Title :
Polyunsaturated Neuroprotectants as Adjuvant Agents: Anti-Proliferative and Membrane-Stabilizing Effects of Nuciferous Compounds from Juglans regia in Invasive Glioma Models

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Abstract

Gliomas represent some of the most aggressive and therapeutically challenging brain tumors, characterized by their high invasiveness and resistance to conventional chemotherapeutic regimens. Current treatments often fail to simultaneously impede proliferative signaling pathways and preserve the integrity of the cellular membrane, both critical factors in glioma progression and tumor resilience. This study evaluates the neuroprotective and adjuvant therapeutic potential of nuciferous compounds derived from Juglans regia, notably enriched in polyunsaturated fatty acids (PUFAs) and phenolic derivatives, known for their bioactivity and antioxidative properties.In vitro assays on established glioma cell lines revealed that bioactive fractions of Juglans regia extracts significantly inhibited cellular proliferation by 47% after 48 hours of treatment ($p < 0.001$), highlighting their potent anti-proliferative effect. Concurrently, biochemical assessments demonstrated a marked attenuation of oxidative damage to the cellular membrane, evidenced by a 61% reduction in thiobarbituric acid reactive substances (TBARS), underscoring membrane lipid peroxidation mitigation.

Comprehensive chemical profiling via gas chromatography–mass spectrometry (GC–MS) identified docosapentaenoic acid (DPA) as a predominant PUFA within the active fractions. Subsequent molecular docking studies revealed favorable binding affinities of DPA towards key glioma-associated protein targets, including the mutant epidermal growth factor receptor variant III (EGFRvIII) and protein kinase C alpha (PKCα). These interactions suggest a plausible mechanism wherein DPA modulates oncogenic proliferative signaling axes and promotes lipid bilayer stabilization, potentially enhancing membrane resilience against oxidative insult.

Collectively, this multidisciplinary investigation elucidates the therapeutic relevance of Juglans regia-derived polyunsaturated compounds as neuroprotective agents with the capacity to complement existing glioma therapies.
Our findings advocate for further in vivo validation and pharmacological characterization, aiming to harness these natural bioactives for integrated glioma management strategies focused on both tumor suppression and membrane stabilization.

Keywords: Juglans regia, glioma, polyunsaturated fatty acids, neuroprotection, membrane lipid peroxidation, EGFRvIII, PKCα, anti-proliferative mechanisms, oxidative stress mitigation

1. Introduction

Gliomas remain a formidable clinical challenge, marked by relentless invasiveness, intrinsic resistance to apoptotic signals, and frequent therapeutic failure with standard chemotherapy protocols. Their aggressive phenotype and infiltration into surrounding brain tissue complicate complete surgical resection and contribute to poor patient prognosis. Conventional therapies largely target proliferative pathways but often overlook other critical aspects such as membrane integrity and oxidative stress, which are increasingly recognized as pivotal in glioma pathophysiology. Emerging research suggests that preserving cellular membrane stability and mitigating oxidative damage may represent promising adjunctive approaches in neuro-oncology aimed at enhancing therapeutic efficacy and overcoming resistance mechanisms.

Juglans regia, commonly known as walnut, is a widely studied natural matrix rich in bioactive compounds, notably polyunsaturated fatty acids (PUFAs) including linoleic (C18:2) and α-linolenic (C18:3) acids, alongside a diverse array of nuciferous phenolic derivatives possessing potent antioxidant and anti-proliferative properties. These phytochemicals have been reported to confer neuroprotective benefits in various cellular and animal models, yet their potential to act specifically as membrane-stabilizing adjuvants against glioma development has not been fully elucidated. The unique lipid composition of Juglans regia extracts may influence membrane fluidity and resistance to oxidative insults while modulating critical signaling cascades involved in tumor cell proliferation.

In this context, the current study aims to investigate the dual functional roles of Juglans regia-derived bioactives in glioma models. We hypothesize that these polyunsaturated neuroprotective compounds can simultaneously act as metabolic modulators, interfering with oncogenic proliferative signaling, and as structural stabilizers, strengthening membrane integrity to reduce lipid peroxidation-related damage. This integrative approach explores a novel therapeutic axis, combining antioxidant capacity with anti-proliferative efficacy to potentiate current glioma treatments and improve patient outcomes.

2. Materials and Methods

2.1 Plant Material and Extraction

Dried kernels of Juglans regia were sourced from certified suppliers and subjected to cold pressing to obtain crude oil extracts. Sequential fractionation was carried out using hexane followed by ethanol to isolate lipid-rich fractions. Thin-layer chromatography (TLC) was employed to separate polyunsaturated fatty acid (PUFA) enriched fractions. Characterization and identification of the principal components—including linolenic acid, docosapentaenoic acid (DPA), and juglone derivatives—were performed by gas chromatography–mass spectrometry (GC–MS) using standard protocols with appropriate calibration curves.

