ANTI-CHOLINESTERASE ACTIVITY OF SELECTED INDIAN MEDICINAL PLANTS AND THEIR MODE OF INHIBITION BY KINETIC STUDIES

Suresh Kumar
University School of Biotechnology
GGS Indraprastha University, Dwarka sector 16C, New Delhi
Annie Ankita
University School of Biotechnology
GGS Indraprastha University, Dwarka sector 16C, New Delhi

Abstract: The central nervous system of mammals contains two major form of cholinesterase enzymes namely acetylcholinestrase (AChE) and butrylcholinestrase (BuChE). AChE is primarily located within neuronal axons and cell bodies whereas BuChE has a neuroglial cell distribution as well as a presence in neuritic plaques and tangles in Alzheimer's disease (AD). Both AChE and BuChE are involved in the breakdown of the neurotransmitter acetylcholine (ACh). The dual inhibition of these enzymes is a primary strategy for the treatment of neurological disorder such as AD, senile dementia, ataxia and myasthenia gravis. Cholinesterase inhibitors are the class of compounds which inhibit cholinesterase enzyme like AChE and BuChE found in the central nervous system. The present study was carried out for determination of BuChE inhibitory activity by Ellman's method. Screening of anticholinesterase activity of ethanolic extracts of *Pistacia vera, Anacardium occidentale, Prunus dulsis, Juglans regia, Convolvulus pluricaulis, Eclipta elba, Terminalia arjuna, Moringa oleifer, and Terminalia chebula* was determined in the present study. The current demonstrated that an ethanolic extract of *Terminalia chebula*, seed showed maximum inhibitory activity of 71.2 ± 0.005 % against BuChE and the IC_{50} value of *Terminalia chebula* is 16.216µg/ml. The Lineweaver-Burk plot of ethanolic extract of *Terminalia chebula* showed mixed and non-competitive inhibition kinetics. This anti-cholinesterase activity shown by *Terminalia chebula* might be useful in future for symptomatic treatment of AD.

Introduction: Acetylcholine (ACh) and Butyrylcholine (BuCh) are the important neurotransmitters of central nervous system (CNS) associated with memory and cognition. Deficit of neurotransmitters levels in CNS leads to conditions such as Alzheimer's disease (AD). AD results in loss of mental ability, severe enough to interfere with normal activities of daily living and decline in cognitive functions such as remembering, reasoning and planning. It is characterized by deficiencies of several neurotransmitters such as acetylcholine (ACh) and butyrylcholine (BuCh), which are essential for the transmission of nerve messages [1]. Cholinesterase represents the group of enzymes namely acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) responsible for the catalytic hydrolysis of ACh and BuCh in the CNS, are needed for the proper functioning of the nervous system. [2] The difference between them is their substrates specificity, AChE hydrolyse ACh whereas BuChE hydrolyse BuCh and thus blocks neural transmission. [3] AChE has a major role in healthy brain but BuChE play a minor role in regulating ACh levels however, the progressive increase of BuChE activity is found in AD whereas, the activity of AChE remains unchanged or declines. [4]

Materials and Methods: ***Chemicals:*** Butyrylcholinesterase, butyrylthiocholine iodide (BTChI), 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB); sodium bicarbonate, Phosphate buffer.
Plant Materials: *Convolvulus pluricaulis, Eclipta elba, Pistacia vera, Anacardium occidentale, Prunus dulcis, Juglans regia, Terminalia arjuna, Moringa oleifera and Terminalia chebula.* Voucher specimen were verified by taxonomist and stored in the laboratory.
Cholinesterase Assay: An assessment of cholinesterase inhibition was carried out in flat-bottom 96-well microtitre plates using the Ellman's method. [5] BuChE inhibition was determined by spectroflurometer which was carried out in flat bottom 96 well microtitre plate using the colorimetric method. A typical run consisted of 5µL of electric eel BuChE solution, at final assay concentrations of 0.03 U/mL; 200µL of 0.1 M phosphate buffer pH 7; 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 test extract .The reactants were mixed and pre incubated for 15 minutes at 30°C. The reaction was initiated by adding 5µL of BuChI at a final concentration of 0.5mM. As a control the inhibitor solution was replaced with buffer. All the reactions were carried out in triplicate (n=3). 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 spectroflurometer, 96-well plate reader for a period of 6 min at 30 °C. The reaction involved in this is hydrolysis of the substrate butyrylthiocholine 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.

Result: Aqueous and ethanolic extracts of selected plants were screened for BuChE inhibitory activity. Most of the plant extracts displayed significant inhibition against BuChE as compared to the controlled sample. The maximum inhibition was shown by *Terminalia chebula* ethanolic extract.

