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Research Article

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

Suresh Kumar\* and Suman Chowdhury

University School of Biotechnology, Guru Gobind Singh Indraprastha University, Dwarka, New Delhi

\*Corresponding author email: [sk222ind@yahoo.com](mailto:sk222ind@yahoo.com)

**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  $76.90 \pm 0.003\%$  in an aqueous extract at  $50 \mu\text{g/ml}$  final concentration. Further studies were conducted to elucidate the mode of AChE inhibition by kinetic studies. Competitive inhibition was observed at lower concentrations ( $12.5 \mu\text{g/ml}$  &  $25 \mu\text{g/ml}$ ) and mixed inhibition was observed at higher concentrations ( $50 \mu\text{g/ml}$  &  $100 \mu\text{g/ml}$ ).

**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 umbellifereae family. It is valued for aroma and its medicinal and therapeutic properties like anti-inflammatory, antibacterial activity, antioxidant and antihyperglycemic effects (Bakhrum *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 amnesic 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, acetylthiocholine 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. cuminum* 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. cuminum* was stored in the herbarium at University School of Biotechnology, Guru Gobind Singh Indraprastha University, Dwarka 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  $-20^{\circ}\text{C}$  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 5  $\mu\text{L}$  of bovine AChE solution, at final assay concentrations of 0.03 U/mL; 200  $\mu\text{L}$  of 0.1 M phosphate buffer pH 8; 5  $\mu\text{L}$  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 5  $\mu\text{L}$  of the cumin seed extract. The reactants were mixed and pre-incubated for 15 min at  $30^{\circ}\text{C}$ . The reaction was initiated by adding 5  $\mu\text{L}$  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  $30^{\circ}\text{C}$ .

#### Determination of dose response curve and kinetic parameters

The concentration of *C. cuminum* extract that inhibited the hydrolysis of substrate by 50% ( $\text{IC}_{50}$ ) was determined by monitoring the effect of various concentrations ranging from 12.5 to 50  $\mu\text{g}/\text{ml}$  (final assay concentration). Each concentration was run equivalent to  $n = 6$ . Dose-response curves were plotted using Microsoft Excel software and  $\text{IC}_{50}$  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  $K_m$  and  $V_{\text{max}}$ .

#### Results

In the present study, the results indicated that an aqueous extract of *C. cuminum* seeds inhibited AChE in a concentration-dependent manner. A maximum inhibition of  $76.90 \pm 0.003\%$  was observed at the final assay concentration of 50  $\mu\text{g}/\text{ml}$ . The  $\text{IC}_{50}$  value (0.437  $\mu\text{g}/\text{ml}$ ) calculated from the equation obtained from the log concentration versus inhibition curve (Fig. 1).

The Lineweaver-Burk plot for aqueous extract of *C. cuminum* showed that at lower concentrations (12.5  $\mu\text{g}/\text{ml}$  and 25  $\mu\text{g}/\text{ml}$ ) competitive inhibition was observed. At higher concentration (50  $\mu\text{g}/\text{ml}$  and 100  $\mu\text{g}/\text{ml}$ ) mixed mode of inhibition was observed (Fig. 2 and 3).

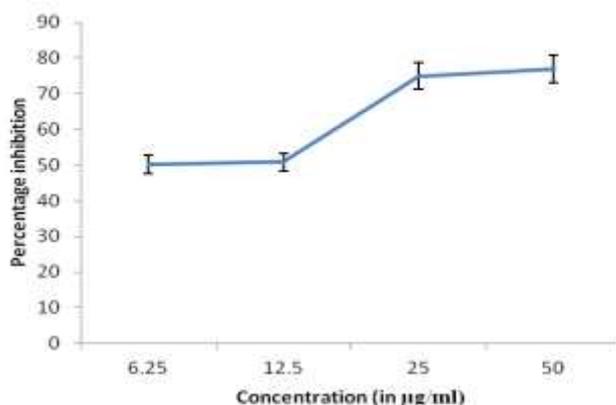


Fig. 1: Concentration-dependent AChE inhibition by an aqueous extract of *C. cuminum* seed.