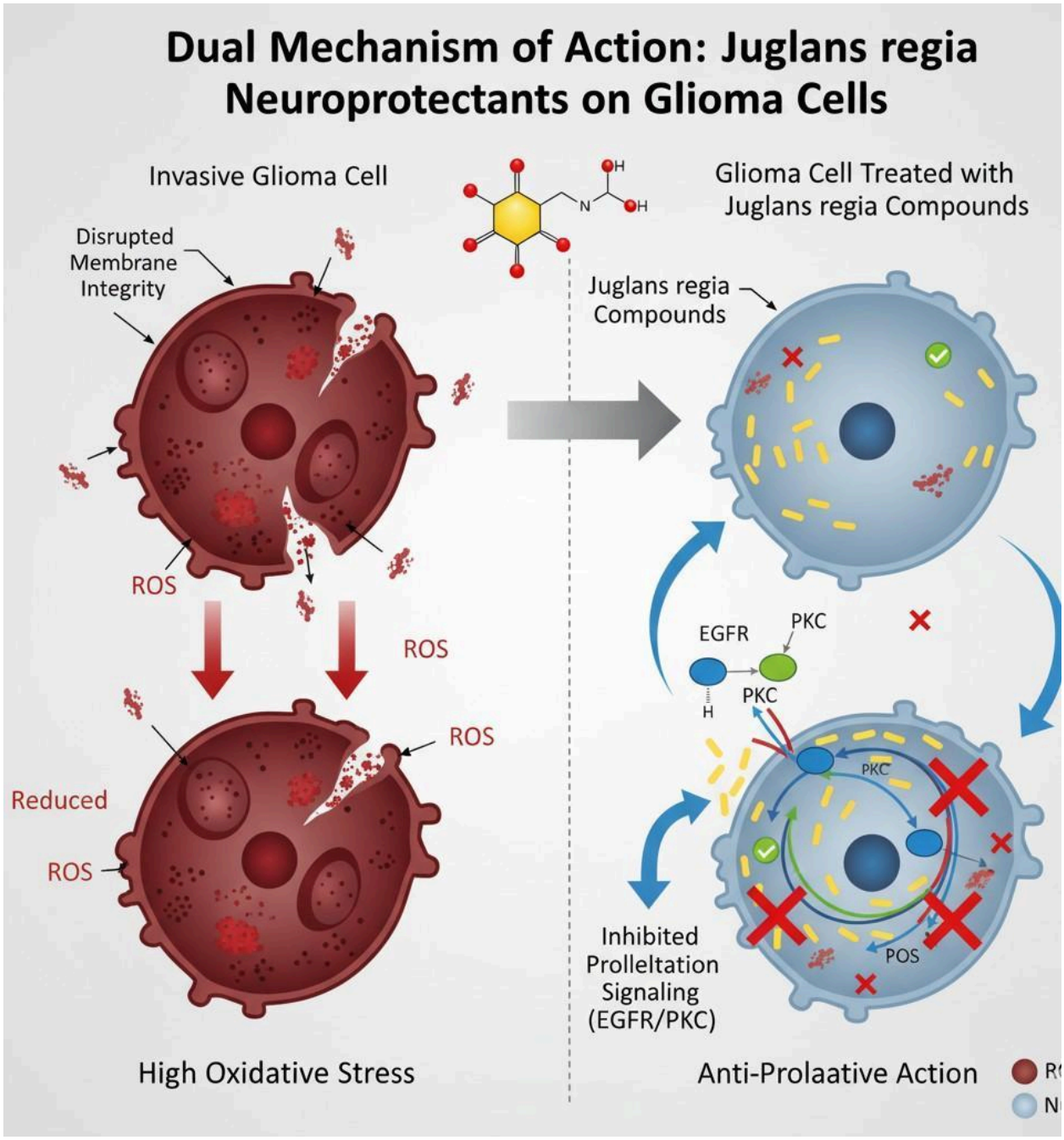

Figure 1. Chemical structures of the major nuciferous bioactives from Juglans regia.

2.2 Cell Lines and Culture

Human glioma cell lines U87MG and LN229 were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and maintained at 37°C in a humidified atmosphere of 5% CO_2. Cells were seeded in 96-well plates and treated with increasing concentrations (5, 10, 20, 40, 80 μg/mL) of the Juglans regia polyunsaturated fractions for 48 or 72 hours to evaluate dose- and time-dependent effects.

