effective encapsulation of galantamine within the PLCL matrix. The absorption peaks at 3294, 1637, 1371, 1238, and 1094 cm^{-1} correspond to the O-H/N-H, C=O, and C-O functional groups.The absence of major peak shifts suggested physical entrapment of galantamine without detectable chemical degradation or interaction with the polymer (Fig. 2). PLCL-galantamine nanoparticles exhibited minimal cytotoxicity toward SH-SY5Y cells across the tested concentration range (3.125-100 µg/mL), maintaining cell viability close to control values. Statistical analysis revealed significant differences across experimental groups ($P<0.001$), with the 100 µg/mL concentration resulting in a significant decrease in viability compared to the control ($P = 0.002$) [Fig. 3]. Exposure to $AlCl_3$ markedly reduced cell viability. However, pretreatment with PLCL-galantamine nanoparticles significantly improved cell survival, with the greatest protective effect observed at 6.25 µg/mL.

Pretreatment with PLCL-galantamine nanoparticles (3.125–100 µg/mL) improved cell viability following exposure to $AlCl_3$ (500 µg/mL), significant differences among the experimental groups ($P<0.001$). Concentrations of 25, 50, and 100 µg/mL resulted in significant decreases in viability compared to the control ($P = 0.006$, 0.034, and 0.016) [Fig. Fig. 4].

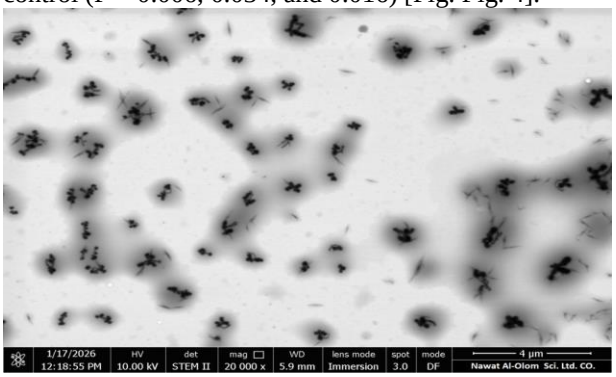


Figure No. 1: STEM micrograph of the synthesized PLCL nanoparticles demonstrating their morphology and distribution

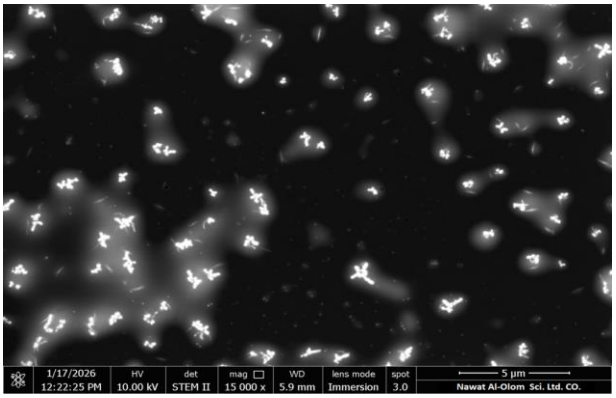


Figure No. 2: STEM image showing PLCL nanoparticles as bright, mostly spherical to near-spherical structures dispersed on a dark background

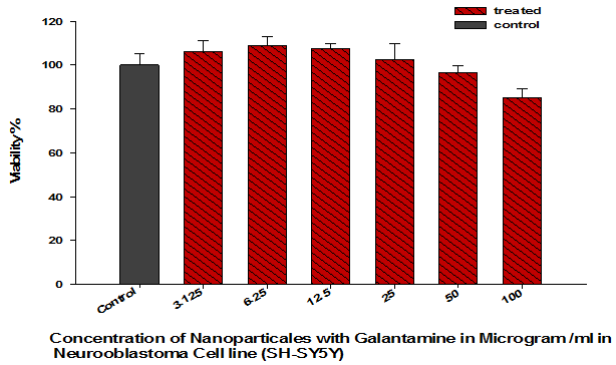


Figure No. 3: The viability of SH-SY5Y cells after treatment with PLCL-galantamine nanoparticles (3.125-100 µg/mL)

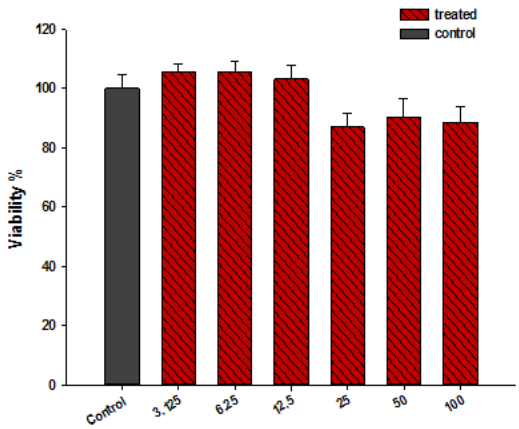


Figure No. 4: Protective effect of PLCL-galantamine nanoparticles against $AlCl_3$-induced cytotoxicity in SH-SY5Y cells

