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Kinetics of Acetylcholinesterase Inhibition by an Aqueous Extract of *Mentha longifolia* Leaves

Chandra Shekhar, Suresh Kumar*

University School of Biotechnology, Guru Gobind Singh Indraprastha University, Sector 16C, Dwarka, Delhi 110075, India

ABSTRACT

Cholinesterase inhibitors are the class of compounds which inhibit cholinesterase enzyme. These are used as drugs for symptomatic treatment of Alzheimer's disease (AD). The present study, evaluate anti-cholinesterase property of an aqueous extract of *Mentha longifolia* leaves, which is an aromatic plant traditionally used for several medicinal properties. Ellman's method was used to determine the acetylcholinesterase (AChE) enzyme inhibitory activity of an aqueous extracts of *Mentha longifolia* leaves which showed concentration dependent AChE inhibition with maximum inhibition of 62.82 ± 0.005% at 25µg/ml final concentrations with IC_{50} value of 8.004µg/mL. The kinetic study using Lineweaver-Burk plot of an aqueous extract of *Mentha longifolia* leaves showed mixed non-competitive mode of inhibition against AChE. The anti AChE enzyme activity exhibited by an aqueous extracts of *Mentha longifolia* leaves extracts might be used in future for symptomatic treatment of AD.

Keywords: Acetylcholinesterase, *Mentha longifolia*, Inhibition, Kinetics, Ellman's method Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) is the most common type of dementia, leads to neurodegeneration over the period of time characterized by loss of memory, thinking akinesia, visuospatial disorientation and aphasia. [1-4] According to cholinergic hypothesis, acetylcholinesterase (AChE) enzymes are responsible for the catalytic hydrolysis of acetylcholine (ACh) into choline and acid in the central nervous system. [5-6] This hypothesis suggests that by blocking the hydrolysis of ACh by cholinesterase (ChE) enzyme will result in increased concentration of ACh in the central nervous system leads to improved cognitive functions. [5-8] ChE inhibitors are the drugs that prolong the existence of ACh after it is released from cholinergic nerve endings by inhibiting AChE. [5] Therefore, inhibition of AChE devised as a suitable strategy for providing symptomatic treatment to AD as well as other forms of dementia such as senile dementia, ataxia, myasthenia gravis and Parkinson's disease. [8] The synthetic drugs approved by FDA used in the treatment of AD such as tacrine, rivastigmine and donepezil success up to certain extent in slowing down neurodegeneration in AD suffering patients, are which includes disturbances in gastrointestinal tract, liver associated toxicity, aggression and depression. [9] Weekly blood monitoring and expensiveness further adds limitations of these drugs. All these limitations prompts an urgent need to look for new lead compound from different sources including plant based natural products, from which variety of phytoconstituents were previously reported for having ChEs inhibitory activities. [10] In the

***Corresponding author: Dr. Suresh Kumar,**
University School of Biotechnology, Guru Gobind Singh Indraprastha University, Sector 16C, Dwarka, New Delhi-110075, India; **E-mail:** sk222ind@yahoo.com
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present study, *Mentha longifolia* leaves were selected to study AChE inhibition and to explore the mode of inhibition by kinetic study. *Mentha longifolia* belongs to Lamiaceae family. Many species of this family are rich source of various natural AChE inhibitors and antioxidants that could be useful in the prevention and treatment of AD. [11] Previous ethnopharmacological study showed that this plant can be used in the treatment of some of the CNS disorders. [12]

MATERIALS AND METHODS

Chemicals and Reagents

Acetylthiocholineiodide (ATChI), acetylcholinesterase from electric eel (AChE) (EC 3.1.1.7), 5, 5- ditiobis [2-nitrobenzoic acid] (DTNB), sodium phosphate dibasic and sodium phosphate monobasic (Sigma Aldrich).

Plant Materials

The leaves samples of plant *Mentha longifolia* (Voucher no. CS/USBT004) were collected and authenticated by Botanist. The voucher specimens of this plant sample are stored in a herbarium at USBT, GGSIP University, Delhi, India.

Equipments and instruments

96-well plate (Corning Inc. NY), eppendorf tubes, centrifuge tubes, tips (Tarsons products Ltd. India), eppendorf tube stand, pipettes (Biomate), weighing balance, aluminium foil, tissue paper, ice box, blotting paper, vortex machine (REMI), magnetic stirrer (REMI) spatula, muslin cloth, spectrofluorometer (SpectraMex) centrifuge machine and lyophilizer (Heto).

Preparation of plant extract

The fresh sample of plant material was air dried at room temperature and powdered using electric grinder. 2 g of sample was weighed and extracted with 40 ml of distilled water. The sample was filtered using muslin cloth, which was then freeze dried in lyophilizer. Finally, the sample was collected and kept in -20°C. Percentage yield of sample extract was 21.5%.

Cholinesterase inhibitory assay

AChE inhibition was determined by the spectrophotometer using the Ellman's method with slight modification in other papers. [13-17] An assessment of cholinesterase inhibition was carried out in flat-bottom 96- well microtitre plates using the colorimetric method. A typical run consisted of 5µl of AChE solution, at final assay concentration of 0.08 U/ml; 200µl of 0.1 M phosphate buffer pH 8; 5µl of DTNB at a final concentration of 0.5mM prepared in 0.1 M phosphate buffer pH 7 containing 0.12 M of sodium bicarbonate; and 5µl of the test extract. The final assay concentration used for an aqueous extract of the plant material was 25µg/ml. The reactants were mixed and pre-incubated for 15 min at 30°C. The reaction was initiated by adding 5µl of ATChI at a final concentration of 0.5mM. As a control the inhibitor solution was replaced with buffer. The control was assayed in triplicate. To monitor any non- enzymatic hydrolysis in the reaction mixture two blanks for each run were prepared in triplicate. One blank consisted of buffer replacing enzyme and a second blank had buffer replacing substrate. Change in absorbance at 412 nm was measured on spectrophotometer, 96 well plate reader for a period of 2 min at 25°C.The reaction involved in this is enzyme hydrolyses the substrate ATChI resulting in the product thiocholine which reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which can be detected.