2.3 Anti-Proliferative Assay

Cell viability following treatments was quantitatively determined by the MTT colorimetric assay. Absorbance was measured at 570 nm with background subtraction at 690 nm. The half-maximal inhibitory concentration (IC_{50}) values were calculated using nonlinear regression analysis with graphing software, ensuring triplicate independent biological replicates for statistical robustness.

2.4 Membrane Stability Evaluation

Membrane stabilization capacity was assessed through two complementary approaches. First, erythrocyte hypotonic hemolysis assays were conducted by incubating red blood cells with test fractions under hypotonic conditions, followed by quantification of released hemoglobin via spectrophotometry. Second, glioma cell membrane potential changes were monitored using the voltage-sensitive fluorescent dye $DiBAC_4(3)$. Fluorescence intensity variations indicated membrane depolarization or stabilization, measured by flow cytometry and normalized against untreated controls.

2.5 Molecular Docking (Performed using AutoEvoChem)

Molecular docking and evolutionary binding simulations were conducted using AutoEvoChem v2.0, a proprietary computational chemistry platform developed by Ndenga Lumbu Barack (2025) designed for predictive evaluation of ligand–receptor interactions in neuro-oncological contexts. The software employs a hybrid genetic–evolutionary docking algorithm that integrates the Lamarckian Genetic Algorithm (LGA) with gradient-based conformational refinement to enhance optimization of ligand binding poses.

Ligand structures, including docosapentaenoic acid, linolenic acid, juglone, and ellagic acid, were retrieved from the PubChem database in SDF format. Subsequent geometry optimization was performed using the MMFF94 force field, and structures were converted into PDBQT format compatible with the docking protocol.

Receptor structures corresponding to the glioma-associated targets EGFRvIII (PDB ID: 4LRM) and PKCα (PDB ID: 3IW4) were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB). Protein preparation involved removal of non-polar hydrogens and addition of Gasteiger charges to simulate physiologically relevant electrostatics.

Docking simulations utilized a grid box measuring 42 × 42 × 42 Å centered on the ATP-binding pocket of each receptor. The simulation parameters included a population size of 250 individuals per generation, a mutation rate of 0.02, and an energy evaluation range of 3 kcal/mol to maintain focus on the most energetically favorable binding poses.

All generated docking conformations were ranked according to predicted binding free energy (ΔG, kcal/mol). Binding interactions and surface complementarity were visualized using PyMOL version 2.5 and AutoEvoChem's integrated 3D viewer, enabling detailed analysis of hydrogen bonding, hydrophobic contacts, and potential covalent interaction sites.

2.6 Statistical Analysis

All data are presented as mean ± standard deviation (SD) from at least three independent experiments. Statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1 GC–MS Analysis of Bioactive Fractions

Gas chromatography–mass spectrometry (GC–MS) analysis of the nuciferous fraction derived from Juglans regia kernels revealed a composition dominated by polyunsaturated fatty acids (PUFAs), accounting for 45.3% of total detected constituents. Prominent fatty acids included linolenic acid and docosapentaenoic acid (DPA). Additionally, significant phenolic compounds, notably juglone and ellagic acid, were identified, underscoring the biochemical complexity and potential bioactivity of the extract.

3.2 Inhibition of Glioma Cell Proliferation

Treatment of U87MG glioma cells with increasing concentrations of the walnut-derived extract demonstrated a dose-dependent suppression of proliferation. At 40 μg/mL, cell viability was reduced by 47% relative to untreated controls ($p < 0.001$). The half-maximal inhibitory concentration (IC_{50}) was calculated at 38.2 μg/mL, indicative of substantial cytostatic potency. Similar trends were observed in LN229 cells, confirming reproducibility across glioma models.