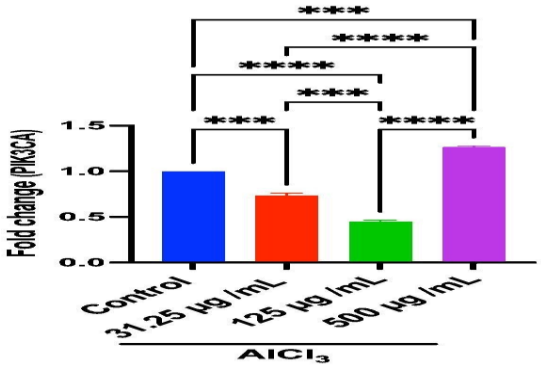


Figure No. 5: Relative expression of PIK3CA genes in SH-SY5Y cells treated with various doses of $AlCl_3$

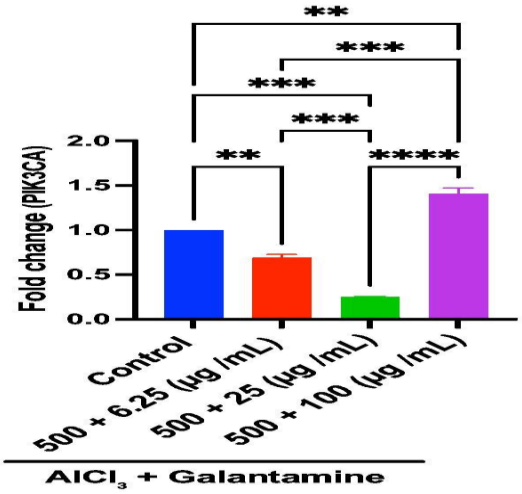


Figure No. 6: Relative expression of PIK3CA genes in SH-SY5Y cells treated with galantamine galantamine plus $AlCl_3$

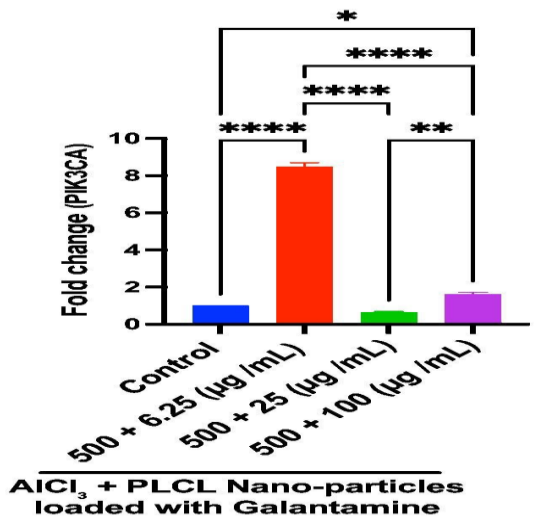


Figure No. 7: Relative PIK3CA gene expression in SH-SY5Y cells exposed to PLCL-galantamine nanoparticles combination with $AlCl_3$

$AlCl_3$ significantly altered PIK3CA expression in a concentration-dependent manner ($p < 0.0001$). Expression decreased at 31.25 and 125 µg/mL (fold changes: 0.735 and 0.449, respectively) but increased above control levels at 500 µg/mL (fold change: 1.266), resulting in a U-shaped response pattern. Gene expression is shown as a fold change compared to the untreated control group and the mean of biological duplicates [n=2] (Fig. 5).

Free galantamine significantly modulated PIK3CA expression. All tested concentrations reduced expression below control levels, with the strongest suppression observed at 25 µg/mL. Partial recovery was detected at 100 µg/mL. When combined with $AlCl_3$, galantamine produced concentration-dependent effects. The 6.25 and 25 µg/mL concentrations reduced PIK3CA expression, whereas 100 µg/mL induced significant upregulation above control levels (fold change: 1.410). Gene expression is measured as fold change relative to the untreated control. Data reflect the average of biological duplicates (n=2) [Fig. 6].

