

Screening of Acetylcholinesterase inhibitors by *Moringa olifera*

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ABSTRACT

Acetylcholinesterase (AChE) is an enzyme involved in the breakdown of the neurotransmitter acetylcholine into acetic acid and choline. Inhibition of Acetylcholinesterase is needed for deceleration of neurodegeneration in Alzheimer's, Parkinson's and other neurodegenerative diseases. Plants are known to contain Acetylcholinesterase inhibitors. The methanolic extracts of *Moringa olifera* leaves were checked for Acetylcholinesterase inhibition on the acetylcholinesterase from brain homogenate of *Danio rerio*. The activity of the enzyme in-vitro and in-vivo conditions. In our investigations the extract from the plant showed a reduction in the Acetylcholinesterase activity. Under in-vitro conditions and in-vivo conditions the activity of Acetylcholinesterase dropped by 29.26% and 34.26% respectively.

Keywords: Alzheimer's Disease, Acetylcholinesterase, Inhibition, Zebrafish, Plant Extracts.

INTRODUCTION

Alzheimer's disease (AD) is a neuro-degenerative disease whose cause is not well understood. It is developed by the deposition of β -amyloid plaques, formation of neurofibrillary tangles containing tau protein and the loss of cholinergic neurons in the cerebral cortex and basal for brain. (Dennis, 2002). The nerve transmissions across the synapses are stimulated by chemical signals carried by acetylcholine. Acetylcholinesterase is a type of hydrolase that discontinues these stimulating signals by hydrolysing acetylcholine into choline and acetic acid (Leuzinger et al., 1969). The mode of enzyme action shown first isolated Acetylcholinesterase from *Electrophorus electricus* Leuzinger and Baker (1967).

Detection of Alzheimer's Disease at the early stages is very difficult and is still under research. One of the current strategies used for the treatment for Alzheimer's Disease involves increasing the levels of acetylcholine in the brain that are depressed in Alzheimer's Disease using Acetylcholinesterase inhibitors. (Nair et al., 2004) (Mark and Cornelius 2002). The approved cholinesterase inhibitors (eserine,

tacrine, donepezil, rivastigmine and galantamine) are known to have unfavourable side effects like diarrhoea, nausea, and vomiting, headache, seizures, insomnia (Rogers et al.,1998) (Mehta et al., 2012) as a result plant extracts can be used to discover more potent Acetylcholinesterase inhibitors.

The *Moringa oliefera* is referred to as the “Miracle tree” in tropics and sub-tropics. Its leaf, seed and flowers have been shown to have potent antioxidant activity 9-119 (Somali et al., 1984), Morton (1991) Sreelatha (2009) reported the leaf extract possess anti-inflammatory, antihypertensive, hypolipidemic, hepatoprotective and antimicrobial activities. It is employed for the treatment of different ailments in indigenous system of medicine, particularly in South Asia (Anwar et al., 2007).

The *Moringa* leaf is a rich source of flavonoids like quercetin, kaempferol, catechin, epicatechin, rutin etc, all are potent antioxidants (Rao and Kamath 2001), (Tsimogiannis and Oreopoulou 2006). The leaves of this plant was used to obtain methanolic extract and test for its inhibitory activity on *Danio rerio*. Many vertebrate models are used to understand the disease and zebrafish (*Danio rerio*) is one of the accepted models used. (Orosco and Panula, 2013). Zebrafish can be used as an effective model for human genetic disease since approximately 70 % of the human genes have at least one orthologous gene in zebrafish (Clark et al., 2013).

MATERIALS AND METHODS

Procurement of Plant Material

The plant material *Moringa oliefera* was obtained from Bhavans College Andheri(W).

Procurement of Zebrafish (*Danio rerio*): Zebrafish were purchased from Aquarium in kurla. The fish were quarantined and stored in a 20L aquarium and fish were fed with shrimp flakes and commercially available feed once every 36 hours. (Jayanth et al., 2014)

Chemicals: Acetylcholine chloride .

Isolation of Acetylcholinesterase (AChE): The brain of healthy zebrafish were dissected and homogenized with 3ml phosphate buffer (PB) (0.05M, pH 7.2) using a pre-chilled mortar and pestle. The homogenate was

diluted with 17ml cold PB and then centrifuged at 5000rpm for 15 minutes at 4°C. The supernatant was collected, which serves as the source of the enzyme. The enzyme source was diluted at 20x concentration

Assay of Acetylcholinesterase: In this assay, to 0.5 ml of the enzyme, 5ml of acetylcholine (100mM) was added and incubated for 5 minutes at room temperature and then the enzyme was deactivated by placing in a boiling water bath for 2-3 minutes. The terminated mixture was then titrated against NaOH (0.05M) with phenolphthalein as the indicator till a pale pink colour obtained. The titre value was noted and the enzyme activity was calculated. (Jayanth and Guruprasad, 2014).

Plant extracts preparation: The leaves of the *Moringa oliefera* were washed and dried. Methanolic extract of the plant was prepared using soxhlet apparatus.

Screening of Acetylcholinesterase inhibitors

In-vitro screening: Different volumes of the plant extract were added to 0.5ml of the enzyme. To this, 5ml of acetylcholine was added and incubated for 10mins at room temperature. The reaction mixture was then deactivated by keeping in a boiling water bath for 3 minutes, cooled, and then titrated with 0.05M NaOH using phenolphthalein as the indicator, and from the titre values obtained, the enzyme activity was calculated. The rate of inhibition was calculated using control.

In-vivo screening: 0.5 ml of the methanolic extract was added into 1 litre of de-chlorinated water having healthy zebra fish. The fishes were monitored for 2 days and then the assay was carried out as mentioned above and the enzyme activity was calculated. The extent of inhibition was found in relation to control group.

