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Original Article

In vitro Acetyl Cholinesterase Inhibitory assay of *Acacia catechu Willd* Ethanolic Seed Extract

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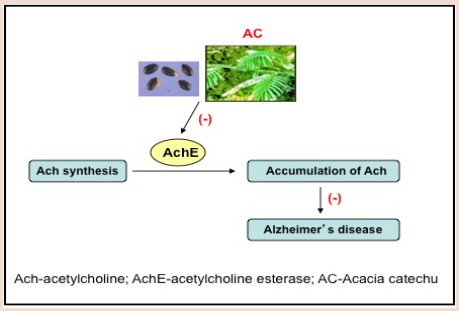
ABSTRACT

Aim & objective: The aim of this study was to evaluate acetyl cholinesterase inhibitory activity of *Acacia catechu* ethanolic seed extract to introduce a new source for management of Alzheimer's disease. **Background:** Alzheimer's disease is a complex, multifactorial, progressive, neurodegenerative disease primarily affecting the elderly population and is estimated to account for 50–60% of dementia cases in persons over 65 years of age. It is likely that the inhibition of acetyl cholinesterase by *Acacia catechu* ethanolic seed extract that is rich in flavonoids and antioxidants may aid in the protection of neurodegenerative disorders and ultimately Alzheimer's disease. **Methods:** Anti cholinesterase activity is determined by adopting *In vitro* standard protocol. **Results:** According to the obtained results, the inhibitory activity (IC_{50} values, µg /ml) of extracts was 204.38 ± 2.54 µg/ml. **Conclusion:** The results indicated and confirmed the traditional use of *Acacia catechu* ethanolic seed extract for management of central nervous system disorders. It showed the moderate activity in inhibition of acetyl cholinesterase at various concentrations. However, further investigations on identification of active components in the extracts are needed.

Key words: *Acacia catechu* seed, Acetyl cholinesterase inhibitor, Alzheimer's disease, Neurodegenerative, Spectrophotometric analysis.

SUMMARY

- *Acacia catechu* Willd. Belongs to the family *fabacaae* Commonly known as khadira in Sanskrit and karungali in Tamil is a very potent medicinal plant with diverse pharmacological actions.
- Alzheimer's disease (AD) is an neurodegenerative disorder affecting people all over the world. It is associated with loss of cholinergic neurons in the brain and decreased level of Ach.
- *Acacia catechu* seed is rich in Catechin, Epicatechin and it also exhibits significant antioxidant property.
- *Acacia catechu* seed inhibits Acetyl cholinesterase level hence, recommended for management of Alzheimer's disease.



Ach-acetylcholine; AchE-acetylcholine esterase; AC-Acacia catechu

PICTORIAL ABSTRACT

Abbreviations used: Ach: Acetyl choline, Ache: Acetyl cholinesterase, Ac: *Acacia catechu.*

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INTRODUCTION

Alzheimer's disease (AD) is an neurodegenerative disorder associated with loss of neurons in the certain areas of brain which leads to cognitive impairment, neurological disturbances, behavioral abnormalities, even leads to death. The pathophysiology of AD is not clear, but it is believed to be associated with cholinergic pathway impairment, leading to reduction in acetylcholine level in certain regions of the brain.[1] Acetylcholine, a neurotransmitter, which is hydrolyzed by acetyl cholinesterase (AChE) and butyryl cholinesterase (BuChE) is considered to play an important role in the pathology of AD.[2]

Neuritic plaques (Neurofibrillary tangles, amyloid plaques) are the major structural abnormality in seen commonly in AD patients. Amino acid Beta-amyloid peptide is an chief protein substance of amyloid in AD individuals.[3] The therapy of AD depends on decreasing the progression of the disease by improving the quality of life. Inhibition of acetyl choline level may aid in control of AD.

