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Original scientific paper

Cholinesterase inhibitory activity and neuronal cytotoxicity of cannabidiol and Δ9-tetrahydrocannabinol: an *in vitro* study

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Abstract

Alzheimer's disease is associated with progressive cholinergic dysfunction, and cholinesterase inhibition remains a principal pharmacological strategy for symptomatic management. Cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC), the two major phytocannabinoids of *Cannabis sativa*, have been proposed as candidate cholinesterase-modulating agents, but their activity has rarely been evaluated alongside neuronal safety under matched conditions. CBD and THC were tested for inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) using Ellman's colorimetric assay (CBD 62.5–2000 µg/mL; THC 31.25–1000 µg/mL), for cytotoxicity in retinoic acid-differentiated SH-SY5Y neuroblastoma cells using the MTT assay (5–500 µg/mL), and for modulation of intracellular reactive oxygen species (ROS) in the same cells using the DCFH-DA assay (5–20 µg/mL). THC showed greater inhibitory potency than CBD against both enzymes (AChE IC_{50} = 182.66 ± 18.86 vs 215.11 ± 3.35 µg/mL; BChE IC_{25} = 122.65 ± 10.35 vs 1316.27 ± 30.07 µg/mL), with CBD reaching an IC_{25} against BChE only near the top of the tested range. Both compounds were severely cytotoxic in SH-SY5Y cells, reducing viability to below 25% from 20 µg/mL upward, roughly an order of magnitude below the concentrations required for measurable cholinesterase inhibition; apparent viabilities above the untreated control at 5 and 10 µg/mL were not interpreted as viability and were excluded from quantitative analysis. In the DCFH-DA assay, neither compound showed a consistent, concentration-dependent antioxidant profile; the irregular response, including complete loss of measurable activity for THC at 20 µg/mL, where the cells were no longer viable, and apparent activity of up to 70% for CBD at the same concentration despite a viability of 9%, was consistent with assay interference rather than genuine ROS attenuation. These findings indicate that while THC and, to a lesser extent, CBD possess measurable cell-free cholinesterase inhibitory activity, their narrow or absent safety margin in neuronal cells, together with unreliable cell-based antioxidant readouts, substantially limit their translational relevance as cholinesterase-targeted agents without further optimisation.

Key words: cannabidiol, tetrahydrocannabinol, cholinesterase inhibition, SH-SY5Y, cytotoxicity, oxidative stress

Introduction

Alzheimer's disease (AD) is the leading cause of dementia worldwide, affecting more than 55 million people, and the cholinergic hypothesis is still the main basis for symptomatic treatment (Alzheimer's Association, 2024; Zhang et al., 2025). Acetylcholinesterase (AChE)

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carries out most of the acetylcholine hydrolysis under normal conditions, but as the disease progresses and cortical AChE activity falls, butyrylcholinesterase (BChE) takes on an increasingly important compensatory role (Ha et al., 2020; Jasiecki et al., 2021). This has motivated growing interest in compounds that act on both enzymes rather than AChE alone.

Cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC), the two principal phytocannabinoids of *Cannabis sativa*, have drawn attention in the context of AD for several reasons, including their interaction with the endocannabinoid system, anti-inflammatory effects, and reports of direct cholinesterase inhibition (Raïch et al., 2025; Puopolo et al., 2022). Several studies using the Ellman colorimetric assay have reported measurable AChE and BChE inhibitory activity for these cannabinoids, generally with THC outperforming CBD, an effect usually attributed to THC's greater lipophilicity and better fit within the hydrophobic AChE active site gorge (Puopolo et al., 2022; Mooko et al., 2022). What is less clear, and what most of these studies do not address, is whether the concentrations needed for this kind of enzymatic activity are actually tolerated by living neurons.

The present study evaluated CBD and THC for AChE and BChE inhibitory activity using Ellman's colorimetric assay, and assessed, under matched conditions, how the same compounds affect the viability and oxidative stress status of retinoic acid-differentiated SH-SY5Y human neuroblastoma cells, using the MTT and DCFH-DA assays respectively. The aim was to determine whether the cholinesterase-inhibiting activity reported for these phytocannabinoids occurs within a concentration range that neurons can tolerate, rather than treating enzymatic potency and cellular safety as separate questions.