Screening of Selected Plant Sample: The present data revealed that the ethanolic and aqueous extracts possessed potent BuChE inhibitory activity at 200µg/ml final concentration. Among the plant screened, an ethanolic extract of *Terminalia chebula* showed the maximum inhibition of 71.2 ± 0.005 % BuChE showed in Table 1 & 2.

Table 1: Percentage inhibition of ethanolic extracts against BuChE

S.No.	Sample Name	% BuChE Inhibition (Ethanolic extract) at 200 µg/ml
1	*Pistacia vera (pista)*	34 ± 0.05
2	*Anacardium occidentale (cashew)*	42.6 ± 0.028
3	*Prunus dulcis (almond)*	41.2 ± 0.0042
4	*Juglans regia (walnut)*	45.3 ± 0.004
5	*Convolvulus pluricaulis (shankpuspi)*	60.7 ± 0.031
6	*Eclipta elba (bhringraj)*	64 ± 0.042
7	*Terminalia arjuna ,leaves (arjuna)*	50 ± 0.029
8	*Terminalia arjuna, stem (arjuna)*	9.49 ± 0.035
9	*Terminalia arjuna, bark (arjuna)*	51 ± 0.004
10	*Moringa oleifer, fruit (drumstick)*	47 ± 0.03
11	*Moringa oleifera, leaves (drumstick)*	53 ± 0.0025
12	*Terminalia chebula*, seed (myrobalan)	71.2 ± 0.005

Data expressed as mean ± SDEV (n=3)

Table 2: Percentage Inhibition of Aqueous Extracts against BuChE

S.No.	Samples	% Inhibition (aqueous extract) at 100 µg/ml
1	*Pistacia vera*	25.8 ± 0.006
2	*Anacardium occidentale*	37.2 ± 0.047
3	*Prunus dulcis*	40 ± 0.053
4	*Juglans regia*	39.6 ± 0.031
5	*Convolvulus pluricaulis*	44.6 ± 0.02
6	*Eclipta elba*	55 ± 0.004

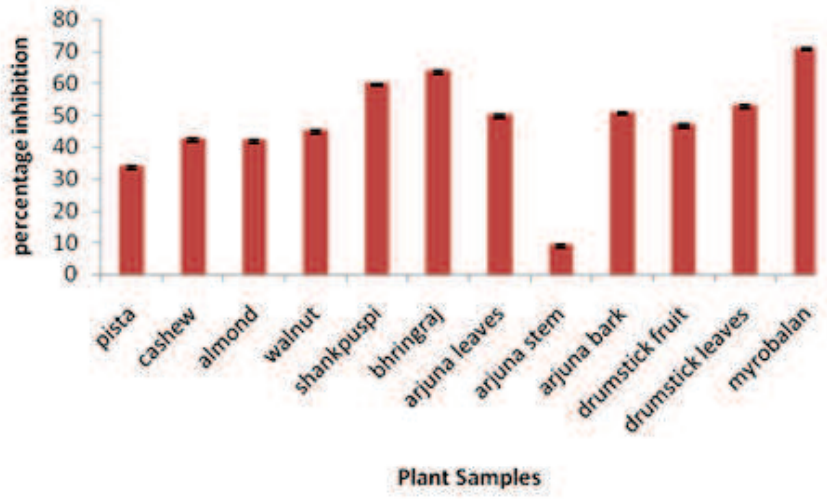

Figure 1: Percentage Inhibition Shown by Plant Extracts against BChE Enzyme

Concentration dependent cholinesterase inhibition by ethanolic extract of *Terminalia chebula:* The results showed that an ethanolic extract of *Terminalia chebula* inhibited BuChE in a concentration-dependent manner and the maximum inhibition was observed at the final assay concentration of 200µg/mL for the enzymes (Table 6).

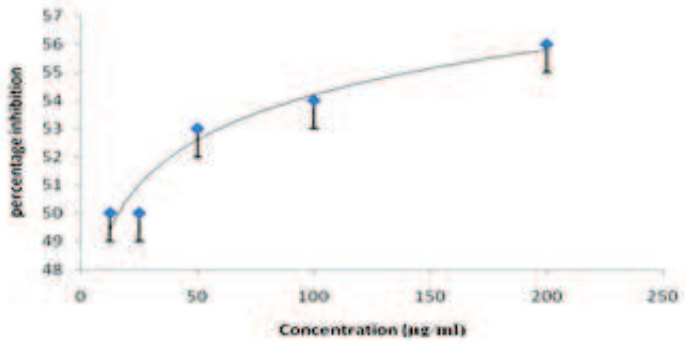

Figure 2: Percentage Inhibition of BuChE Activity at Different Concentration of *Terminalia Chebula* for BuChE [y=2.3083In(x) +43.57 and R^2 = 0.9412]

Study the Mode of Enzyme Inhibition by Lineweaver - Burk Plot: The Lineweaver- Burk plot of *Terminalia chebula* shows that extracts followed non competitive inhibition kinetics (Figure: 4) and their Km and Vmax value are mentioned in Table 3.