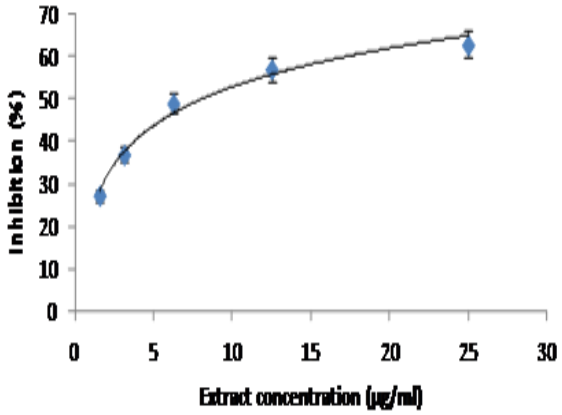

Fig 1: Percentage inhibition of AChE activity of different concentration of an aqueous extract of *Mentha longifolia* leaves. [The equation of the line is y=13.157ln(x) + 22.522; R^2=0.9844].

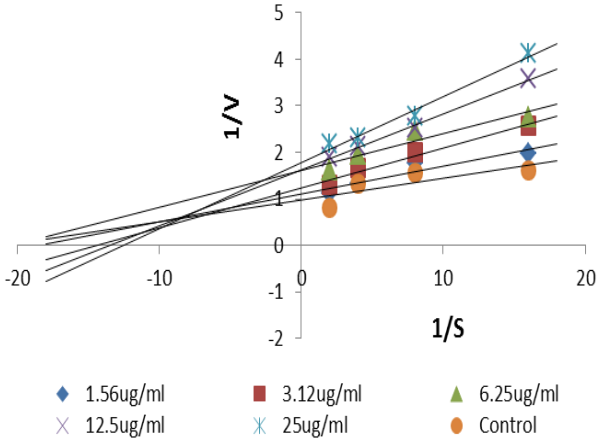

Fig 2: Lineweaver-Burk plot representing the reciprocal of initial enzyme velocity versus the reciprocal of acetylthiocholine iodide (ATChI) concentration in the presence and absence (control) of different concentrations of an aqueous extract of *Mentha longifolia* leaves.

RESULTS

The results showed that an aqueous extract of *Mentha longifolia* leaves showed concentration dependent inhibition against AChE at concentration ranging from 1.56 to 25µg/mL. The maximum inhibition of 62.82±0.005% was observed at 25µg/mL final assay concentration. The IC_{50} value calculated from the equation obtained from the concentration versus percentage inhibition curve was 8.004µg/mL (Figure 1). The mode of enzyme inhibition was derived from the Lineweaver-Burk (LB) plot between the reciprocal of substrate concentration on x-axis and reciprocal of velocity on y-axis. [15-17] The LB plot of an aqueous extract of *Mentha longifolia* leaves showed mixed non-

competitive inhibition kinetics as the intersection of lines occurred neither on x-axis or y-axis but nearby x-axis in the second quadrant (Figure 2).

DISCUSSION

Cholinesterase inhibitors are used as drugs for symptomatic treatment of AD. The limitations associated with FDA approved drugs such as tacrine, donepezil and rivastigmine are their side effects such as diarrhoea, nausea, vomiting, fatigue, insomnia, muscles cramps, loss of appetite and hepatotoxicity. [18] This prompted us to look for novel and safer compounds from natural sources which might have lesser side effects. In this regard, the present study showed that an aqueous extract of *Mentha longifolia* leaves significantly inhibited AChE enzyme in a concentration dependent manner. The mechanism of inhibition demonstrated by LB plot showed mixed non-competitive mode of inhibition. The results of present study is complementary to the previous studies that demonstrated anti-cholinesterase activity by an ethanolic extract of *Mentha longifolia* but the mode of inhibition kinetics was demonstrated for the first time by our study using an aqueous extract of this plant. Earlier reports also suggest that the variety of phytochemicals present in the medicinal plant extract also might be responsible for mixed inhibition kinetic behaviour. [19] Other species of *Mentha* demonstrated neuroprotective and neurochemical properties *in vivo.* [20] Various studies shows that AChE has beta-amyloid (Aβ) aggregating property which can be inhibited by mixed or non-competitive type of inhibitors due to their ability to bind to the peripheral anionic site of AChE, therefore, these can be used as a model candidate for inhibiting the AChE induced Aβ aggregation. [21] The mechanism of inhibition also revealed that the extract might compete with substrate for binding at substrate binding site of AChE or combined with enzyme (AChE) or with enzyme-substrate complex (AChE-ATChI). In case of high concentration of substrate the extract may bind to the secondary binding site of AChE. The AChE inhibition kinetics in the present study indicates a putative mechanism by which the aqueous extract may have a novel therapeutic potential for AD.

In conclusion, an aqueous extract of *Mentha longifolia* leaves showed significant anti acetylcholinesterase activity. Further studies are required to identify, isolate and characterize the phytoconstituents from an aqueous extract to find novel molecule which might be useful in alleviating the symptoms associated with AD.

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