Material and methods

Preparation of CBD and THC solutions

CBD and THC were used as commercial olive oil-based preparations with cannabinoid contents of 692.72 mg/g and 650.93 mg/g, respectively, determined in-house. For determination of *in vitro* cholinesterase inhibitory activity, the preparations were dissolved in dimethyl sulfoxide (DMSO; Carlo Erba, Italy) to achieve a stock concentration of 2 mg/mL. The DMSO stock solutions were serially diluted two-fold with 50 mM Tris–HCl buffer (pH 8) to achieve six working concentrations of the extracts (62.5–2000 µg/mL for CBD and 31.25–1000 µg/mL for THC, the latter series being started from a further two-fold dilution of the stock). Eserine, a ChE-specific inhibitor, was dissolved in DMSO to achieve a stock concentration of 10 mg/mL, diluted to 0.1 mg/mL and serially diluted in the same manner to achieve eight working concentrations (0.78125–100 µg/mL). Since the dilution series were prepared directly from the DMSO stock solutions, the solvent content of the wells was halved at each dilution step together with the sample concentration, whereas the control reaction received buffer instead of sample and contained no DMSO. For determination of *in vitro* cytotoxicity and antioxidant activity, a CBD preparation and a cannabis distillate (57.61% and 70.93%, w/w) were dissolved in DMSO (Carlo Erba, Italy) to achieve stock concentrations of 10 mg/mL of active cannabinoid, and the working concentrations (5–500 µg/mL) were achieved by dilution in low-serum medium. The solvent content of the cell-based assays ranged from 0.05% (*v/v*) at 5 µg/mL to 5% (*v/v*) at 500 µg/mL, with a DMSO vehicle control of 0.1% (*v/v*).

Enzyme inhibition assay (AChE and BChE)

The neuroprotective activity of the preparations was determined through inhibition of AChE (from Electrophorus electricus) and BChE (from equine serum) using Ellman's method. The cholinesterase (ChE) activity was measured indirectly by quantification of the 5-thio(2-nitrobenzoic acid) (TNB) anion formed in the reaction between 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and thiocholine. Thiocholine is a product of substrate (acetylthiocholine iodide/S-butyrylthiocholine chloride) hydrolysis by the ChE activity.

The reaction mixture consisted of sample (30 µL), 45 µL of 50 mM Tris–HCl buffer (pH 8) with bovine serum albumin, 3 mM DTNB (125 µL) and 25 µL of AChE/BChE, and was incubated at room temperature for 5 min. The two-fold dilution series was prepared in the plate, the first well of each series receiving 30 µL of the sample solution and the remaining five wells 30 µL of buffer, with 30 µL transferred from each well to the next and discarded from the last; since the 30 µL aliquot is diluted into the 250 µL reaction volume, the concentrations in the reaction mixture are 8.33-fold lower than the reported working concentrations. The reaction was initiated by addition of 15 mM substrate (25 µL) and the increase in absorbance was monitored at 405 nm, for 5 min with readings every minute for AChE and for 10 min with readings every 2 min for BChE. In parallel, a control was prepared with replacement of the sample by 75 µL of buffer, and this reaction represents 100% enzyme activity. The percentage of enzyme inhibition was calculated as $I\ (\%) = [1 - (\Delta As/\Delta Ac)] \times 100$, where ΔAs is the change in absorbance of the assay and ΔAc the change in absorbance of the control. All measurements were performed in triplicate (n = 3).

Cell culture cultivation, differentiation, and MTT cytotoxicity assay

SH-SY5Y human neuroblastoma cells (ATCC CRL-2266) were cultured in 1:1 (*v/v*) MEM/Ham's F-12 medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 2 mM L-glutamine at 37 °C under 5% CO_2. Prior to the cytotoxicity assay, cells were differentiated to a post-mitotic neuronal phenotype using a 10-day retinoic acid protocol (10 µM all-trans retinoic acid

in low-serum medium), with medium renewal every three days. All sample dilutions for the cell-based assays were prepared in the same low-serum medium (1:1 MEM/Ham's F-12 with 1% fetal bovine serum, 1% penicillin-streptomycin and 2 mM L-glutamine) used for seeding.

