OPEN ACCESS

International Journal of Applied Sciences and Biotechnology

A Rapid Publishing Journal

ISSN 2091-2609

Available online at:

http://www.ijasbt.org

&

http://www.nepjol.info/index.php/IJASBT/index

Indexing and Abstracting

CrossRef, Google Scholar, Global Impact Factor, Genamics, **Index Copernicus**, **Directory of Open Access Journals**, International Society of Universal Research in Sciences (EyeSource), Journal Seeker, Socolar, BioRes, Indian Science, Jadoun Science, Jour Informatics, Journal Directory, JournalTOCs, Academic Journals Database, Journal Quality Evaluation Report, PDOAJ, Science Central, Journal Impact Factor, NewJour, Open Science Directory, DRJI, Global Institute of Scientific Information, Open Access Library, IIJS, Sciseek, Cabell's Directories, Scientific Indexing services, CiteFactor, Unisa, InfobaseIndex, Infomine, Getinfo, Open Academic Journals Index etc.

SEM-Biotech Publishing

IC Value: **4.37**

For any type of query and/or feedback don't hesitate to email us at: editor.ijasbt@gmail.com

Kumar and Chowdhury (2014) Int J Appl Sci Biotechnol, Vol 2(1): 64-68
DOI: 10.3126/ijasbt.v2i1.9348

ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt

International Journal of Applied Sciences and Biotechnology (IJASBT)

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

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: Acetylcholinestrase; *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 (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, acetylthiolcholine 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 Indraprasha 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°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% (IC_{50}) 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 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 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 IC_{50} 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).

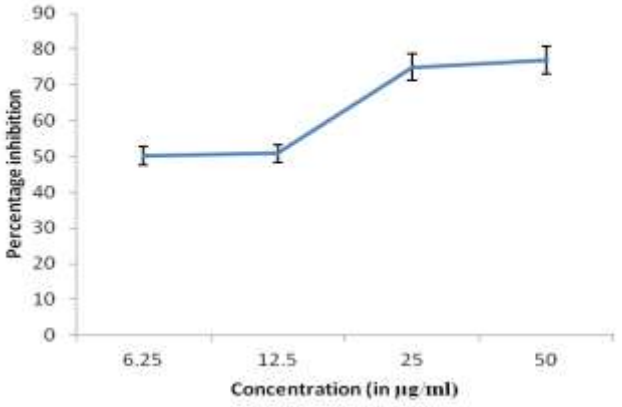


Fig. 1: Concentration-dependent AChE inhibition by an aqueous extract of *C. cyminum* seed.

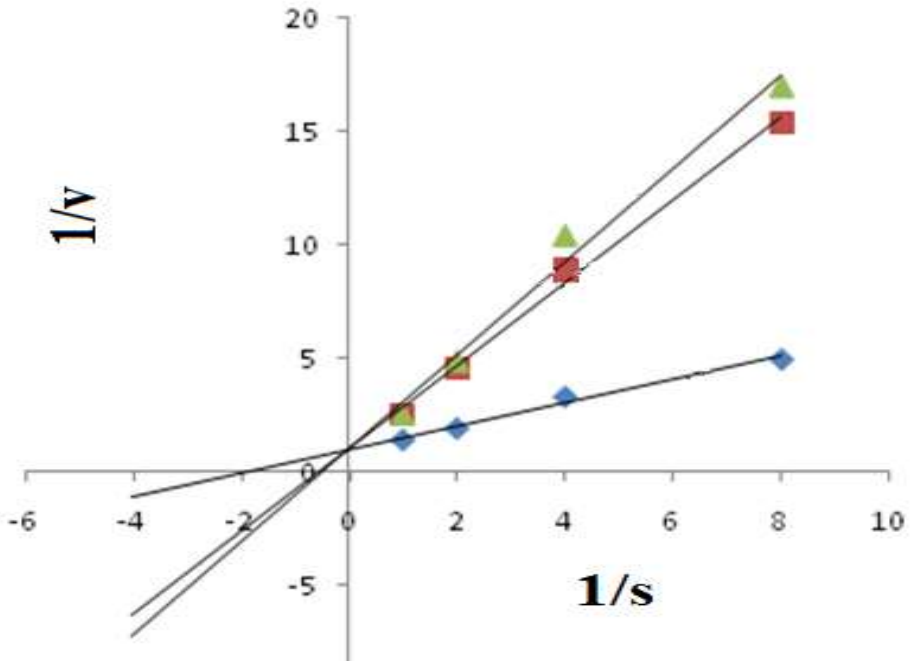


Fig. 2: Lineweaver-burk plot at 12.5µg/ml 25µg/ml concentrations of *C. cyminum* aqueous extract representing Anticholinesterase activity.