Cognitive enhancers are used in management of AD, Few of the cognitive enhancers are only approved by FDA in united states.[4] few drugs that have received regulatory approval currently includes donepezil, rivastigmine and galantamine, these drugs acts through increasing the concentration of acetylcholine at the neurotransmitter sites or directs by regulating activity at nicotinic receptors.[5]

Various side effects of medications reported in clinical trials causes nausea, vomiting, diarrhea, syncope and bradycardia.[6] Absolutely, there requires a fundamental need for an alternative to anti-cholinesterase compounds with fewer side effects that leads to investigation on plants as a novel source of treatment of AD.[7]

Natural sources have been used since antiquity in the treatment of various diseases including cognitive disorders, such as AD.

Considering the importance of plant based compounds in drug discovery, the present study was undertaken to evaluate the anti-cholinesterase activity of a number of selected medicinal plants with various ethno botanical uses, aiming to discover new compounds for anti-cholinesterase activity to be used in management of AD.[8-10]

Acacia catechu (Family: *Fabacaae*) is an indigenous tree grown in all the parts of the world. Commonly known as karungali in Tamil and khadira in Sanskrit. Similarly to Neem, Turmeric, *Aloe Vera*, People in Kerala used this karungali leaves in boiling water, extracted juice for management of digestive disorders.[11] It has been used in Ayurveda (Indian Medicinal System) for years extensively as an anti-inflammatory agent. It has been proven to possess antioxidant, anticancer, antiulcer, hepato protective, anti diabetic effects.[12-17] It also has a role in dye industry the heartwood of khadira is employed for this purpose. Study reported that

Table 1: Anti cholinesterase inhibitory assay of *Acacia catechu* ethanolic seed

AchE inhibitory assay	
Concentration (ug/ml)	% inhibition
50	5
100	10
150	18
200	25
250	35
300	45
IC50 *Acacia catechu* = 204.38 = ± 2.54 (ug/ml)	-
IC_{50} Galantamine = 2.727 = ± 0.08 (ug/ml)	-

Acacia catechu Catechin produced promising result in cognitive enhancement in AD patients.

Hence, Ethanolic extract of *Acacia catechu* seed is investigated for its anti-cholinesterase inhibitory activity for the first time.

MATERIALS AND METHODS

Plant material

Acacia catechu seeds were collected from Hosur, Tamilnadu and was authenticated by Dr. H.B Singh, NISCAIR, New Delhi and the voucher specimen were stored for further use in Green Chem lab, Bangalore.

Ethanolic Extraction

Seeds were shade dried for a week. Dried seeds were milled to fine powder. Powder was passed through 100 mesh sieve and stored in a sealed polythene bag. 2.5 kg of powdered *Acacia catechu* seeds were extracted with 10 liters of Ethanol, at 65°C temperature, for 1 hour, in a 20 liter round bottom flask with Graham condenser attached. Condenser was cooled circulating with chilled water. After 1 hour of extraction, round bottom flask was cooled to room temp and the extract were filtered and collected. The Marc was extracted repeatedly with 10 liters of Ethanol, twice. The extracts were filtered and collected. The combined extracts was evaporated to dryness under reduced pressure in a Buchi Rotary Evaporator (Switzerland) at 65°C, to obtain 150 g of powder extract. The w/w yield of the prepared extract was 6%. The extract were stored at 4°C until used.

Chemicals

Acetylthiocholine iodide (ATCI), 5,5'–dithio-bis-2-nitrobenzoic acid (DTNB), bovine serum albumin (BSA) were purchased from Sigma (USA). Buffers and other chemical were of extra pure analytical grade. The following buffers were used: Buffer A:50 mM Tris–HCl, pH 8, containing 0.1% BSA; Buffer B:50 mM Tris-HCl, pH 8 containing 0.1 M NaCl, 0.02 M $MgCl_2 \times 6H_2O$.

Anti-cholinesterase inhibitory assay

The AChE activity was measured according to the method developed by Eldeen *et al.*[18,19] with slight modifications. To various concentrations of sample extracts (50–250 µg/ml), 25 µl of 15 mM acetylthiocholine iodide (ATCI), 125µl of 3 mM DTNB (5-5'-thiobis-2-nitrobenzoic acid) in Buffer C (50 mM Tris-HCl, pH 8, containing 0.1 M NaCl and 0.02 M $MgCl_2.6H_{20}$) and 50 µl of Buffer B (50 mM Tris-HCl, pH 8, containing 0.1% bovine serum albumin) were added. Thereafter, AChE (0.2 U/ml) was added and the final mixture was read at 405 nm immediately. Galantamine was used as a standard drug.