Differentiated cells were seeded at 1×10^4 cells per well in 96-well plates and allowed to adhere for 48 h. Cells were treated with CBD or THC at 5, 10, 20, 50, 100, 200, and 500 µg/mL for 24 h; the 5 to 20 µg/mL range was additionally assessed after 48 h. Viability was assessed by MTT reduction: cells were incubated with MTT solution (5 mg/mL in PBS, 20 µL per well) for 3 h at 37 °C in the dark, formazan crystals were dissolved in 200 µL of DMSO per well, and absorbance was read at 570 nm with a reference wavelength of 630 to 690 nm. Viability was expressed as a percentage of untreated control (n = 4).

DCFH-DA intracellular ROS assay

Differentiated cells seeded in black 96-well plates were loaded with DCFH-DA at a final concentration of 10 to 20 µM in serum-free medium for 30 min at 37 °C in the dark, then washed twice with PBS. CBD or THC (5, 10, or 20 µg/mL) and H_2O_2 (100 µM final) were added simultaneously. Fluorescence was recorded at 30, 45, 60, 90, and 120 min (excitation 485 nm, emission 528–535 nm). Antioxidant activity was calculated as [(FH_2O_2 − Fsample) / FH_2O_2] × 100, where FH_2O_2 is the mean fluorescence of the wells treated with hydrogen peroxide alone and Fsample the mean fluorescence of the treated wells; negative values indicate fluorescence above that of the challenge alone. Each condition was measured in four replicate wells (n = 4).

Statistical analysis

The enzyme assays were performed in triplicate (n = 3) and the cell-based assays in four replicate wells (n = 4), and results are expressed as mean ± SD. Statistical analysis was conducted using ordinary one-way ANOVA followed by Tukey's multiple comparisons test. A p-value < 0.05 was considered statistically significant. IC_{50} and IC_{25} values were determined by nonlinear regression of inhibitor concentration against normalized response with variable slope, Y = 100 / (1 + (IC_{50}/X)^HillSlope), with the plateaux fixed at 0 and 100% inhibition and IC_{50} constrained to positive values. Statistical calculations were performed using GraphPad Prism (version 9.3.0).

Results and discussion

Acetylcholinesterase inhibition

Both CBD and THC inhibited AChE in a concentration-dependent manner across the ranges tested (62.5 to 2000 µg/mL for CBD and 31.25 to 1000 µg/mL for THC). THC was the more potent of the two (IC_{50} = 182.66 ± 18.86 µg/mL), compared with 215.11 ± 3.35 µg/mL for CBD, and this difference reached statistical significance (p = 0.0027, Tukey's HSD). Neither compound came close to the reference inhibitor eserine, which was roughly fifteen to twenty times more potent (IC_{50} = 11.81 ± 0.67 µg/mL) and differed significantly from both cannabinoids (Table 1).

THC outperforming CBD against AChE is broadly consistent with previous reports, though the literature is not entirely uniform on this point. Puopolo et al. (2022) tested CBD alongside the related isomer Δ8-THC and several other cannabinoids and found broadly comparable, moderate activity for both compounds rather than a clear separation; their IC_{50} values were close (91.7 µM for Δ8-THC versus 102.8 µM for CBD), so the THC advantage, where present, was modest. Mooko et al. (2022) reported the opposite pattern: working with both whole *Cannabis sativa* extracts and the isolated cannabinoids, they found CBD to be the better AChE inhibitor of the two, while THC and the crude extracts themselves barely reached 41% inhibition even at the highest concentration tested. These inconsistencies suggest that the relative ranking of CBD and THC against AChE depends substantially on the THC isomer used, the source and purity of the material, and the specific assay conditions, underscoring the value of direct, matched comparisons such as the one performed here rather than comparisons across studies with differing methodologies.

Butyrylcholinesterase inhibition

The gap between the two compounds widened considerably at BChE. THC inhibited the enzyme with an IC_{25} of 122.65 ± 10.35 µg/mL, whereas CBD reached an IC_{25} only near the top of the tested range (1316.27 ± 30.07 µg/mL), a difference of more than an order of magnitude. Eserine remained far more potent than either cannabinoid (IC_{25} = 0.11 ± 0.06 µg/mL) and differed significantly from both (Table 2).