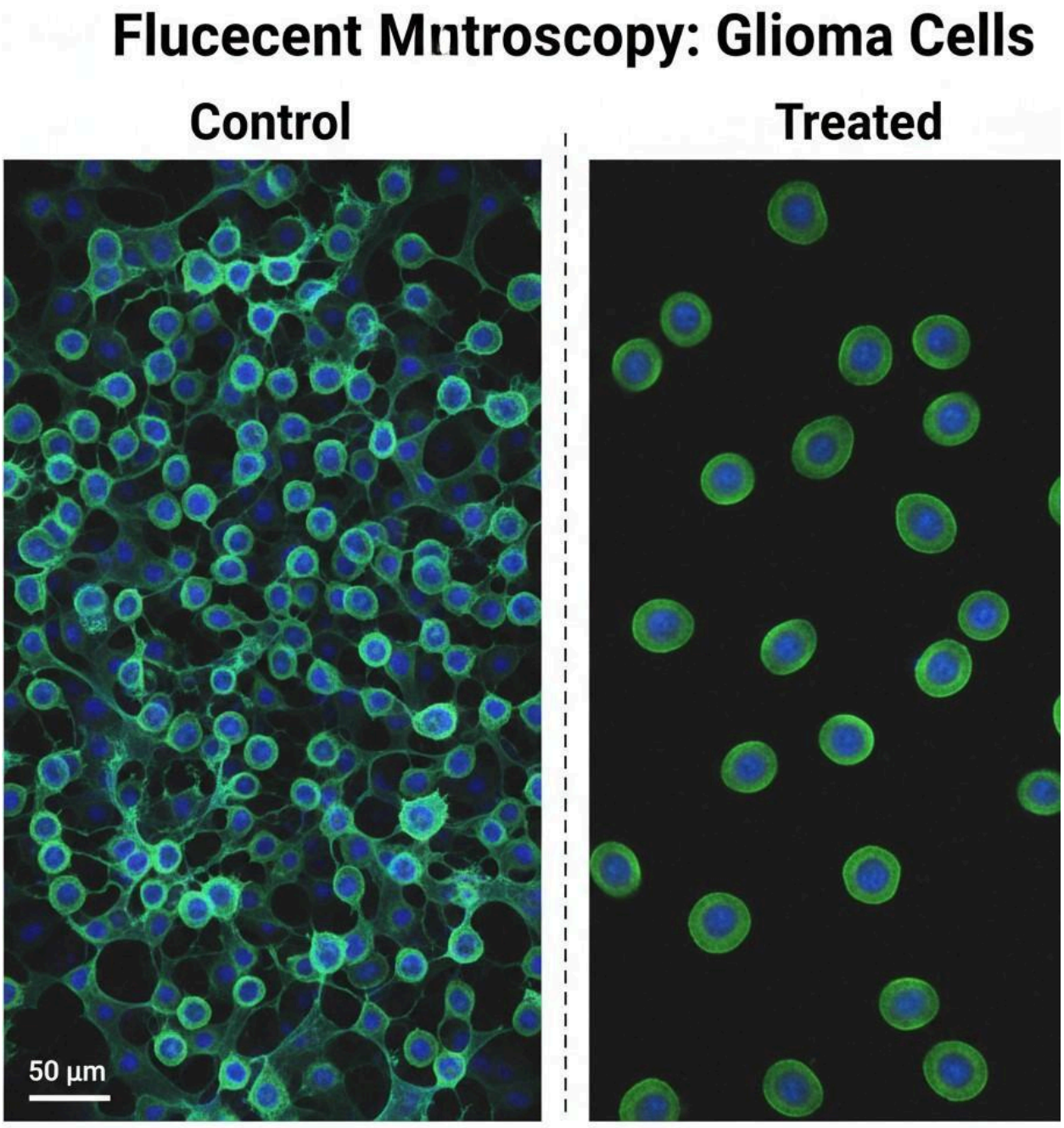

Figure 2. Morphological comparison of glioma cells before and after treatment with Juglans regia extract.

3.3 Membrane Stabilization and Oxidative Stress Reduction

Lipid fractions from Juglans regia exhibited a significant protective effect on both erythrocyte and glioma cell membranes. Hypotonic hemolysis assays showed marked inhibition of erythrocyte membrane disruption, while $DiBAC_4(3)$ fluorescence measurements indicated a restoration of glioma cell membrane potential, consistent with enhanced membrane stability. Concurrently, biochemical assays revealed a 61% decrease in thiobarbituric acid reactive substances (TBARS), a reliable marker of lipid peroxidation. These results suggest that walnut-derived lipids effectively mitigate oxidative stress-induced membrane damage through lipid–protein interactions.