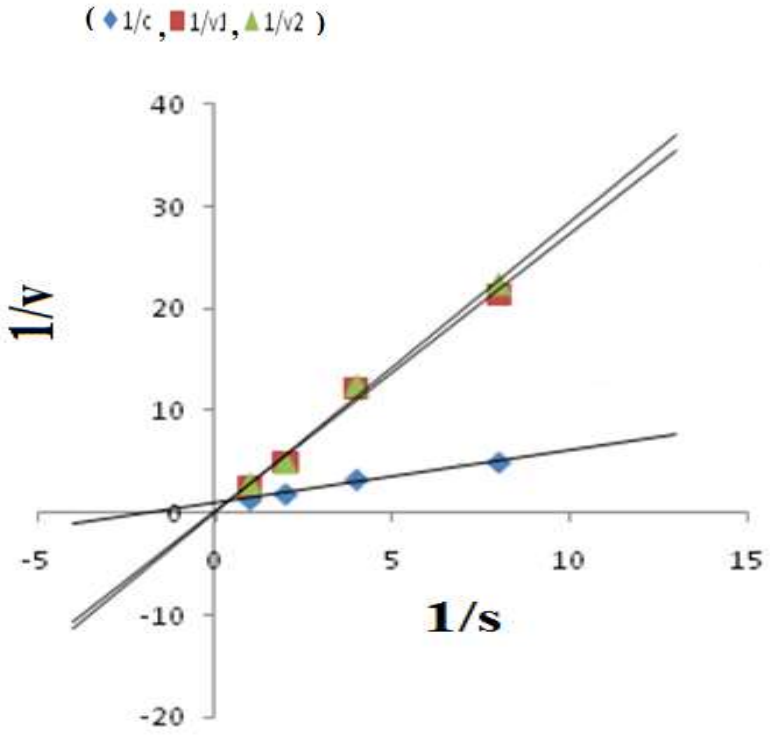
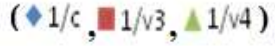


Fig. 3: Lineweaver-burk plot at 50µg/ml and 100µg/ml concentrations of *C. cyminum* aqueous extract representing anticholinesterase activity.



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 β- 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 β-amyloid aggregation due to their ability to bind peripheral sites (Bartolini *et al.,* 2003). This hypothesis supports that the higher concentration (50µg/ml & 100µg/ml) of *C. cyminum* might be useful in inhibiting AChE induced β-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.

Acknowledgement

The study was supported by grants from University Grant Commission (UGC, MRP), India.

References

Agnihotri S and Vaidya (1996) ad: A Novel Approach to Study Antibacterial Properties of Volatile Components of Selected Indian Medicinal Herbs. *Ind .J. Exp. Biol.* **134**: 712–715.

Alzheimer's Association (2013) Alzheimer's Disease Facts and Figures. *Alzheimer's & Dementia*, **9**: 2.

Alzheimer's Association (2012) Alzheimer's Disease Facts and Figures, *Alzheimer's & Dementia,* **8**: 2.

Ames DJ, Bhathal PS, Davies BM and Fraser JR (1988) Hepatotoxicity of tetrahydroaminoacridine. Lancet. **1**: 887. DOI: 10.1016/S0140-6736(88)91636-4

Bakhru HK (2001) Herbs that heal: Natural Remedies for good health. orient paperbacks.Vision books pvt ltd., New Delhi, India.

Bartolini M, Bertucci C, Cavrini V and Andrisano V (2003) Beta-Amyloid aggregation induced by human acetylcholinesterase: inhibition studies. *Biochemical Pharmacology*, **65**: 407-416. DOI: 10.1016/S0006-2952(02)01514-9

Bores GM, Huger FP, Petko W, Mutalib AE, Camacho F, Rush DK, Selk DE, Wolf V, Kosley RW, Davis L and Vargas HM (1996) Pharmacological evaluation of novel Alzheimer's disease therapeutics: acetylcholinesterase inhibitors related to galanthamine. *J. Pharmacol. Exp. Ther.* **277**: 728-738.

Brenner T, Nizri E, Irony-Tur-Sinai M, Hamra-Amitay Y and Wirguin I (2008) Acetylcholinesterase inhibitors and cholinergic modulation in Myasthenia Gravis and neuroinflammation. *Journal of Neuroimmunology*. **15**: 121-127. DOI: 10.1016/j.jneuroim.2008.05.022

Coelho Filho JMJMC and Birks J (2001) Physostigmine for dementia due to Alzheimer's disease. *Cochrane Database of Systematic Reviews.* **2**.