Table 1. AChE IC_{50} values (µg/mL) for CBD, THC, and eserine. Values are mean ± SD (n = 3). Different letters denote statistically significant differences (p < 0.05, Tukey's HSD)

Sample	AChE IC_{50} (µg/mL) ± SD	Statistical group
THC	182.66 ± 18.86	b
CBD	215.11 ± 3.35	a
Eserine	11.81 ± 0.67	c

Table 2. BChE IC_{25} values (µg/mL) for CBD, THC, and eserine. Values are mean ± SD (n = 3). Different letters denote statistically significant differences ($p < 0.05$, Tukey's HSD)

Sample	BChE IC_{25} (µg/mL) ± SD	Statistical group
THC	122.65 ± 10.35	b
CBD	1316.27 ± 30.07	a
Eserine	0.11 ± 0.06	c

The much weaker BChE inhibition by CBD, set against its comparatively modest disadvantage at AChE, is a notable contrast that the present data cannot fully explain. The ranking at one enzyme clearly does not generalise to the other, and would require a dedicated structural investigation to clarify properly. CBD does not, however, appear to be a structural dead end for this purpose: Zhang et al. (2024) showed that chemically modifying the CBD scaffold can substantially improve its cholinesterase selectivity, indicating that the parent molecule may require structural modification rather than direct application to become a competitive BChE inhibitor.

Cytotoxicity in differentiated SH-SY5Y cells

The cytotoxicity results differ markedly from the enzymatic findings. Both CBD and THC were severely toxic to differentiated SH-SY5Y neurons from 20 µg/mL upward. At 20 µg/mL, viability fell to 9.1 ± 0.6% for CBD and 22.1 ± 5.0% for THC at 24 h, and CBD viability remained below 13% across the whole 50 to 500 µg/mL range. At 5 and 10 µg/mL both preparations returned apparent viabilities well above the untreated control (203.4 ± 19.4% and 261.1 ± 26.5% for CBD, 105.4 ± 43.5% and 116.9 ± 43.9% for THC), and THC returned apparent viabilities rising from 14.8% at 50 µg/mL to 50.2% at 500 µg/mL. Neither pattern is biologically plausible for a cytotoxic preparation, and neither can be read as a measure of cell viability; these values are therefore excluded from quantitative interpretation, and no mechanism is proposed for them here. A reliable cytotoxic IC_{50} could not therefore be determined, but it lies below 20 µg/mL for both compounds, roughly an order of magnitude below the concentrations required for meaningful cholinesterase inhibition in the enzyme assay (Table 3).

This relationship between cholinesterase-active and cytotoxic concentrations is the central finding of this study, and one that the cannabinoid-cholinesterase literature rarely addresses directly. Puopolo et al. (2022), Mooko et al. (2022), and Suttithumsatid et al. (2023) all report enzyme inhibitory activity for CBD and THC, but none of these studies includes a matched cell-based safety assessment, leaving it unclear whether the reported cell-free potency could be achieved inside a living neuron without cytotoxic consequences. Under the matched conditions used here, the concentration window in which CBD and THC inhibit cholinesterases does not overlap with the window in which neurons remain viable.

Table 3. Cell viability (% of untreated control) in retinoic acid-differentiated SH-SY5Y cells after 24 h of treatment. Values at 20 µg/mL are mean ± SD (n = 4); values at 50 and 100 µg/mL are means (n = 4). Readings at 5 and 10 µg/mL exceeded the untreated control and are excluded as assay interference

Sample	20 µg/Ml	50 µg/mL	100 µg/mL	Notes
CBD	9.1 ± 0.6	9.5	11.9	Severe cytotoxicity from 20 µg/mL; viability below 13% to 500 µg/mL
THC	22.1 ± 5.0	14.8	24.7	Severe cytotoxicity from 20 µg/mL; apparent rise to 50.2% at 500 µg/mL attributed to interference

Part of this toxicity likely reflects the lipophilicity of these molecules: at micromolar-to-low-millimolar concentrations, cannabinoids are known to disrupt cell membranes, and this same property has been shown to interfere directly with the MTT reagent in other plant-derived compounds, producing falsely elevated viability readings (Karakaş et al., 2017). The rising apparent viability of THC between 50 and 500 µg/mL is consistent with such an artefact at the upper end of the range, where the material is present in bulk. The fall in viability at and above 20 µg/mL is nevertheless too large and too consistent across both compounds to be explained by the artefact alone. CBD's neuroprotective profile in the literature, by contrast, derives from a markedly different concentration regime: Raïch et al. (2025) used CBD at 200 nM in primary neuronal cultures and 10 mg/kg given orally in a transgenic mouse model, reporting reduced amyloid-beta and tau pathology along with improved memory. These concentrations are several orders of magnitude below those tested here, indicating that CBD's neuroprotective reputation and its behaviour as a cholinesterase inhibitor occupy distinct dose ranges that should not be conflated.