Concentration of an ethanolic extract of *Terminalia chebula*	Maximum velocity, Vmax (mM)	Michealis-Menton constant, Km (mM)
200 µg/ml	0.385	0.431
100 µg/ml	- 0.371	- 0.839
50 µg/ml	- 0.180	-1.258
25 µg/ml	- 0.099	-0.922
12.5 µg/ml	- 0.062	-0.904

Table 3: Kinetic Constants (Km and Vmax) at Different Concentrations (For BuChE)

In the present study, the IC_{50} value of *Terminalia chebula* is 16.216µg/ml which represents the concentration of sample at which 50% of enzyme activity is inhibited. Lineweaver plot analysis to determine the kinetic constants Km and Vmax. The Lineweaver-Burk plot of ethanolic extract of *Terminalia chebula* showed mixed and non competitive inhibition kinetics as the Vmax value decreases with decreasing concentration of

ethanolic extract of *Terminalia chebula* and the value of Km varies with decreasing value of ethanolic extract of *Terminalia chebula*

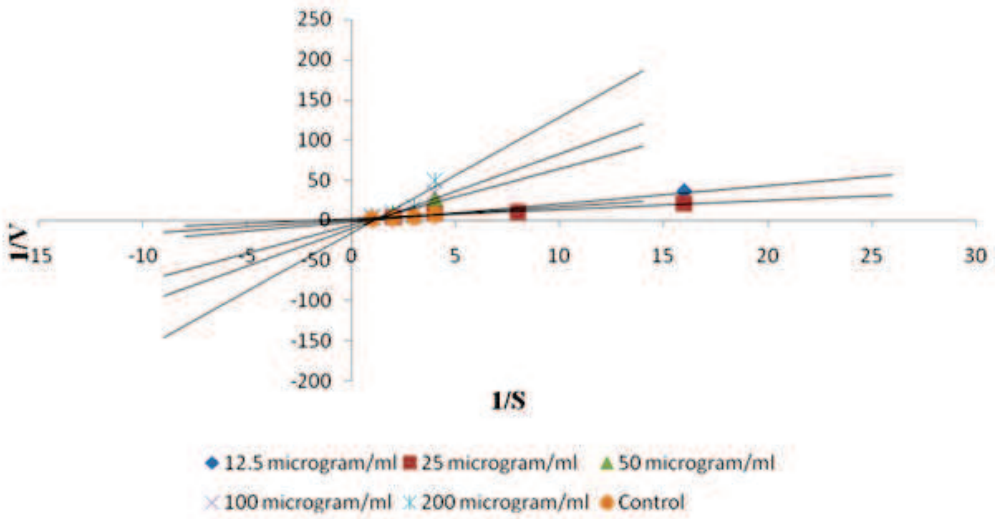

Figure: 3 Lineweaver-Burk Plot Representing the Reciprocal of Initial Enzyme Velocity Versus the Reciprocal of BuChI Concentration in the Presence and Absence (Control) of Different Concentrations of an Ethanolic Extract of *Terminalia Chebula*

Biochemical Test: The preliminary biochemical test shows the presence of various phytoconstituents present in different plant samples (Table 4).

Table 4: Biochemical Estimation

Phytoconstituents	Plant Name						
	Pistacia Vera	*Anacardium Occidentale*	*Prunus Dulcis*	*Juglans Regia*	*Convolvulus Pluricaulis*	*Eclipta Elba*	*Terminalia Chebula*
Alkaloids	+	+	+	+	+	+	+
Saponins	-	+	-	+	-	-	-
Proteins	+	+	+	+	+	+	-
Flavonoids	+	-	-	+	+	+	-
Glycosides	-	+	-	+	-	-	+

Discussion and Conclusion: As the increasing number of people suffering AD now days and there is an urgent need to find some new cure for this disease. Cholinesterase inhibitors (ChEIs) which is currently available for symptomatic treatment of AD are synthetic drugs such as rivastigmine, tacrine, donepezil have associated with number of side effects leads to poor usage of these drugs. [6] Therefore there is an immediate need of new ChEIs as drugs which is non toxic, safer, more tolerable and has improved bioavailability than the conventional drugs. Indian medicinal plants have been used from earlier days for different purposes and presently investigated for anti-cholinesterase activity against AD. Pistachio nuts are a rich source of phenolic compounds, and have recently been ranked among the first 50 food products highest in antioxidant potential. Introduction of pistachios in our daily diet will protect human health and against cancer, inflammatory diseases, cardiovascular pathologies and, more generally, pathological conditions related to free radical overproduction. On the other hand, pistachio skins could be successfully employed in food and cosmetic.[7] In the present study, an ethanolic extract and aqueous extract of *Pistacia vera* showed inhibitory activity is 34 ± 0.05 % and 25.8 ± 0.006 % respectively against BuChE. An ethanolic extract and aqueous extract of *Anacardium occidentale* showed inhibitory activity is 42.6 ± 0.028 % and 37.2 ± 0.047 % respectively against BuChE. This plant is also reported for several medicinal properties.[8] *Prunus dulcis* (Almonds) both bitter and sweet, contains 50% fatty oil and is made up of glycerides. The total phenols and flavonoids contents has a correlation for radical scavenging activity, reducing power, inhibition of β-carotene bleaching and inhibition of lipid peroxidation in brain tissue. [9] In the present study, an ethanolic extract and aqueous extract of *Prunus dulcis* showed inhibitory activity is 41.2 ± 0.0042 % and 40 ± 0.053 % respectively against BuChE. *Juglans regia* has