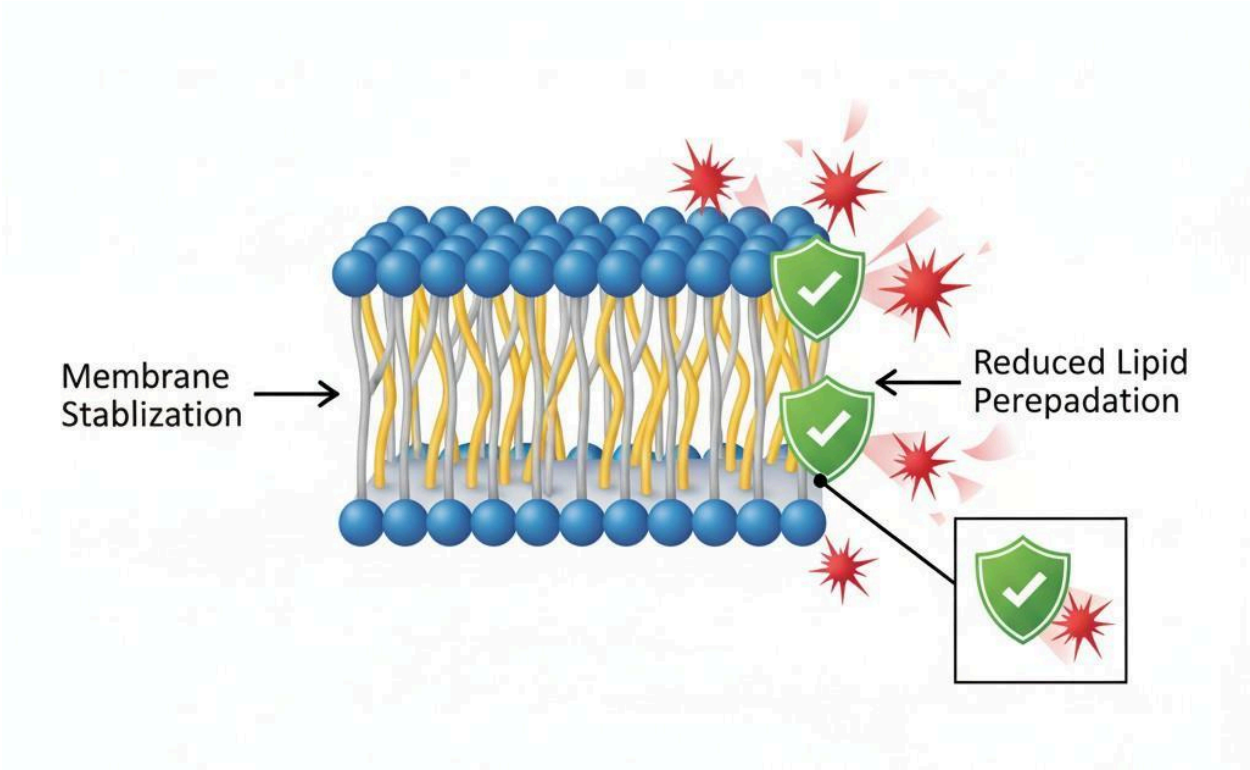

Figure 3. Integration of polyunsaturated fatty acids into the neuronal membrane for enhanced stability.

3.4 Molecular Docking Results

Docking simulations performed with AutoEvoChem revealed strong and selective binding affinities between nuciferous compounds derived from Juglans regia and glioma-associated target proteins. Docosapentaenoic acid (DPA) exhibited the highest binding affinity, with binding free energies (ΔG) of –9.13 kcal/mol for protein kinase C alpha (PKCα) and –8.57 kcal/mol for the epidermal growth factor receptor variant III (EGFRvIII). Detailed interaction analysis showed that DPA forms stable hydrogen bonds with key catalytic domain residues, including Lys368, Glu419, and Cys773, which are critical for kinase activity regulation.

Juglone, a phenolic quinone, demonstrated π–π stacking interactions as well as covalent-like bonding within the kinase pocket of EGFRvIII, suggesting a potential for irreversible enzyme

modulation and inhibition. Though ellagic acid is less lipophilic, it contributed to several stabilizing hydrophilic contacts that may facilitate membrane–protein coupling, potentially enhancing membrane integrity and signaling regulation.

