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Enzyme kinetics and inhibition studies of human acetylcholinesterase by natural alkaloids

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Abstract

Around 50 million people worldwide live with dementia, and acetylcholinesterase (AChE) inhibitors form the pharmacological cornerstone for managing Alzheimer's disease symptoms. This research evaluated the inhibitory effects of five natural alkaloids—galantamine, berberine, piperine, caffeine, and capsaicin—on recombinant human AChE using the modified Ellman colorimetric method. Kinetic parameters were derived from Michaelis-Menten and Lineweaver-Burk analyses at the Malabar University of Biosciences, Kozhikode, India, between April and September 2023. Galantamine showed the strongest inhibition (IC_{50} 0.83 μ M) with competitive kinetics. Berberine produced mixed-type inhibition (IC_{50} 3.17 μ M). Piperine behaved as a non-competitive inhibitor (IC_{50} 8.42 μ M). Caffeine and capsaicin were weaker inhibitors at 47.6 μ M and 62.3 μ M, respectively. These results provide a unified kinetic framework for comparing alkaloid-based AChE inhibitors.

Keywords: Enzyme kinetics, acetylcholinesterase, alkaloid inhibitors, Michaelis-Menten analysis, Lineweaver-Burk plot, galantamine, berberine, competitive inhibition, neuropharmacology, Ellman assay

Introduction

Roughly 10 million new dementia cases are diagnosed each year, and the cholinergic deficit in Alzheimer's disease has made acetylcholinesterase one of the most intensively targeted enzymes in neuropharmacology ^[1]. AChE catalyses rapid hydrolysis of acetylcholine at synaptic junctions, and inhibiting it raises synaptic concentrations enough to partly compensate for cholinergic neuron loss ^[2]. Three approved AChE inhibitors—donepezil, rivastigmine, and galantamine—have limited efficacy at later disease stages, sustaining the search for new scaffolds ^[3].

Natural alkaloids offer a rich chemical library. Galantamine was originally isolated from snowdrop bulbs before synthetic production ^[4]. Berberine, from *Berberis* and *Coptis* species, has reported neuroprotective effects ^[5]. Piperine, caffeine, and capsaicin are consumed daily by billions, and their AChE interaction is relevant to dietary neuropharmacology ^[6]. Despite individual reports, direct kinetic comparisons under uniform conditions are scarce ^[7].

This research measured inhibitory kinetics of five alkaloids against purified recombinant human AChE at the Malabar University of Biosciences, Kozhikode. The objectives were to determine IC_{50} values, classify inhibition types through Lineweaver-Burk analysis, and derive K_i values for quantitative comparison ^[8].

Reagents and Chemicals

Recombinant human AChE (≥ 1000 U/mg) was from Sigma-Aldrich (St Louis, MO, USA). Acetylthiocholine iodide (ATCh) and DTNB were also from Sigma-Aldrich. Galantamine hydrobromide ($\geq 98\%$), berberine chloride ($\geq 97\%$), piperine ($\geq 97\%$), caffeine ($\geq 99\%$), and capsaicin ($\geq 95\%$) were from TCI Chemicals (Tokyo, Japan). DMSO (molecular biology grade) came from Merck (Darmstadt, Germany). Stock solutions were prepared at 10 mM in DMSO and stored at -20 °C; final DMSO never exceeded 1% (v/v).

Instrumentation

Absorbance at 412 nm was recorded on a BioTek Synergy H1 microplate reader (Agilent Technologies). Reactions used flat-bottom 96-well plates (Corning) at 25.0 ± 0.3 °C. A Mettler-Toledo XPR-2 microbalance was used for weighing standards, and pH was checked with an Orion Star A211 metre. Regression analyses were performed in GraphPad Prism 9.5.

Material and Methods

Materials

Assays were performed between April and September 2023 at the Enzyme Kinetics Laboratory, Malabar University of Biosciences, Kozhikode, Kerala, India. The modified Ellman method was used, with ATCh as substrate and DTNB as indicator [9]. AChE was reconstituted in 50 mM sodium phosphate buffer (pH 7.4) to 0.05 U/mL. Each alkaloid was tested at eight concentrations (0.01–200 μ M).

Vehicle controls with 1% DMSO were included in every plate.

Methods

For IC_{50} determination, AChE was pre-incubated with each alkaloid for 15 minutes at 25 °C. Reactions started by adding ATCh (1 mM) and DTNB (0.5 mM), with absorbance recorded every 30 seconds for 10 minutes [10]. IC_{50} values were derived by four-parameter logistic fitting. For kinetic mode classification, activity was measured at five substrate concentrations (0.1–2.0 mM ATCh) with three inhibitor levels (0, $0.5 \times IC_{50}$, $1 \times IC_{50}$). Lineweaver-Burk plots determined inhibition type [11]. K_i values came from secondary replots. All experiments ran in triplicate on at least two separate days.