been regarded as healthy food rich in omega-3 fatty acids, beneficial for brain function and has both therapeutic and preventive effects. The evaluation of walnut on learning and memory in male rats has also been reported in earlier studies. A significant improvement in learning and memory of walnut treated rats compared to controls was observed in previous studies.[10] In the present study, an ethanolic extract and aqueous extract of *Juglans regia* showed inhibitory activity is 45.3 ± 0.004 % and 39.6 ± 0.031 % respectively against BuChE. *Terminalia chebula* is a traditional medicinal known for homeostatic, laxative, diuretic, and cardiotonic activities. [11] It exhibits *in vitro* antioxidant and free radical-scavenging activities.[12] *Terminalia chebula* helps in inhibiting the enzymes like AChE in the nervous tissue of freshwater snail *Lymnaea acuminata*.[13] In the present study, an ethanolic extract of *Terminalia chebula,* seed showed maximum inhibitory activity of 71.2 ± 0.005 % against BuChE and the IC_{50} value of *Terminalia chebula* is 16.216µg/ml The Lineweaver-Burk plot of ethanolic extract of *Terminalia chebula* showed mixed and non competitive inhibition kinetics.

Conclusion: In conclusion, *T chebula* demonstrated the most potent BuChE inhibitory activity among the various plants screened for anti-BuChE activity and can be explored further for isolation, purification of active phytoconstituents for potential source of novel compound that might alleviate the symptoms associated with Alzheimer's disease.

Reference:

1. Dale, Loewi, Stedman E 1932. Cholinesterase: An enzyme present in blood serum of human. Biochem 26(6): 2056-66
2. Mendel B, Rudney H. 1943 On the type of cholinesterase present in brain. Science 98 (2539) : 201-2
3. Greig NH. Debomoy K. Lahiri DK. and Sambamurti K. 2002. Butyrylcholinesterase: An important new target in AD. International Psychogeratics, 14:77-91
4. Hardy J, Selkoe DJ (2002) The Amyloid Hypothesis of Alzheimer's Disease:
5. Progress and Problems on the Road to Therapeutics. Science 297: 353–356.
6. Ellman G.L., Courtney D., Andres V. and Featherstone R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology; 7: 88–95.
7. Jann M.W., Shirley K.L. and Small G.W. (2002). Clinical pharmacokinetics and pharmacodynamics of cholinesterase inhibitors. Clinical Pharmacokinetics; 41: 719-739.
8. A Tomaino, M Martorana, T Arcoraci, D Monteleone, C Giovinazzo, A Saija: Antioxidant activity and phenolic profile of pistachio (Pistacia vera L., variety Bronte) seeds and skins. Biochimie. 2010 Sep; 92(9):1115-22.
9. V. R Kannan, C.S. Sumathi, V. Balasubramanian and N. Ramesh Elementary Chemical Profiling and Antifungal Properties of Cashew (Anacardium occidentale L.) Nuts, Botany Research International 2 (4): 253-257, 2009]
10. J C Barreira , I C Ferreira , M B Oliveira, J A Pereira. Antioxidant potential of chestnut (Castanea sativa L.) and almond (Prunus dulcis L.) by-products: Food Sci Technol Int. 2010 Jun;16(3):209-16. Epub 2010 Aug 12.
11. Barthakur N.N. and Arnold N.P . (1991). Nutritive value of the chebulic myrobalan (Terminalia chebula retz.) and its potential as a food source. Food Chemistry; 40: 213–219
12. H.Y Cheng, T.C Lin, Y.U Kuo-Hua, C.M Yang, and C.C Lin. Antioxidant and Free Radical Scavenging Activities of Terminalia chebula: Biol. Pharm. Bull. 26(9) 1331—1335 (2003).
13. A Upadhyay, D.K Singh. Inhibition kinetics of certain enzymes in the nervous tissue of vector snail Lymnaea acuminata by active molluscicidal components of Sapindus mukorossi and Terminalia chebula.

***