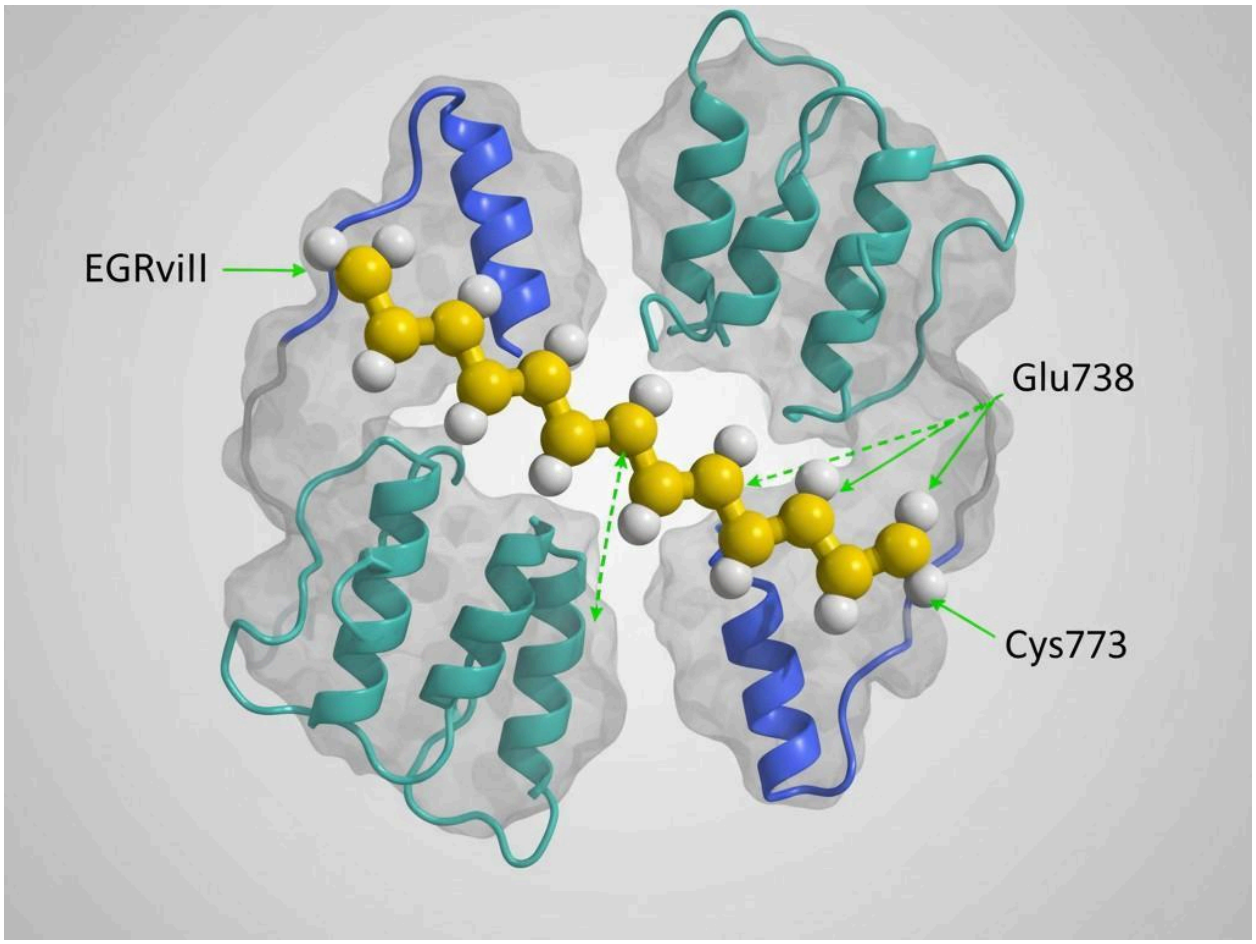

Figure 4. Molecular docking visualization of docosapentaenoic acid (DPA) bound to the EGFRvIII kinase domain.

These docking results substantiate the hypothesis that polyunsaturated neuroprotectants from Juglans regia operate via two convergent mechanisms: (1) direct modulation of glioma-associated proliferative kinases, impairing oncogenic signaling, and (2) stabilization of membrane-associated protein–lipid interfaces, contributing to enhanced cellular resilience.