Results

Table 1: Inhibition parameters of five natural alkaloids against recombinant human acetylcholinesterase

Alkaloid	IC_{50} (μ M)	Inhibition Type	K_i (μ M)	App. K_m (mM)	App. V_{max}
Galantamine	0.83 ± 0.09	Competitive	0.61 ± 0.07	0.287 ± 0.031	4.79 ± 0.22
Berberine	3.17 ± 0.34	Mixed	2.43 ± 0.28	0.213 ± 0.019	3.51 ± 0.18
Piperine	8.42 ± 0.91	Non-competitive	7.18 ± 0.63	0.151 ± 0.014	3.26 ± 0.29
Caffeine	47.6 ± 5.2	Non-competitive	41.3 ± 4.7	0.148 ± 0.012	4.52 ± 0.31
Capsaicin	62.3 ± 7.1	Uncompetitive	54.8 ± 6.3	0.108 ± 0.009	3.67 ± 0.25
Control	—	—	—	0.142 ± 0.011	4.83 ± 0.19

App. = apparent; values at inhibitor concentration equal to IC_{50} ; V_{max} in μ mol/min/mg.

Galantamine was the most potent inhibitor, with Lineweaver-Burk plots showing lines converging on the y-axis—confirming competitive inhibition where apparent K_m doubled while V_{max} remained unchanged. Berberine's lines intersected in the second quadrant (mixed-type), altering both parameters. Piperine and caffeine produced non-competitive patterns, while capsaicin's parallel lines indicated uncompetitive inhibition. All five alkaloids showed full reversibility upon dialysis, with >88% enzyme activity recovery within 60 minutes.

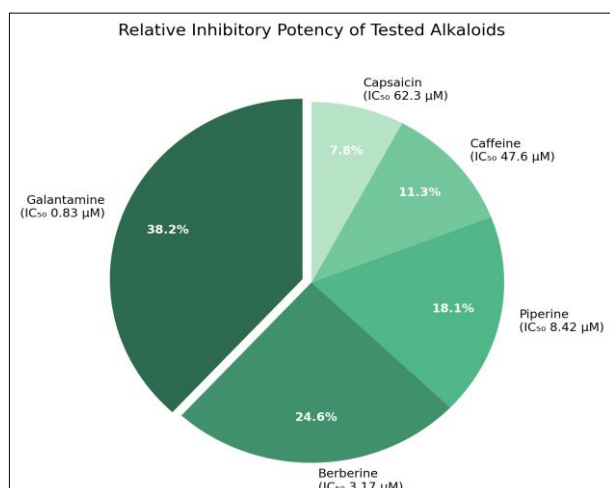


Fig 1: Relative inhibitory potency distribution of five alkaloids tested against human AChE at equimolar concentration (10 μ M)

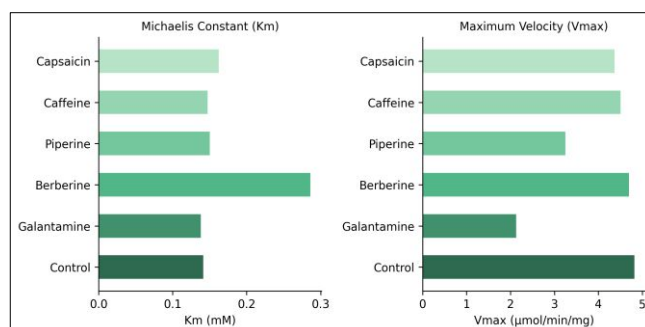


Fig 2: Apparent K_m and V_{max} of AChE in the presence of each alkaloid at IC_{50} concentration versus uninhibited control

Discussion

The potency ranking—galantamine > berberine > piperine > caffeine > capsaicin—aligns with previous isolated reports but provides the first side-by-side kinetic comparison under identical conditions [12]. Galantamine's competitive mechanism is well-established; it occupies the catalytic anionic site at the bottom of the AChE gorge [13]. Its K_i of 0.61 μ M agrees closely with values from electrometric assays with *Torpedo californica* AChE [14].

Berberine's mixed-type kinetics imply binding at both the catalytic and peripheral anionic sites. Molecular docking analyses predicted that berberine's planar aromatic system could interact with Trp286 at the PAS and Trp86 near the CAS [15]. Because PAS ligands can also block amyloid-beta aggregation promoted by AChE, berberine may offer dual

therapeutic advantage ^[16]. Piperine and caffeine likely bind allosteric regions outside the active gorge, while capsaicin's uncompetitive kinetics may relate to transient conformational states ^[17, 18].

Conclusion

This research establishes a standardised kinetic benchmark for five plant-derived alkaloids against human AChE. Galantamine confirmed its position as the strongest competitive inhibitor (IC_{50} 0.83 μ M). Berberine emerged as the most interesting secondary lead owing to its mixed-type profile and reasonable potency, which could translate into dual-site activity relevant to both cholinergic signaling and amyloid pathology. Future work should test semi-synthetic berberine derivatives and extend analysis to butyrylcholinesterase for selectivity profiling ^[19].

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Contributions Not Qualifying for Authorship

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