Coyle JT, Price DL and DeLong MR (1983) Alzheimer's disease: a disorder of cortical cholinergic innervations. *Science.* **219**: 1184–1190. DOI: 10.1126/science.6338589

De Ferrari GV, Canales MA, Shin I, Weiner LM, Silman I and Inestrosa NC (2001) A structural motif of acetylcholinesterase that promotes amyloid beta-peptide fibril formation. *Biochemistry*. **40**: 10447-57. DOI: 10.1021/bi0101392

Dhandapani S, Subramanian VR, Rajagopal S, and Namasivayam N(2002) Hypolipidemic effect of *Cuminum cyminum* L. on alloxan-induced diabetic rats. *Pharmacol Res.* **46:** 251–255. DOI: 10.1016/S1043-6618(02)00131-7

Ellman GL, Courtney KD, Andres V Jr and Feather-Stone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology.* **7**: 88-95. DOI: 10.1016/0006-2952(61)90145-9

Forette F, Anand R and Gharabawi G (1999) A phase II study in patients with Alzheimer's disease to assess the preliminary efficacy and maximum tolerated dose of rivastigmine (ExXelon). *Eur. J. Neurol.* **6**: 423-429. DOI: 10.1016/0006-2952(61)90145-9

Hirano S, Shinotoh H, Arai K, Aotsuka A, Yasuno F, Tanaka N, Ota T, Sato K, Fukushi K, Tanada S, Hattori T and Irie T (2008) PET study of brain acetylcholinesterase in cerebellar degenerative disorders. *Movement Disorders*. **23**: 1154-1160. DOI: 10.1002/mds.22056

Inestrosa NC, Dinamarca MC and Alvarez A (2008) Amyloid-cholinesterase interactions- Implications

for Alzheimer's disease. *FEBS Journal*. **275**: 625-632. DOI: 10.1111/j.1742-4658.2007.06238.x

Inestrosa NC, Alvarez A, Pérez CA, Moreno RD, Vicente M, Linker C, Casanueva OI, Soto C and Garrido J (1996) Acetylcholinesterase accelerates assembly of amyloid-beta-peptides into Alzheimer's fibrils: possible role of the peripheral site of the enzyme. *Neuron.***16**: 881-891. DOI : 10.1016/S0896-6273(00)80108-7

Inglis F (2002) The tolerability and safety of cholinesterase inhibitors in the treatment of dementia. *International Journal Clinical Practice Supplement*. **127**: 45-63.

Jann MW, Shirley KL and Small GW (2002) Clinical pharmacokinetics and pharmacodynamics of cholinesterase inhibitors. *Clinical Pharmacokinetics*. **41**: 719-739. DOI: 10.2165/00003088-200241100-00003

Koppula S and Choi DK (2011) *Cuminum cyminum* extract attenuates scopolamine-induced memory loss and stress- induced urinary biochemical changes in rats: a non-invasive biochemical approach. *Pharm. boil*. **49**:702-8. DOI: 10.3109/13880209.2010.541923

Kumar S, Brijeshlata and Dixit S (2012) Screening of traditional Indian spices for inhibitory activity of Acetylcholinesterase and Butyrylcholinesterase enzymes, *International Journal of Pharma and Bio Sciences* **3**:59.

Liston DR, Nielsen JA, Villalobos A, Chapin D, Jones SB, Hubbard ST, Shalaby IA, Ramirez A, Nason D, White WF (2004) Pharmacology of selective acetylcholinesterase inhibitors: implications for use in Alzheimer's disease. *Eur. J. Pharmacol*. **486**: 9–17. DOI: 10.1016/j.ejphar.2003.11.080

Melzer D (1998) New drug treatment for Alzheimer's disease: lessons for healthcare policy. *British Medical Journa*. **316**: 762-764. DOI: 10.1136/bmj.316.7133.762

Mukherjee PK, Kumar V, Mal M and Houghton PJ (2007) Acetylcholinesterase inhibitors from plants. *Phytomedicine.* **14**: 289-300.

Okello EJ, Savelev SU and Perry EK (2004) *In vitro* anti-beta-secretase and dual anti-cholinesterase activities of *Camellia sinensis* L. (tea) relevant to treatment of dementia. *Phytotherapy Research.***18**: 624-627. DOI: 10.1002/ptr.1519

Perry E, Walker M, Grace J, Perry R (1990) Acetylcholine in mind: a neurotransmitter correlate of consciousness? *TINS*, **22**: 273–280. DOI: 10.1016/S0166-2236(98)01361-7

Roman-Ramos R, Flores-Saenz JL, and Alarcon-Aguilar FJ(1995) Antihyperglycemic effect of some edible plants. *J. Ethnopharmacol.* **48:** 25–32. DOI: 10.1016/0378-8741(95)01279-M

Satyanarayana S, Sushruta K, Sarma GS, Srinivas N and Subba Raju G V (2004) Antioxidant activity of the aqueous extracts of spicy food additives–evaluation and comparison with ascorbic acid in *in vitro* systems. *Journal of Herbal Pharmacotherapy* **4**: 1–10.

Shaji KS, Jotheeswaran AT, Girish N, Srikala Bharath, Amit Dias, Meera Pattabiraman and Mathew Varghese (2010) The Dementia India Report: prevalence, impact, costs and services for Dementia: Executive Summary. ARDSI, New Delhi. ISBN

Watkins PB, Zimmerman HJ, Knapp MJ, Gracon SI, and Lewis KW (1994) Hepatotoxic effects of tacrine administration in patients with Alzheimer's disease. *JAMA* **271**: 992–998. DOI: 10.1001/jama.1994.03510370044030