Intracellular ROS modulation (DCFH-DA assay)

In the DCFH-DA assay, CBD returned apparent antioxidant activity at all three concentrations tested (17.5–34.5% at 5 µg/mL, 25.6–50.7% at 10 µg/mL and 33.4–70.2% at 20 µg/mL across the 30–120 min window), even though only 9.1% of the cells remained viable at 20 µg/mL. THC showed lower and more erratic apparent activity at 5 and 10 µg/mL (10.5–25.7% and 26.4–45.3% respectively) and no measurable activity at all at 20 µg/mL, where the cells were no longer viable. Neither compound produced a coherent concentration-response relationship, and neither profile follows the temporal pattern of a genuine scavenger (Table 4).

Neither profile is consistent with a genuine, dose-dependent radical scavenger. The apparent antioxidant activity recorded for CBD at 20 µg/mL, a concentration at which the MTT assay showed only 9.1% viability, cannot be read as radical scavenging in viable cells, and the complete absence of signal for THC at the same concentration reflects cell death rather than an absence of antioxidant capacity. One plausible explanation for the CBD readings is assay interference: Nelson et al. (2020), in a detailed chemistry-focused critique of CBD research, describe the compound as showing considerable "bioassay promiscuity" because of its lipophilicity and tendency to disrupt membranes, and specifically identify redox cycling and membrane damage as mechanisms that can distort cell-based readouts. Given that 20 µg/mL lies within the cytotoxic range identified in the MTT assay, membrane disruption or some direct interaction with the fluorescent probe could plausibly distort the signal at that concentration. The antioxidant literature on CBD is not unanimous, however: several independent groups have reported genuine, receptor-independent antioxidant activity for CBD at comparable or lower concentrations, so assay interference cannot be confirmed as the explanation without further investigation. Without an acellular control or a second, orthogonal antioxidant assay run in parallel, the DCFH-DA results in this study are best treated as descriptive rather than conclusive.

Three limitations of the present design should be stated explicitly. First, in the cholinesterase assays the dilution series was prepared in the plate directly from the DMSO stock, so the solvent content of the sample wells was halved at each step in parallel with the sample concentration, while the control wells received buffer and contained no solvent. The assay was therefore not solvent-matched, and a contribution of DMSO to the inhibition measured at the upper end of the series cannot be excluded. The two enzymes are not equally exposed: Novales and Schwans (2022) reported that 1% (*v/v*) DMSO reduced acetylcholinesterase activity by approximately 75% but butyrylcholinesterase activity by only about 20%. Both AChE IC_{50} values reported here fall in the middle of the series, where the solvent load is intermediate, so they may

Table 4. Percentage antioxidant activity (% inhibition of H_2O_2-induced ROS, relative to the H_2O_2-only control) for CBD and THC at 5, 10, and 20 µg/mL over 30–120 min in retinoic acid-differentiated SH-SY5Y cells. Values are mean (n = 4). Values of 0.00 for THC at 20 µg/mL denote confirmed cell death. All values are subject to the assay interference described in the text and are reported for completeness only.

Sample	Conc. (µg/mL)	30 min (%)	60 min (%)	90 min (%)	120 min (%)
CBD	5	17.52	26.63	27.97	34.47
CBD	10	25.57	45.10	50.70	48.71
CBD	20	33.38	37.99	70.23	69.09
THC	5	10.49	18.16	25.71	23.95
THC	10	26.40	34.04	33.33	45.26
THC	20	0.00	0.00	0.00	0.00

overstate the potency of the preparations. The reference inhibitor was subject to the same scheme, since its stock was also prepared in DMSO and diluted in the plate in the same way, and its AChE IC_{50} falls at a comparable point of its own series; the three samples are therefore compared on a common solvent footing, but every inhibition value is referenced to a control that contained none. Second, in the cell-based assays the solvent content reached 0.5 to 5% (*v/v*) at 50 µg/mL and above, and the measurements were made in replicate wells on a single plate rather than in independent biological repeats; the high-concentration data are therefore best read as indicating the direction of the concentration-response relationship rather than its magnitude, and the cellular results support descriptive comparison rather than formal inferential statistics. Third, the enzyme concentrations reported are those of the dilution series rather than of the reaction mixture. On the latter basis the AChE IC_{50} values correspond to 21.9 µg/mL THC and 25.8 µg/mL CBD, which lie at rather than above the concentration at which viability had already collapsed, so the relationship between enzyme-active and cytotoxic concentrations is best read as the absence of any usable window rather than as a defined fold-difference.