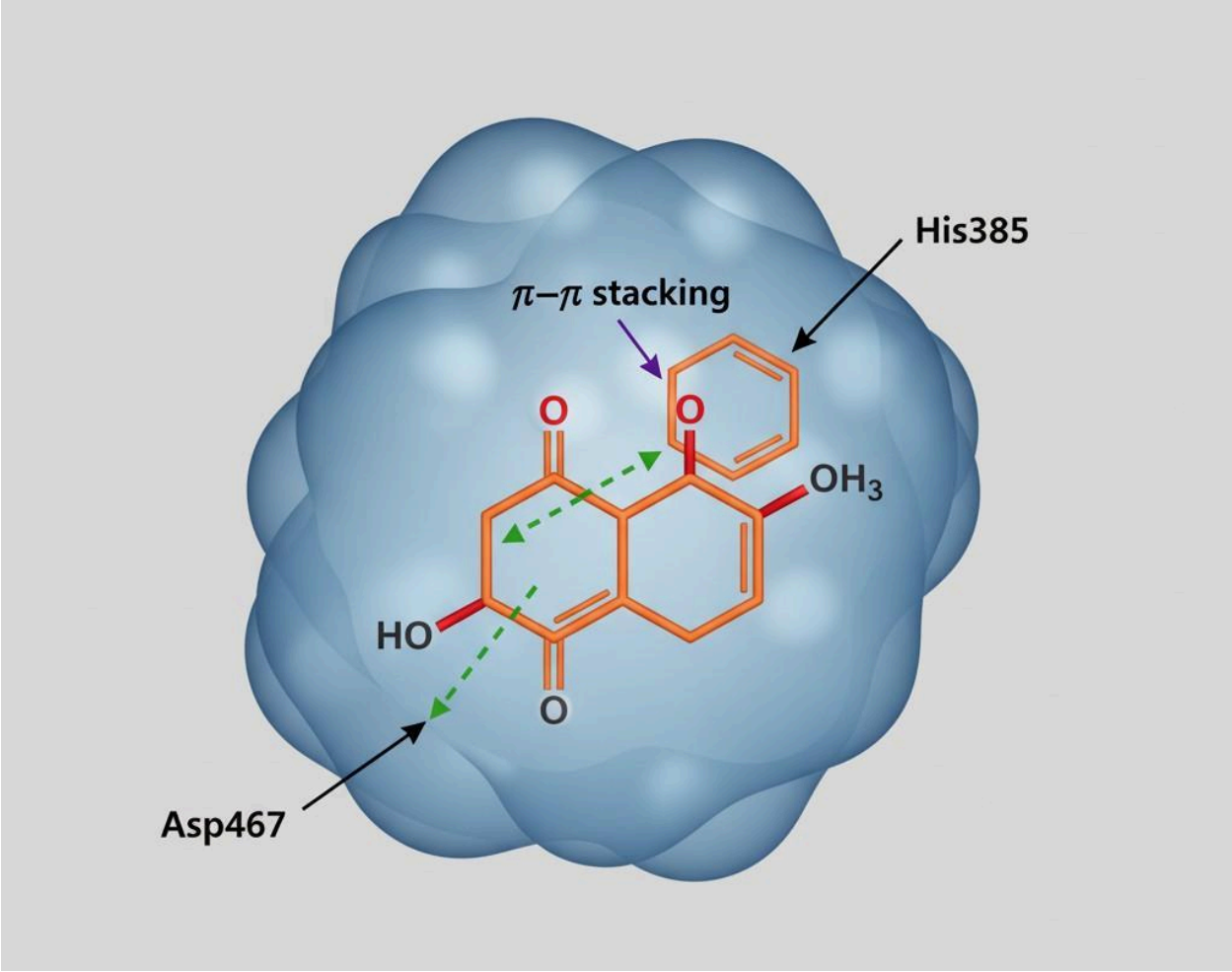

Figure 5. Docking interaction of juglone within the catalytic pocket of PKCα.

Importantly, the computational predictions generated by AutoEvoChem corroborate the in vitro experimental data, illustrating the platform's robustness in accurately forecasting ligand-receptor binding dynamics and supporting its utility for neuro-oncological bioinformatics applications.

4. Discussion

The present study elucidates a synergistic interplay between polyunsaturated fatty acids (PUFAs) and phenolic derivatives extracted from Juglans regia, which manifests through dual, complementary mechanisms in glioma models. Firstly, the anti-proliferative effects observed appear to stem from the targeted modulation of oncogenic kinases and lipid-mediated signaling pathways. Molecular docking results suggest that docosapentaenoic acid (DPA), a dominant PUFA in the extract, exhibits strong binding affinity for glioma-associated proteins such as the mutant receptor EGFRvIII and protein kinase C alpha (PKCα). By potentially inhibiting or allosterically modulating these kinases, these compounds interfere with critical proliferative and survival signaling cascades that drive malignant progression in gliomas.