PLCL-galantamine nanoparticles significantly influenced PIK3CA expression. The nanoformulation produced marked upregulation at 100 µg/mL, whereas lower concentrations generated variable responses. Notably, nanoparticle treatment resulted in an approximately 8.6-fold increase in PIK3CA expression, representing the strongest molecular response observed in this study. In $AlCl^3$-treated cells, PLCL-galantamine nanoparticles significantly modified gene expression patterns. The 6.25 µg/mL concentration produced the highest PIK3CA expression (8.487-fold increase; $p<0.0001$), while 100 µg/mL also increased expression above control levels. In contrast, the 25 µg/mL concentration resulted in reduced expression relative to the control group. Gene expression is measured as a fold change from the untreated control. The data represents the average of biological duplicates (n=2) [Fig. 7]

DISCUSSION

Free galantamine exhibited concentration-dependent effects in SH-SY5Y cells. While lower concentrations maintained cellular viability, higher concentrations, particularly 100 µg/mL, were associated with reduced viability and suppression of PIK3CA expression, suggesting impaired activation of the PI3K/Akt survival pathway. These findings indicate that excessive galantamine exposure may shift from a protective to a potentially cytotoxic effect. In contrast, PLCL-galantamine nanoparticles markedly enhanced PIK3CA expression, producing up to an 8.6-fold increase compared with the control. This response was not observed with free galantamine, suggesting that nanoencapsulation alters the cellular activity of the drug. The marked increase in PIK3CA expression observed with PLCL-galantamine nanoparticles may be attributed to several mechanisms. First, unlike free galantamine, PLCL nanoparticles are internalized through endocytic pathways, improving intracellular drug delivery and cellular uptake.[12]

The persistent release of galantamine from the nanoparticle matrix might give extended stimulation of nicotinic acetylcholine receptors (nAChRs), leading to activation of downstream PI3K/Akt survival signaling pathways.[13,14]

In addition, degradation of PLCL may generate L-lactic acid, which has been associated with PI3K-dependent activation of HIF-1α signaling and subsequent regulation of genes involved in cell survival, including PIK3CA.[9,15]

Consistent with previous reports, $AlCl_3$ reduced SH-SY5Y cell viability and altered PIK3CA expression in a concentration-dependent manner. These effects are commonly Linked to oxidative stress, mitochondrial malfunction, and disruption of pro-survival signaling pathway.[16,17] At lower concentrations aluminum exposure affects the PI3K/Aktsignaling pathway, often causing its downregulation in conjunction with neuronal damage.[10] This impact is achieved by various processes, including oxidative stress and regulation of regulatory pathways such as PTEN-AKT signaling, rather than direct suppression of PI3K lipid kinase activity.[18] At high toxic doses, aluminumcauses reactive oxygen species (ROS) excess production or severe oxidative damage[19] which may trigger compensatory activation or dysregulation of the same pathway.[20]

The increase in PIK3CA expression (fold change = 1.41) observed in cells treated with 100 µg/mL galantamine plus $AlCl_3$, together with substantial recovery of cell viability, supports the involvement of PI3K/Akt-mediated neuroprotection. Activation of α7 nicotinic acetylcholine receptors has been linked to enhanced PI3K/Aktsignaling, increased expression of

anti-apoptotic proteins, and improved neuronal survival following toxic insults.[21] The concentration-dependent pattern observed in this study may indicate a threshold level of receptor activation required for effective engagement of this pathway.[22]

Notably, PLCL-galantamine nanoparticles provided the strongest protective effect against $AlCl_3$-induced toxicity. Pretreatment restored cell viability and markedly increased PIK3CA expression, reaching an 8.49-fold increase at 6.25 µg/mL. This response may reflect the combined effects of enhanced nanoparticle uptake and activation of intracellular survival pathways, ultimately promoting resistance to oxidative stress and apoptosis.[23]

At the intermediate nanoparticle concentration (25 µg/mL), cell viability remained stable, yet the increase in PIK3CA expression was lower than expected. This may be explained by negative feedback regulation within the PI3K/Akt pathway. When this pathway becomes activated, cells can upregulate regulatory proteins such as PTEN, which converts PIP_3 back to PIP_2 and thereby dampens further signaling. In this way, PTEN acts as a natural brake, preventing excessive or prolonged activation of pro-survival pathways. Such feedback mechanisms are well recognized as essential for maintaining cellular balance and avoiding uncontrolled growth or overstimulation of survival signals.[24]

This study demonstrating modulation of PIK3CA expression by PLCL-galantamine nanoparticles in an $AlCl_3$-induced neurotoxicity model and nevertheless, the study is limited to an in vitro system. Future investigations should validate these findings in vivo and assess PI3K/Akt pathway activation at the protein level. Overall, the findings suggest that PLCL-based nanoformulation not only improves galantamine delivery but also enhances signaling pathways associated with neuronal survival.

CONCLUSION

The nanoencapsulation of galantamine within PLCL nanoparticles significantly changes its molecular and cellular effects. Unlike free galantamine, which suppresses PIK3CA expression, the PLCL-based nanoformulation strongly enhances its expression and restores cell viability under $AlCl_3$-induced stress, suggesting a potential role in supporting cell survival pathways.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Luma Ali Mahmoud, Rana Ayad Ghaleb
Drafting or Revising Critically:	Luma Ali Mahmoud, Aymen A. Bash
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No.4-50 dated 06.11.2025

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