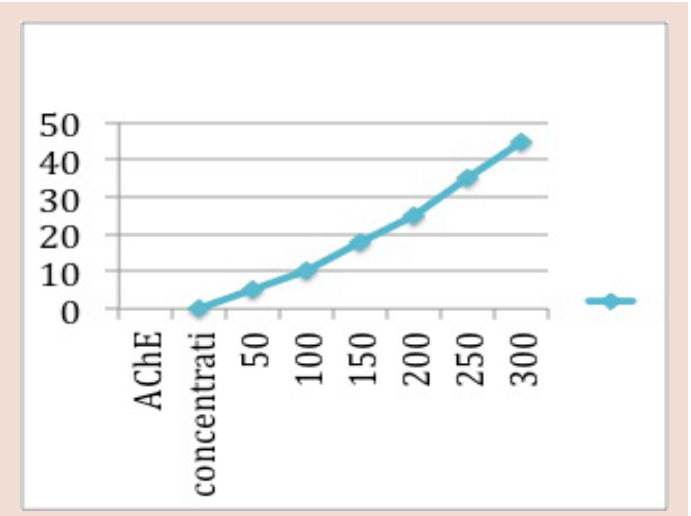

Figure 1: Acetyl choline inhibitory assay of *A. catechu* seed extract

Determination of IC_{50}

The percentage inhibition was then calculated using the following formula;

% inhibition = [(Control OD – Sample OD)/Control OD] x 100

The experiment was done in triplicate and concentrations of the test extract that inhibit the hydrolysis of the substrate (acetylcholine) by 50% (IC_{50}) were determined by linear regression analysis between the inhibition percentage versus the extract concentration.

RESULT AND DISCUSSION

Neurodegenerative disease is a term applied to a variety of conditions arising from a chronic breakdown and deterioration of the neurons, particularly those of the central nervous system.

Alzheimer's disease is associated with loss of cholinergic neurons in the brain and the decreased level of AChE. The major therapeutic target in the AD treatment strategies is the inhibition of brain AChE.[20] Cholinesterase inhibitor drugs, inhibiting AChE activity, maintain ACh level by decreasing its breakdown rate.[21] Although the underlying patho physiological mechanisms are not clear, AD is firmly associated with impairment in cholinergic pathway, which results in reduced level of acetylcholine (Ach) that is hydrolysed by cholinesterase (ChE) in certain areas of brain.

Medications currently approved by regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) to treat the cognitive manifestations of AD and improve life quality of the patients are Donepezil, rivastigmine and galantamine as reversible AChE inhibitors, and memantine as an NMDA receptor antagonist.

Tacrine was the first of the AChE inhibitors approved for the AD treatment in 1993, but its use has been abandoned because of a high incidence of side effects including hepatotoxicity.[22-24]

Plant based compounds have been used as cognition enhancers herbs like *Withania somnifera*, *Centella asiatica*, *Curcuma longa, Bacopa monnieri, Convolvulus pluricaulis, Celastrus paniculatus, Nardostachys jatamansi* have been reported to be used in management of cognitive impairment in Ayurvedic system of medicine.[25,26]

Acetyl cholinesterase inhibitory activity is dose dependently increasing at a concentration ranging from 50 µg/ml to 300 µg/ml and when compared to standard Galanthamine the inhibitory activity is moderate even though the tested extract shows significant activity. The results are depicted in Table 1 and Figure 1 and can be used for treating neurodegenerative disorders.

It was reported that the presence of Catechin, Epicatechin in *Acacia catechu* is responsible for treating cognitive impairment and the observed anti-cholinesterase activity found in the study could be due to the presence of Catechins, Quercetin and Epicatechin.[27]

CONCLUSION

The results concluded that *Acacia catechu* ethanolic seed extract exhibited potential acetyl cholinesterase inhibitory activity, further *In vivo* studies are required to prove its cognitive efficacy.

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