



EXTRACTION OF ACETYLCHOLINE ESTERASE INHIBITORS FROM *MORINGA OLEIFERA* AND ANALYZING ITS ACTIVITY WITH ZEBRAFISH

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ABSTRACT

Alzheimer disease is neuro-degenerative disorder where acetylcholine esterase (AChE) plays key role, in the progression of the disease. The enzyme AChE plays a major role in the dissociation of the neurotransmitter acetylcholine (Ach) in to acetic acid and choline and thereby affecting the nerve transmission. One of the current scenarios for the treatment of Alzheimer disease is to inhibit the activity of AChE, which decrease the degree of neuro-degeneration. AChE inhibitors are found to be naturally present in the plants. Hence the methanolic extract of the leaves of *moringa oleifera* was screened for AChE inhibitors for the brain homogenate of the zebrafish (*Danio*

rerio). Through the titri-metric analysis of the AChE in in-vitro and in-vivo conditions the activity was recorded. We have found that the methanolic extract of *moringa oleifera* reduces the activity of AChE. Under in-vitro and in-vivo conditions the activity of the AChE reduce from 18% - 15.8%.

KEYWORDS: Alzheimer Disease, Neuro-degenerative Disorder, Acetylcholine Esterase, *Moringa oleifera*.

INTRODUCTION

Alzheimer's disease (AD) is one of the most common forms of dementia found in people, of age 65 and above. It is a neuro-degenerative disease causing nerve cell death and tissue damage, observed as brain shrinkage with the progression of time.^[1,3] This causes damages to cortex and hippocampus areas of brain, result in thinking, planning and memory defects.^[2] In this process the beta-amyloid protein clumps together forming plaques and neurofibrillary

tangles containing tau protein which causes damages to neurons. The Alzheimer's disease interferes with the cell signal transmission across the synapsis by affecting the activity of neurotransmitter "Acetylcholine". The level of acetylcholine is controlled by acetylcholine esterase (E.C3.1.1.7),^[26,27]

The function of acetylcholine esterase is to break down acetylcholine, once the nerve transmission is completed. It keeps the synaptic cleft clear, so that to avoid mixing of messages.^[28]

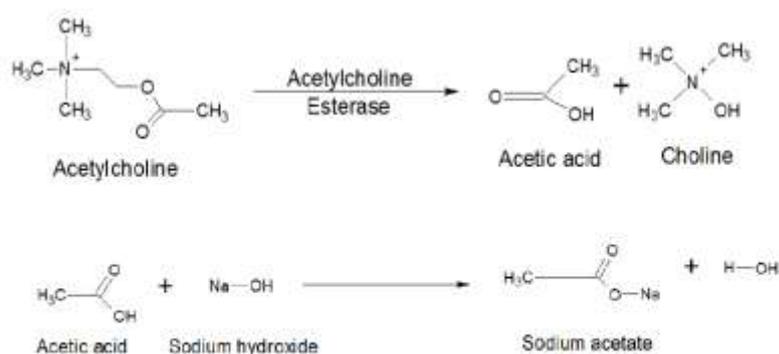


Figure-1 Conversion of acetylcholine to sodium acetate

The current scenario related to treatment of Alzheimer's disease including the increased level of acetylcholine in the affected brain by using acetylcholine esterase inhibitors.^[4,5,6] Tacrine is the first drug approved for the treatment of Alzheimer in 1993.^[7] The current available drugs for Alzheimer's disease are Galantamine, Rivastigmine, Donepezil and Memantine.^[8,9] These drugs are known to have side effects including, vomiting, dizziness, diarrhea, seizures and syncope and insomonia.^[11,12]

Introduction to Plant Source

To reduce these effects, we can incorporate plant extract containing potent AChE inhibitors.



Figure. 2: *Moringa oleifera* plant.

