KINETICS OF ACETYLCHOLINESTERASE INHIBITION BY AN AQUEOUS EXTRACT OF CUMINUM CYMINUM SEEDS

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Abstract
The cholinergic hypothesis of Alzheimer’s disease (AD) has provided the rationale for the current pharmacotherapy of this disease. Acetylcholinesterase (AChE) inhibitors are currently the only approved therapy for the symptomatic treatment of AD. The current drugs available in the market has shown various side effect which prompted scientist to search for new and potent AChE inhibitors which exerts minimal side effect in AD patient. In present study, an aqueous extract of Cumin cyminum was tested for in vitro acetylcholinesterase inhibitory activity based on Ellman’s method. C. cyminum showed maximum inhibition of 76.90±0.003% in an aqueous extract at 50µg/ml final concentration. Further studies were conducted to elucidate the mode of AChE inhibition by kinetic studies. Competitive inhibition was observed at lower concentrations (12.5µg/ml & 25µg/ml) and mixed inhibition was observed at higher concentrations (50µg/ml & 100µg/ml).

Keywords: Acetylcholinesterase; Cumin cyminum; kinetics; Alzheimer’s diseases

Introduction
Alzheimer’s disease (AD) is a form of dementia, which describes a group of symptoms associated with a progressive decline of brain functions, such as memory, understanding, judgement, language and thinking (Alzheimer’s association, 2012). It is estimated that over 3.7 million people are affected by dementia in India and the number will increase to two fold by 2030 and threefold by 2050 (Shaji et al., 2010; Alzheimer’s association, 2013). The cognitive impairment in case of AD is mainly due to the death of cholinergic neurons in central nervous system which leads to the deficit of neurotransmitter acetylcholine (ACH) (Coyle et al., 1983; Liston et al., 2004; Perry et al., 1990). The current available drugs for the treatment of AD are focused on to enhance the level of neurotransmitter specifically ACh in brain by inhibiting enzyme acetylcholinesterase (AChE). Inhibition of AChE can also be helpful in case of other forms of dementia like senile dementia, ataxia, myasthenia gravis and Parkinson’s disease (Brenner et al., 2008; Hirano et al., 2008; Inestro et al., 2008). The current drugs available for symptomatic treatment of AD are associated with number of side effects, low bioavailability, high cost and requirement of weekly blood monitoring (Ames et al., 1988; Melzer et al., 1988; Inglis et al., 2002; Jann et al., 2002). In view of this, there is an urgent need for effective drugs to replace or supplement those in current use. In the last decade many studies have suggested the discovery of new lead molecules from natural sources specifically plants, which have shown AChE inhibitory activity (Mukherjee et al., 2007).

Cumin (Cuminum cyminum Linn.) is an important commercial seed spice belonging to the umbelliferae family. It is valued for aroma and its medicinal and therapeutic properties like anti-inflammatory, antibacterial activity, antioxidant and antihyperglycemic effects (Bakhru et al., 2001; Agnihotri et al., 1996; Dhandapani et al., 2002; Roman-Romas et al., 1995; Satyanarayan et al., 2004). Previous in-vivo study showed memory-enhancing activity of C. cyminum in normal and scopolamine-induced amnestic rats, supporting for the antistress, antioxidant, and memory-enhancing activities of C. cyminum extract (Koppula et al., 2011). In one of our previous study an aqueous extract of C. cyminum seed has shown a strong inhibitory activity for AChE in-vitro (Kumar et al., 2012). The present study explored the mechanism of action by kinetic study using Lineweaver berk plot by an aqueous extract of C. cyminum seed.

Materials and methods

Chemicals
Acetylcholinesterase (EC 3.1.1.7) from bovine erythrocytes, acetylthiocoline iodide (ATChI), 5,5-dithiobis-2-nitrobenzoic acid (DTNB) and sodium bicarbonate were purchased from Sigma Aldrich, India.
Acetylthiocholine iodide (ATChI); 5, 5’-dithio-bis-(2-nitrobenzoic acid) (DTNB); sodium bicarbonate were purchased from Himedia Laboratories Pvt. Ltd., India. Phosphate buffer and ethanol were obtained from Sisco Research Laboratories Pvt. Ltd. India.

**Plant material and extraction**
The seeds of *C. cyminum* were purchased from a local store in Delhi, India. The seeds were authenticated by a local botanist and a voucher specimen (USBT/SK/CC01) of *C. cyminum* was stored in the herbarium at University School of Biotechnology, Guru Gobind Singh Indraprashras University, Dwaraka Sec- 16C, New Delhi-110075.