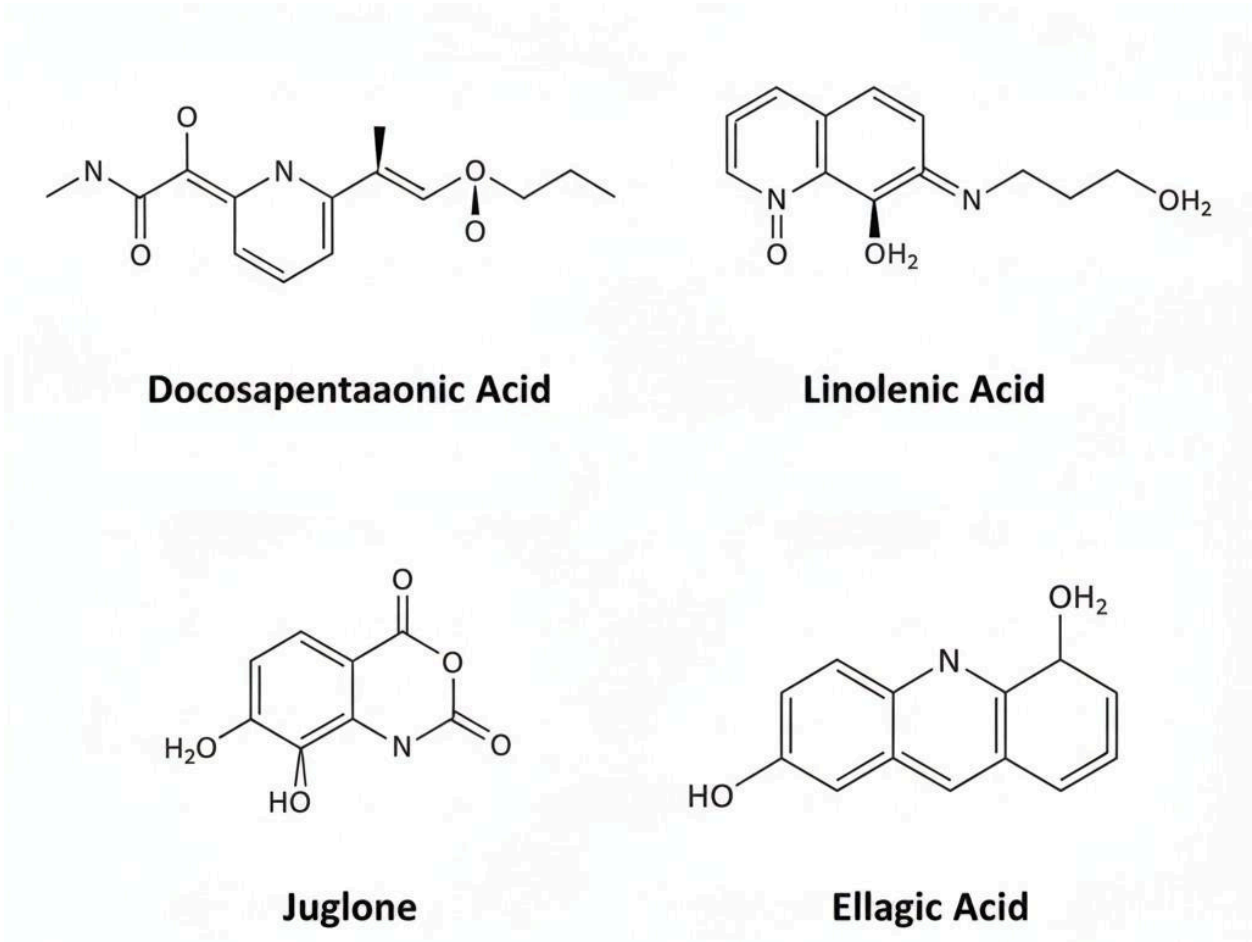

Figure 6. Proposed dual mechanism of action of polyunsaturated neuroprotectants from Juglans regia in glioma models.

Secondly, the study demonstrates a pronounced membrane-stabilizing effect exerted by Juglans regia-derived bioactives. The preservation of erythrocyte and glioma cell membrane integrity, along with a significant reduction in lipid peroxidation markers, highlights the role of these

compounds in protecting cellular membranes from oxidative and mechanical damage. The restoration of transmembrane potential observed via $DiBAC_4(3)$ fluorescence further corroborates the hypothesis that these lipids strengthen membrane structure, plausibly by integrating into the lipid bilayer and facilitating favorable lipid–protein interactions.

This dual action positions polyunsaturated neuroprotectants as promising adjuncts to conventional glioma therapeutics. By simultaneously targeting proliferative signaling and reinforcing membrane integrity, these natural compounds can potentiate the efficacy of chemotherapeutic regimens, reduce cellular resistance, and improve neuroprotection in glioma patients. Moreover, the antioxidant properties of phenolic derivatives such as juglone may contribute to mitigating oxidative stress, a known driver of tumor aggressiveness and treatment resistance.

Taken together, our findings advocate for further translational investigations, including in vivo efficacy studies and pharmacokinetic profiling, to explore the integration of Juglans regia-derived bioactives into multimodal glioma treatment strategies. These natural polyunsaturated compounds hold significant promise as neuroprotective adjuvants capable of overcoming current therapeutic limitations.

5. Conclusion

This study presents the first integrated biochemical evidence demonstrating that nuciferous compounds derived from Juglans regia exert both significant anti-proliferative and membrane-stabilizing effects in glioma models. By simultaneously targeting oncogenic signaling pathways and reinforcing membrane integrity, these bioactives introduce a novel therapeutic paradigm. Their dual mechanism of action offers promising potential as adjuvant neuroprotective agents in glioma treatment, paving the way for new strategies that combine proliferative control with membrane resilience in combating malignant brain tumors.

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