Moringa oleifera (Figure-2) contain largely of the shrubs of flowering plants which are growing throughout the tropical region of the world.^[14] This plant is very well known for religious and cosmetic values and tremendous potential to be used as medical agent to cure infections, digestive diseases etc. It has anti inflammatory, anti pyretic, anti tumor potential and anti oxidant properties etc.^[16,17,18] This plant contains Tannins, Alkaloid, Flavanoids, Iridoids etc.^[15]

Methanolic extraction of dried leaves was obtained and used to check the inhibitory activity for AChE in *Danio rerio* (zebrafish). Zebrafish can be used as an ideal model for the study of human genetic diseases because there is approximately 70% similarity with human genes.^[21,22,23]

MATERIALS AND METHODS

Selection of Plant material: The Plant leaves of *moringa oleifera* were obtained from Yelahanka, Bangalore district of Karnataka, India.

Selection of zebrafish: The mutant variety was obtained from A-Z Aquarium in Kumaraswamy Layout, Bangalore 560068. The fish were quarantined and stored in 20L aquarium and were fed with shrimp flakes and commercially available feed once every 24 hours.^[10]

Isolation of Acetylcholine esterase (AChE)

Healthy mutant zebrafish were selected and anaesthetized, and the fish brains were dissected, weighed and were homogenized with 5ml of 0.05M phosphate buffer (PB) of pH 7.2 using pre-chilled mortar and pestle. The homogenate was diluted by addition of 15ml of phosphate buffer and then centrifuged for 10 minutes and 5000 rpm at 4°C. The supernatant was collected. This was used as the source for the enzyme. The mixture was titrated against NaOH solution using phenolphthalein as indicator. The titration was stopped at the reach of persistent pale pink colour, via neutralization reaction of acetic acid hence by increasing the alkaline content by using NaOH. (The reactions were drawn by using chem-sketch free ware).

Plant extract preparation

The *moringa oleifera* plant leaves were cleaned, dried and made in to a power and methanolic extract of the plant was prepared using soxhlet apparatus. The dried plant extract was subjected for gas chromatography to find out different compound present in the extract.^[24,25]

Chromatogram of the methanolic extraction of *moringa oleifera*

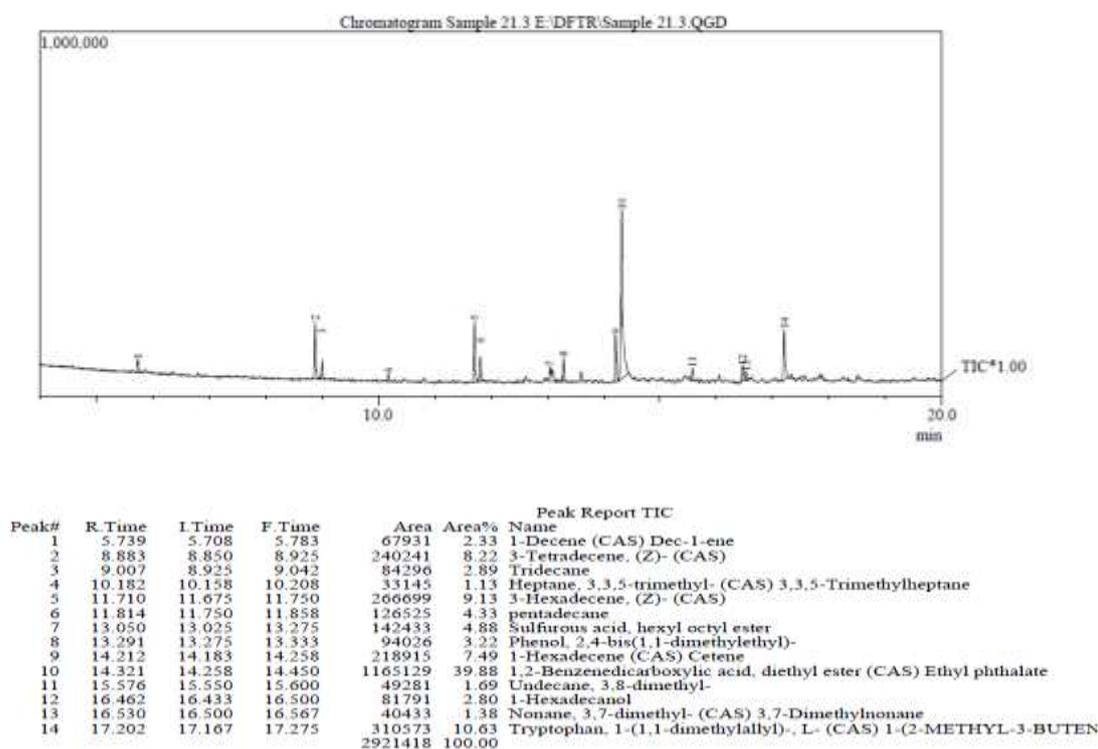


Figure. 3: GCMS of *moringa oleifera* plant.

