Macedonian Pharmaceutical Bulletin, 72 (Suppl 1) 601 - 602 (2026)
Online ISSN: 1857-8969
DOI: 10.33320/maced.pharm.bull.2026.72.03.290
Short communication

Neuroprotective potential of a multi-ingredient nootropic formulation in SH-SY5Y cells

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Introduction

Daily exposure to cognitive workload, mental fatigue and stress has increased interest in nootropic formulations that do not rely on a single pharmacological target, but combine nutrients, amino acids and botanical extracts with complementary effects on neuronal metabolism, neurotransmission and redox balance.

Among nutritional neuroactive compounds, CDP-choline/citicoline is recognized for its involvement in phospholipid metabolism, membrane repair, cholinergic neurotransmission and neuroinflammatory modulation (Cavalu et al., 2024). In parallel, amino acid-derived ingredients such as L-carnitine, L-tyrosine and L-theanine are commonly associated with mitochondrial energy metabolism, neurotransmitter precursor availability and stress-related cognitive support. Botanical nootropic ingredients, including *Hericium erinaceus* and *Bacopa monnieri*, have also been investigated for their antioxidant, anti-inflammatory and neuroprotective properties, supporting their use in multi-component cognitive-support formulations (Contato et al., 2025; Gościniak et al., 2025).

Therefore, the main aim was to evaluate the biological profile of six nootropic ingredients (CDP-choline, L-carnitine, L-tyrosine, L-theanine, Lion's mane extract and Bacopa extract) and their combined formulation P-04. The study assessed both cytocompatibility and antioxidant activity in SH-SY5Y neuroblastoma cells, with particular emphasis on whether the complete blend provides a stable protective response against H_2O_2-induced oxidative stress compared with the individual components.

Materials and methods

Materials

MTT, DCFH-DA and H_2O_2 were obtained from Sigma-Aldrich. SH-SY5Y human neuroblastoma cells were maintained in DMEM/F12 medium supplemented with 10% FBS, 1% penicillin–streptomycin and 1% L-glutamine at 37 °C and 5% CO_2 and passaged at 70–80% confluence using 0.05% trypsin–EDTA (purchased from Thermo Fisher Scientific). All tested components and extracts were commercially sourced and all other reagents were of analytical grade.

MTT cell viability assay

Cells were seeded in 96-well plates at 1×10^4 cells/well and incubated for 48 h before treatment with individual components or P-04 at 5, 50 and 100 µg/mL. After 24 h, MTT solution was added and plates were incubated for 3 h at 37 °C in the dark. Formazan crystals were dissolved in DMSO, and

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absorbance was measured at 570 nm. Cell viability was expressed as percentage of untreated control cells, considered as 100% viability. Experiments were performed in six replicates.

DCFH-DA oxidative stress assay

Intracellular ROS levels were assessed using the DCFH-DA assay. SH-SY5Y cells were seeded in black 96-well plates at 1×10^4 cells/well and incubated for 48 h. Cells were loaded with 10 µM DCFH-DA for 30 min at 37 °C in the dark, washed with PBS, and then exposed simultaneously to 100 µM H_2O_2 and test samples at 5, 50, or 100 µg/mL. Untreated cells served as the negative control, while H_2O_2-treated cells served as the oxidative stress control. Fluorescence was recorded at 485/535 nm over 120 min. Antioxidant activity was expressed as percentage reduction of H_2O_2-induced fluorescence relative to control conditions.

Results and discussion

All tested components and the combined formulation P-04 preserved SH-SY5Y cell viability after 24 h exposure at 5–100 µg/mL, with no sample reducing viability below the commonly accepted cytotoxicity threshold. CDP-choline, L-carnitine, Bacopa extract, L-theanine and Lion's mane extract maintained or increased apparent metabolic activity across most concentrations, while L-tyrosine showed a moderate reduction only at 50 µg/mL.

The P-04 formulation remained non-cytotoxic, with viability values of 110.7%, 107.2%, and 90.5% at 5, 50, and 100 µg/mL, respectively, indicating that the multi-component blend was well tolerated by SH-SY5Y cells under the tested conditions.

The DCFH-DA assay showed that all tested samples reduced H_2O_2-induced intracellular oxidative stress to varying degrees. Among the individual components, L-theanine and L-tyrosine produced the strongest antioxidant responses, reaching 86.7% and 87.8% protection at 100 µg/mL, respectively. L-carnitine also showed consistent activity, with protection values above 60% across all concentrations, while Bacopa extract maintained a stable antioxidant effect of approximately 59–61%.

CDP-choline and Lion's mane extract showed moderate antioxidant activity at lower concentrations, but their protective effect decreased at 100 µg/mL, suggesting that higher concentrations did not further enhance antioxidant response in this acute oxidative stress model.

Notably, P-04 demonstrated one of the most balanced antioxidant profiles, with protection values of 76.3%, 72.9%, and 76.4% at 5, 50, and 100 µg/mL, respectively. Unlike some individual components, the formulation maintained stable activity across the full concentration range while preserving cell viability. This supports the rationale for using a multi-component nootropic blend rather than relying solely on isolated ingredients. Although individual compounds may show strong activity in specific conditions, the combined formulation appears to provide a broader and more consistent response, likely reflecting complementary and potentially synergistic interactions between its constituents.

Conclusion

The tested nootropic components and their combined formulation P-04 were non-cytotoxic to SH-SY5Y neuroblastoma cells at 5–100 µg/mL after 24 h exposure. P-04 formulation demonstrated stable antioxidant protection of approximately 73–76% across all concentrations while maintaining cell viability above 90%. These preliminary *in vitro* findings indicate that P-04 has a favorable neuroprotective screening profile and warrants further investigation in more mechanistically defined neuronal oxidative stress models.

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