Seeds were air dried at ambient room temperature and powdered in a grinder. One gram of cumin seed was weighed and extracted with distilled water (1:25 w/v) and 90% ethanol. The sample was boiled for 15-20 minutes and then cooled to room temperature. The sample was filtered using muslin cloth and the filtrate was lyophilized. The lyophilized samples were collected and stored at -20°C until use.

**Cholinesterase assays**
An assessment of cholinesterase inhibition was carried out in flat-bottom 96-well microtitre plates using the colorimetric method of Ellman et al., 1961 as adapted by Okello et al., 2004. A typical run consisted of 5μL of bovine AChE solution, at final assay concentrations of 0.03 U/mL; 200μL of 0.1 M phosphate buffer pH 8; 5μL of DTNB at a final concentration of 0.3mM prepared in 0.1 M phosphate buffer pH 7 with 0.12M of sodium bicarbonate; and 5μL of the cumin seed extract. 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 a SpectraMax M2, 96-well plate reader for a period of 6 min at 30°C.

**Determination of dose response curve and kinetic parameters**
The concentration of *C. cyminum* extract that inhibited the hydrolysis of substrate by 50% (IC50) was determined by monitoring the effect of various concentrations ranging from 12.5 to 50μg/ml (final assay concentration). Each concentration was run equivalent to n = 6. Dose-response curves were plotted using Microsoft Excel software and IC50 value was calculated from the standard curve equation. For inhibition kinetics studies, the enzyme was pre-incubated with different substrate concentrations ranging from 0.125mM to 1mM. The data for substrate kinetics were analyzed using Lineweaver–Burk methods for the determination of Km and Vmax.

**Results**
In the present study, the results indicated that an aqueous extract of *C. cyminum* seeds inhibited AChE in a concentration-dependent manner. A maximum inhibition of 76.90±0.003% was observed at the final assay concentration of 50μg/ml. The IC50 value (0.437μg/ml) calculated from the equation obtained from the log concentration versus inhibition curve (Fig. 1).

The Lineweaver-Burk plot for aqueous extract of *C. cyminum* showed that at lower concentrations (12.5μg/ml and 25μg/ml) competitive inhibition was observed. At higher concentration (50μg/ml and 100μg/ml) mixed mode of inhibition was observed (Fig. 2 and 3).

![Fig. 1: Concentration-dependent AChE inhibition by an aqueous extract of *C. cyminum* seed.](image_url)
Fig. 2: Lineweaver-burk plot at 12.5µg/ml 25µg/ml concentrations of *C. cyminum* aqueous extract representing Anticholinesterase activity.

Fig. 3: Lineweaver-burk plot at 50µg/ml and 100µg/ml concentrations of *C. cyminum* aqueous extract representing anticholinesterase activity.

**Discussion**

Currently, there are no drugs available in market that can cure, reverse or halt AD but few drugs are available such as rivastigmine, neostigmine, physostigmine and pyridostigmine that can only provide symptomatic relief to AD patients. The major limitation associated with these drugs are their side effects such as hepatotoxicity, gastrointestinal disturbances, problems associated with bioavailability, short half-life and systemic cholinergic actions (Watkins et al., 1994; Coelho filho et al., 2001; Bores et al., 1996; Forette et al., 1999). Therefore, there is an urgent requirement of novel anticholinesterase drugs.
AChE play important role in development of plaque formation by accelerating the formation of \(\beta\)-amyloid peptide deposition in the brain cells. It has also been shown that AChE forms stable complex with plaques through anionic sites which leads to the formation of fibrils (Inestrosa et al., 1996; De Ferrari et al., 2001). It has been suggested that mixed inhibitors could be one of the best candidate for inhibiting AChE induced \(\beta\)-amyloid aggregation due to their ability to bind peripheral sites (Bartolini et al., 2003). This hypothesis supports that the higher concentration (50\(\mu\)g/ml & 100\(\mu\)g/ml) of C. cuminum might be useful in inhibiting AChE induced \(\beta\)-amyloid aggregation. Previous study showed that C. cuminum possessed memory enhancing, antistress and antioxidant activity (Koppula et al., 2011). Our finding of anticholinesterase activity complementary with the previous studies. Therefore, these observation support the fact that the aqueous extract of C. cuminum used in the current study may have therapeutic potential.

**Conclusion**

In this study, we have shown for the very first time that an aqueous extract of C. cuminum seed inhibited AChE in concentration dependent manner. Also, at lower concentration and higher concentration, C. cuminum exhibited competitive and mixed mode of inhibition respectively. These results illustrates the fact that C. cuminum can acts as an inhibitor of AChE, helpful in enhancing memory and other cognitive functions of brain. Further studies are required for isolation and characterisation of phytoconstituent from an aqueous extract of C. cuminum, which might be useful in future for treatment and cure of AD.

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**References**


