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Short communication

In vitro evaluation of rosemary and *Cannabis sativa* extracts loaded nanoliposomal formulations for potential treatment of Alzheimer's disease

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Introduction

Targeting progressive neurodegenerative disorders like Alzheimer's disease with bioactive plant extracts is severely bottlenecked by poor aqueous solubility, structural instability, and obstruction by the blood-brain barrier (BBB) (Alvi et al., 2022). Encapsulating these phytocompounds within surface-PEGylated nanoliposomes (NLs) offers a protective matrix that shields fragile molecules from metabolic degradation, improves bioavailability, and confers a "stealth" character that prolongs circulation times (Mihailova et al., 2022; Shalabalija et al., 2021). This extended circulation safely facilitates localized intracellular delivery within neurons while mitigating systemic toxicity. The objective of this study was to evaluate the cytocompatibility and neuroprotective potential of the PEGylated nanoliposomal formulations of Rosemary (RE) and *Cannabis sativa* (CE) extracts against H_2O_2 and $A\beta_{1-42}$ -induced neurotoxicity using human SH-SY5Y neuroblastoma cells.

Materials and methods

Materials

Soybean lecithin (Vitalia), LIPOID PE 18:0/18:0-PEG 2000 (Lipoid), cholesterol and DPBS (Sigma-Aldrich), RE, CE (Institute of Pharmacognosy, Skopje), cell culture media (Merck KGaA), and the SH-SY5Y cell line (LGC Standards) and all chemicals, reagents, and solvents used were of the highest analytical or purity grade commercially available.

Methods

Preparation of extract-loaded NLs: NLs dispersions loaded with Rosemary and Cannabis sativa extracts were prepared via a previously optimized modified lipid film hydration technique detally described in Shalabalija et al. (2021) and Mihailova et al. (2022), respectively.

Culturing, subculturing and differentiation of SH-SY5Y cell line: SH-SY5Y human neuroblastoma cells were cultured in DMEM/F12 supplemented with 10% FBS, 1% penicillin–streptomycin, and 1% L-glutamine at 37°C in a humidified 5% CO_2 atmosphere. The medium was renewed every 3 days, and cells were passaged at 70–80% confluence using 0.05% trypsin–EDTA.For neuronal differentiation, cells were cultured for 10 days in DMEM/F12 containing 2% FBS, 1% non-essential amino acids, 10 µM retinoic acid, and 50 ng/mL BDNF, with medium changes every 3 days. Differentiated neuron-like cells were used between days 7 and 10.

MTT Assay: Cells were seeded in 96-well plates (1×10^4 cells/well) and allowed to attach for 48 h

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before treatment with NLs (5, 10, 20, 50 and 100 µg/mL) for 24 h. Subsequently, MTT solution (5 mg/mL) was added and cells were incubated for 4 h at 37°C. Formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability was expressed as a percentage relative to untreated controls.

Antioxidant – DCFH-DA assay: Intracellular ROS levels were assessed using the DCFH-DA assay. Cells were seeded (1×10^4 cells/well) and treated with NLs (5–100 µg/mL) for 24 h. Subsequently, cells were incubated with DCFH-DA (10–20 µM) for 1 h and exposed to 100 µM H_2O_2 to induce oxidative stress. Fluorescence was measured at Ex/Em 485/528–535 nm every 15 minutes over a period of 2 h, Antioxidant activity was expressed as the percentage reduction in ROS-induced fluorescence relative to H_2O_2-treated control cells.

Anti-Amyloid Beta assay: SH-SY5Y cells seeded on 96-well plates (1×10^4 cells/well) were pretreated with NLs (10 or 20 µg/mL) for 24 h and then exposed to $A\beta_{1-42}$ oligomers for an additional 24 h. Untreated cells served as controls, while cells treated with $A\beta_{1-42}$ alone represented the neurotoxicity group. Post-treatment cell viability was evaluated using the MTT assay. Neuroprotective efficacy was determined by comparing the NLs-treated groups against the $A\beta_{1-42}$ neurotoxic control.

Results and discussion

The biocompatibility of the formulations in differentiated SH-SY5Y cells over 24 h showed that RE NLs maintained high cell viability (80–100%) across the tested concentration range, indicating excellent cellular compatibility even at the highest dose. Similarly, CE NLs were well tolerated at lower concentrations (90% cell viability); however, concentrations ≥20 µg/mL induced a noticeable reduction in cell viability (15%), suggesting the onset of concentration-dependent cellular stress.

Intracellular ROS production following hydrogen peroxide exposure revealed distinct antioxidant profiles for the two formulations. RE NLs exhibited a sustained, concentration-dependent antioxidant effect, reaching a maximum protection rate of 52.67% at 20 µg/mL after 120 min, following a decrease for the higher concentrations (50-100 µg/mL), which may be attributed to the saturation of cellular uptake mechanisms, thus leading to reduced bioavailability of the antioxidant components. Similarly, CE NLs demonstrated a rapid antioxidant response at low concentrations, with 5 µg/mL producing 42.58% protection within 15 min and maintaining approximately 40% protection throughout the observation period. The neuroprotective capacity test demonstrated that both formulations increased neuronal survival compared to the non-treated control. RE NLs improved protection from 16.40% (10.0 µg/mL) to 21.82% (20.0 µg/mL), while CE NLs achieved 25.38% neuronal preservation at 10.0 µg/mL. In contrast, the non-encapsulated RE and CE extracts showed no significant anti-amyloid activity at the same concentrations. Therefore, the enhanced effect of the NLs can be attributed to improved stability, solubility, bioavailability, and cellular penetration and uptake of the encapsulated phytochemicals, enabling more effective interaction with amyloid-β-induced toxicity pathways (Alvi et al., 2022).

Conclusion

This study demonstrates that nanoliposomal encapsulation significantly enhances the biological activity of plant-derived extracts, supporting their potential as effective delivery systems for targeted intervention in complex neurodegenerative disorders, including Alzheimer's disease.

References

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