These findings indicate that neither CBD nor THC, in their current crude oil-based form, is a realistic candidate for direct use as a cholinesterase-targeted agent, since the concentrations needed for meaningful enzymatic inhibition are not tolerated by neurons. The chemical derivatisation work by Zhang et al. (2024), which achieved improved potency and selectivity for a CBD-derived compound against cholinesterases through structural modification, suggests one possible route forward: separating the desired enzymatic activity from the cytotoxic concentration range through medicinal chemistry rather than relying on the parent molecules directly.

Conclusion

THC inhibited AChE and BChE more strongly than CBD under the matched conditions used here, with CBD reaching an IC_{25} against BChE only near the top of the tested range. However, both compounds proved severely toxic to differentiated SH-SY5Y neurons well below the concentrations required for any meaningful cholinesterase inhibition, and neither produced a reliable, dose-dependent antioxidant signal in the DCFH-DA assay, a pattern more consistent with assay interference than with genuine radical scavenging. This narrow-to-absent safety margin, together with the unreliable cell-based antioxidant readout, represents the principal limitation to the use of CBD and THC as cholinesterase-targeted agents, and both should be addressed explicitly in future pharmacological work on these phytocannabinoids in the context of Alzheimer's disease.

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References

Ha, Z.Y., Mathew, S., Yeong, K.Y., 2020. Butyrylcholinesterase: a multifaceted pharmacological target and tool. Curr. Protein Pept. Sci. 21, 99–109. https://doi.org/10.2174/1389203720666191107094949

Alzheimer's Association, 2024. 2024 Alzheimer's disease facts and figures. Alzheimers Dement. 20, 3708–3821. https://doi.org/10.1002/alz.13809

Jasiecki, J., Targońska, M., Wasąg, B., 2021. The role of butyrylcholinesterase and iron in the regulation of cholinergic network and cognitive dysfunction in Alzheimer's disease pathogenesis. Int. J. Mol. Sci. 22, 2033. https://doi.org/10.3390/ijms22042033

Karakaş, D., Arı, F., Ulukaya, E., 2017. The MTT viability assay yields strikingly false-positive viabilities although the cells are killed by some plant extracts. Turk. J. Biol. 41, 919–925. https://doi.org/10.3906/biy-1703-104

Mooko, T., Bala, A., Tripathy, S., Kumar, C.S., Mahadevappa, C.P., Chaudhary, S.K., Matsabisa, M.G., 2022. Cannabis sativa L. flower and bud extracts inhibited in vitro cholinesterases and β-secretase enzymes activities: possible mechanisms of cannabis use in Alzheimer disease. Endocr. Metab. Immune Disord. Drug Targets 22, 297–309. https://doi.org/10.2174/1871530321666210222124349

Nelson, K.M., Bisson, J., Singh, G., Graham, J.G., Chen, S.N., Friesen, J.B., Dahlin, J.L., Niemitz, M., Walters, M.A., Pauli, G.F., 2020. The essential medicinal chemistry of cannabidiol (CBD). J. Med. Chem. 63, 12137–12155. https://doi.org/10.1021/acs.jmedchem.0c00724

Novales, N.A., Schwans, J.P., 2022. Comparing the effects of organic cosolvents on acetylcholinesterase and butyrylcholinesterase activity. Anal. Biochem. 654, 114796. https://doi.org/10.1016/j.ab.2022.114796

Puopolo, T., Liu, C., Ma, H., Seeram, N.P., 2022. Inhibitory effects of cannabinoids on acetylcholinesterase and butyrylcholinesterase enzyme activities. Med. Cannabis Cannabinoids 5, 85–94. https://doi.org/10.1159/000524086