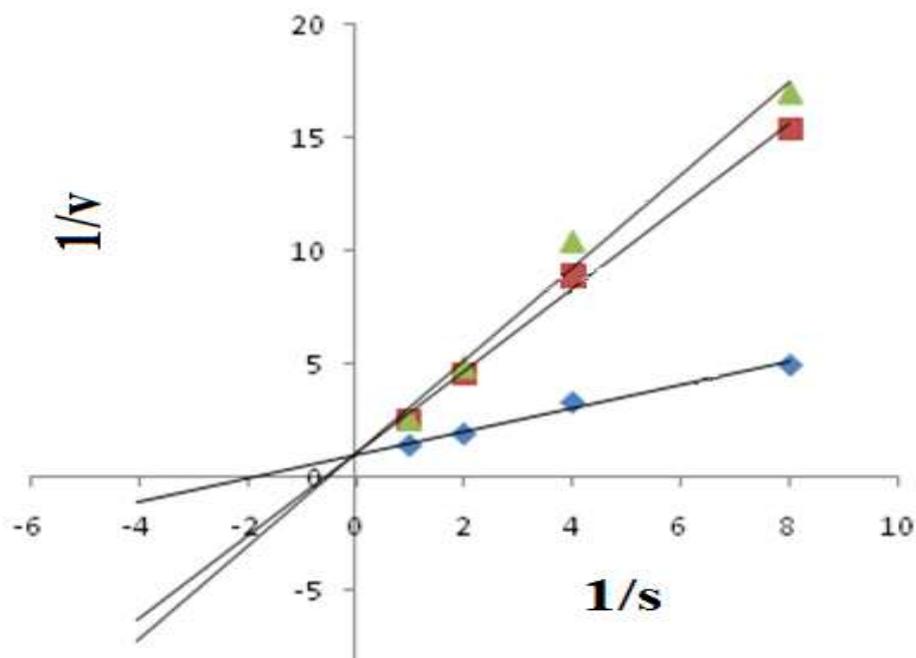


Fig. 2: Lineweaver-burk plot at 12.5µg/ml 25µg/ml concentrations of *C. cynimum* aqueous extract representing Anticholinesterase activity.

(♦ 1/c, ■ 1/v1, ▲ 1/v2)

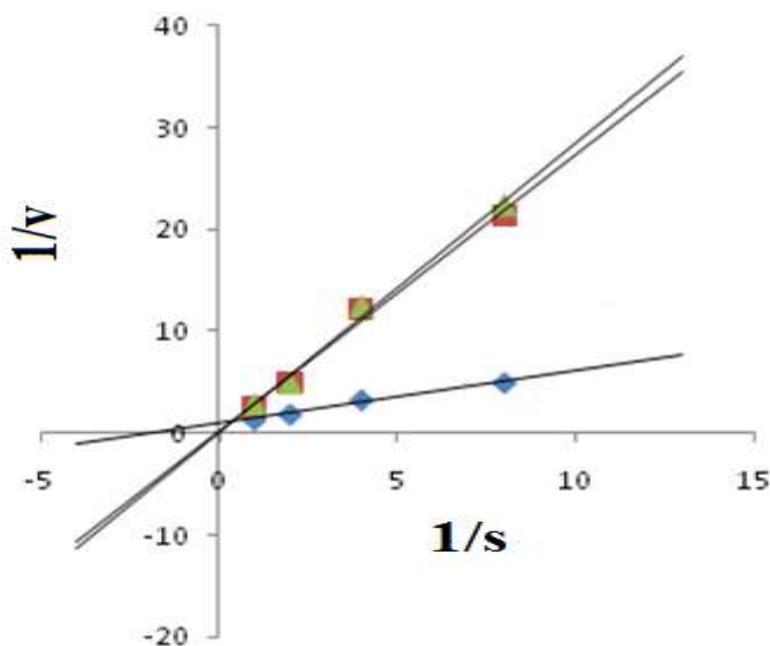


Fig. 3: Lineweaver-burk plot at 50µg/ml and 100µg/ml concentrations of *C. cynimum* aqueous extract representing anticholinesterase activity.

(♦ 1/c, ■ 1/v3, ▲ 1/v4)

### 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. cyminum* might be useful in inhibiting AChE induced  $\beta$ -amyloid aggregation. Previous study showed that *C. cyminum* 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. cyminum* 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. cyminum* seed inhibited AChE in concentration dependent manner. Also, at lower concentration and higher concentration, *C. cyminum* exhibited competitive and mixed mode of inhibition respectively. These results illustrates the fact that *C. cyminum* 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. cyminum*, which might be useful in future for treatment and cure of AD.

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