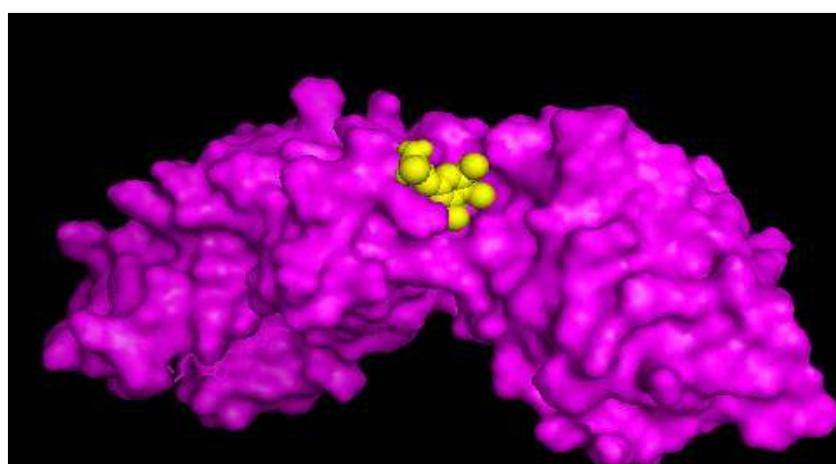


Figure. 5: Acetylcholine esterase docked with the ligand.

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-4.9	0.000	0.000
2	-4.4	2.069	3.707
3	-4.2	21.353	23.118
4	-3.9	26.806	28.958
5	-3.7	24.350	25.874
6	-3.7	19.772	20.750
7	-3.6	27.073	29.211
8	-3.6	24.800	26.308
9	-3.6	23.733	24.632

Figure. 6: Binding Energy.

Assay of acetylcholine esterase

In this assay 0.5ml of enzyme (AChE) and 2ml of (acetylcholine) substrate (10mM) was added and incubated for 5 minutes at room temperature followed by placing in a boiling water bath 1-2 minutes for the inactivation of enzyme to terminate the reaction. The mixture was titrated against 0.1N NaOH using phenolphthalein as the indicator. The titration was stopped at the reach of persistent pale pink colour (endpoint). The protocol was repeated thrice. The titrate value is recovered and enzyme activity was calculated.^[20]

Screening of the Acetylcholine esterase inhibitors

In vitro screening

To 0.5ml of enzyme (AChE) different volume of plant extract were added, and then incubated for 10minutes at room temperature, followed by the addition 2ml of substrate (AChE) and incubate for 5minutes at room temperature. And then the enzyme was deactivated by placing in a boiling water bath for 1minute, cooled and titrated against 0.1N NaOH using phenolphthaline as indicator. The titration was stopped at the reach of persistent pale pink colour (endpoint). The titrate value was calculated. The level of inhibition was compared with the control value.

Screening of acetylcholine esterase on Alzheimer induced fish

The enzyme (AChE) was extracted from induced Alzheimer fish and assay was performed using 0.1N NaOH with phenolphthaline as indicator. The titrate value was recorded and the enzyme activity was calculated.

In-vivo screening of (AChE)

A selected set of Alzheimer induced zebrafish were contained in 1L de-chlorinated water container. 1ml of plant extract was added to it (with known concentration). The fish were observed for 3 days and their assay was calculated. The level of inhibition was found by comparing with the control group.

RESULT AND DISCUSSION

In-vitro assessment of acetylcholine esterase activity

Through the in-vitro assay (**Figure-6**) of methanol extract of *moringa oleifera*, we have observed reduced enzyme activity^[13] of AChE upon addition of 0.1ml of 100 microgram/ml of the plant extract, the enzyme activity was found to be 1.313 micromole/min, when compared to control showing 1.559 micromole/min.