Raïch, I., Lillo, J., Rebassa, J.B., Griñán-Ferré, C., Bellver-Sanchis, A., Reyes-Resina, I., Franco, R., Pallàs, M., Navarro, G., 2025. Cannabidiol as a multifaceted therapeutic agent: mitigating Alzheimer's disease pathology and enhancing cognitive function. Alzheimers Res. Ther. 17, 109. https://doi.org/10.1186/s13195-025-01756-0

Suttithumsatid, W., Kara, J., Lomlim, L., Nualsri, C., Panichayupakaranant, P., 2023. Acetylcholinesterase inhibitory activity of standardized cannabinoids-rich fractions. Pharm. Biomed. Res. 9, 173–182. https://doi.org/10.32598/PBR.9.3.1054.1

Zhang, K., Zhao, M., Wang, D., Zhao, Y., Li, J., Zhang, S., Zhang, W., Shi, Z., 2024. Synthesis and evaluation of a highly selective cannabidiol amide cholinesterase inhibitor. Results Chem. 7, 101492. https://doi.org/10.1016/j.rechem.2024.101492

Zhang, N., Chai, S., Wang, J., 2025. Assessing and projecting the global impacts of Alzheimer's disease. Front. Public Health 12, 1453489. https://doi.org/10.3389/fpubh.2024.1453489

Резиме

Инхибиција на холинестераза и невронална цитотоксичност на канабидиол и Δ9-тетрахидроканабинол: *In Vitro* студија

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Клучни зборови: канабидиол, тетрахидроканабинол, инхибиција на холинестераза, SH-SY5Y, цитотоксичност, оксидативен стрес

Алцхајмеровата болест е поврзана со прогресивна холинергична дисфункција, а инхибицијата на холинестеразата останува главна фармаколошка стратегија за симптоматски третман на оваа болест. Канабидиолот (CBD) и Δ9-тетрахидроканабинолот (THC), двата главни фитоканабиноиди на *Cannabis sativa*, се предложени како кандидати за агенси кои го модулираат нивото на холинестераза, но нивната активност ретко се оценува заедно со нивната безбедност во соодветни услови. Во ова истражување беше тестирана инхибиторната активност на CBD и THC врз ацетилхолинестераза (AChE) и бутирилхолинестераза (BChE) со употреба на Елмановата колориметриска метода (CBD 62,5–2000 μg/mL; THC 31,25–1000 μg/mL). Дополнително беше направено испитување за цитотоксичност врз диференцирани SH-SY5Y невробластома клетки со употреба на MTT тестот (5–500 μg/mL), како и тест за модулација на интрацелуларни реактивни кислородни видови (ROS) во истите клетки - DCFH-DA тест (5–20 μg/mL). THC покажа поголема инхибиторна потентност од CBD и кај двата ензима (AChE IC_{50} = 182,66 ± 18,86 наспроти 215,11 ± 3,35 μg/mL; BChE IC_{25} = 122,65 ± 10,35 наспроти 1316,27 ± 30,07 μg/mL), при што CBD достигна IC_{25} за BChE дури при највисоките тестирани концентрации. И двете соединенија беа цитотоксични врз SH-SY5Y клетките, намалувајќи ја вијабилноста под 25% при концентрации повисоки од 20 μg/mL, приближно за еден ред на големина под концентрациите потребни за мерлива инхибиција на холинестеразата. Во DCFH-DA тестот, ниту едно од испитуваните соединенија не покажа конзистентен и концентрационо-зависен антиоксидативен ефект. Забележаниот нерегуларен одговор, вклучително и целосниот губиток на мерлива активност кај THC при концентрација од 20 μg/mL, при која клетките веќе не покажуваа соодветна виталност, како и привидната активност од приближно 70% кај CBD при истата концентрација, и покрај клеточната виталност од само 9%, најверојатно се должи на интерференција со тестот, а не на реално намалување на нивото на интрацелуларните ROS.

Овие резултати покажуваат дека, иако THC, а во помала мера и CBD, поседуваат мерлива инхибиторна активност кон холинестеразите, Дополнително, несигурноста на клеточно-базираните антиоксидативни мерења дополнително го ограничува толкувањето на нивниот потенцијален заштитен ефект. Оттука, добиените наоди значително го ограничуваат транслациското значење на THC и CBD како потенцијални агенси насочени кон холинестеразите и укажуваат на потребата од дополнителна оптимизација и валидација на нивниот фармаколошки профил.