When 0.2ml of the plant extract of 100 microgram/ml is used, the enzyme activity was found to be 0.848 micromole/min, when compared to the control showing 1.559 micromole/min.

We have also found that the addition of plant extract displayed high rate of inhibition. The in-vitro analysis showed an average inhibition of 45.6% of the enzyme activity.^[20]

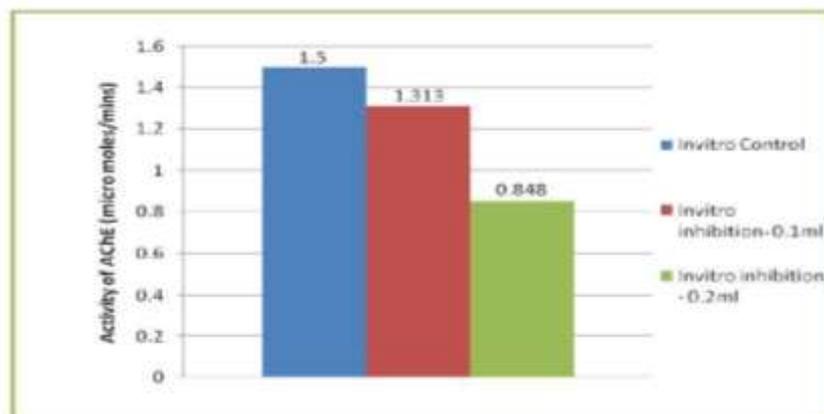


Figure. 6: Invitro assessment of AChE activity.

In-vivo assessment of acetylcholine esterase activity

In the in-vivo assay, of induced Alzheimer by the treatment of the chemical, the enzyme activity AChE showed 1.970 micromole/min, when compared to the control, showing 1.559 micromole/min (Figure-7).



Figure-7: In-vivo assessment of AChE activity of fish with induced Alzhiemer. In treated Alzheimer fish, with plant extract.

In the *in-vivo* assay of treated AD fish, the enzyme activity of the AChE showed 1.28 micromole/min, when compared to the control showing 1.559 micromole/min.

The *in-vitro* analysis showed an average inhibition of 17.90% enzyme activity (Figure-8).

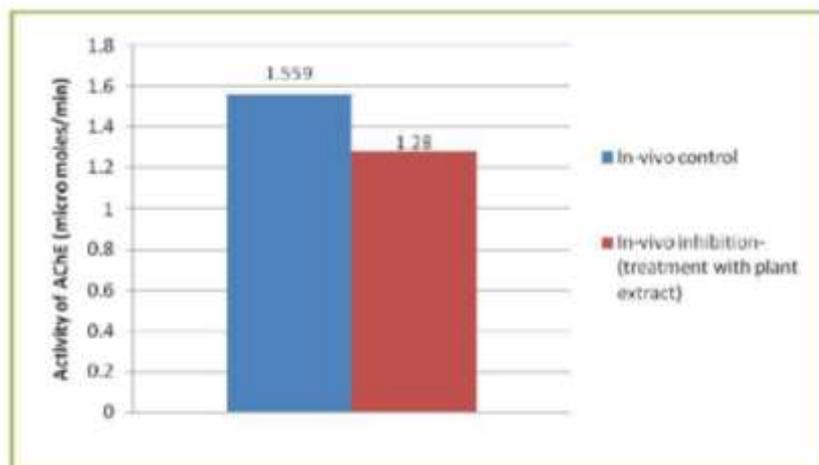


Figure. 8: *In-vivo* assessment of AChE activity in treated Alzhiemer fish.

CONCLUSION

The plant extract (methanol extract) of *moringa oleifera* was analysed for the inhibitory activity for acetylcholine esterase enzyme, obtained from the brain homogenate of *Danio rerio*, we found that the plant source acting as an inhibitor for (AChE), by enzyme assay performed at different stages. In the neuro-degenerative diseases, such as Parkinson's, Alzheimer's & others, the (AChE) level proved to be increased. Hence the *moringa oleifera* methanolic extract can be used to treat the neuro-degenerative disorders. Further studies are

required to identify the specific compounds or molecules, that act as inhibitors for acetylcholine esterase enzyme.

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