RESULTS AND DISCUSSION

In-vitro assessment of for Acetylcholinesterase activity: The in-vitro assay showed that the methanolic extract of *Moringa oliefera* reduced enzyme activity. Upon addition of 0.2ml of the extract, enzyme activity was found to be 0.934 ± 0.0057 micromoles/min when compared to control showing $4.1.168 \pm 0.001$ micromoles/min. (Fig1) Addition of

0.4ml of the extract showed enzyme activity of 0.992 ± 0.00057 micromoles/min when compared to the control 1.284 ± 0.001 micromoles/min (Fig 2). When 0.6ml of the extract was added enzyme activity was seen to be 1.051 ± 0.0057 micro moles/min in

comparison the control showed 1.401 ± 0.00057 micromoles/min (Fig 3). We also found that addition of 0.2ml of the extract showed higher rate of inhibition. The in-vitro analysis showed an average inhibition of 29.26% of the enzyme activity.

Table 1: In-vitro assesment of acetylcholinesterase enzyme.

Volume/Amount of plant extract (ml)	Test group	Acetylcholinesterase enzyme $\mu\text{mol}/\text{min}$ (mean value)
0.2	Control	1.168 ± 0.001
0.2	Inhibitor	0.934 ± 0.0057
0.4	Control	1.284 ± 0.001
0.4	Inhibitor	0.993 ± 0.00057
0.6	Control	1.401 ± 0.00057
0.6	Inhibitor	1.051 ± 0.0057

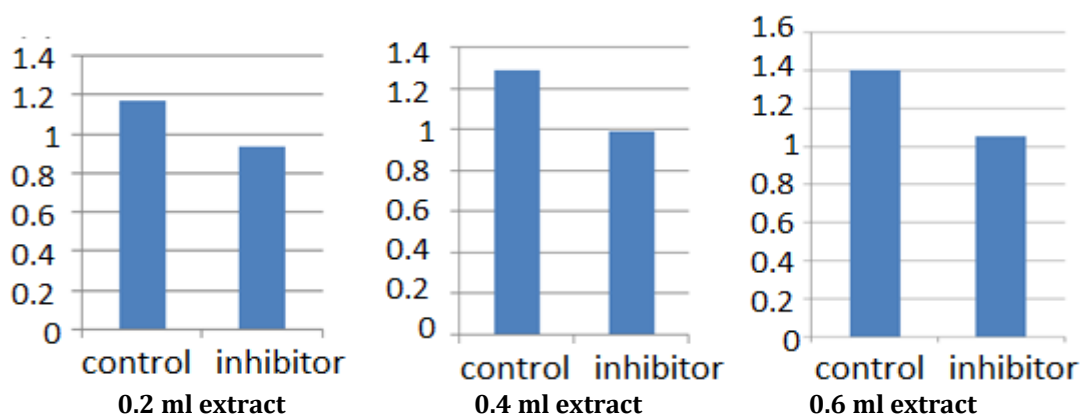
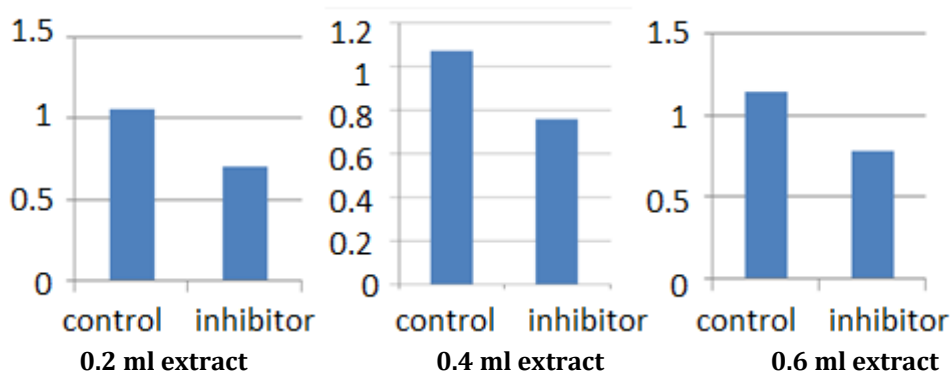


Table 2: In-vivo assesment of acetylcholinesterase enzyme I

Volume/Amount of plant extract ml	Test group	Acetylcholinesterase enzyme $\mu\text{mol}/\text{min}$ (mean value)
0.2	control	1.051 ± 0.00057
0.2	inhibitor	0.700 ± 0.00057
0.4	control	1.074 ± 0.001
0.4	inhibitor	0.759 ± 0.001
0.6	control	1.144 ± 0.00057
0.6	inhibitor	0.78 ± 0.00057



In-vivo assessment of for Acetylcholinesterase activity

The in-vivo assay showed that the methanolic extract of *Moringa olifera* reduced the enzyme activity. Upon addition of 0.2ml of the extract showed an enzyme activity of 1.8347 ± 0.0046 micromoles/min when compared to the control 2.824 ± 0.0682 micromoles/min. (Fig 1) Addition of 0.4ml of the extract showed an enzyme activity of 2.0842 ± 0.0565 micromoles/min when compared to the control 3.3421 ± 0.0973 micromoles/min. (Fig 2) When 0.6ml of the extract was added enzyme activity was seen to be 2.3543 ± 0.1454 micromoles/min when compared to the control 4.2023 ± 0.1471 micromoles/min. (Fig 3).

On comparison we found that addition of 0.6ml of the extract showed higher rate of inhibition. The in-vivo analysis showed an average inhibition of 34.26% of the enzyme activity.

Further we would like to investigate the mode of inhibition of the plant extracts to understand what the components of the extract are doing to the enzyme to inhibit or decelerate its activity.

CONCLUSION

The methanolic extracts from leaves of *Moringa olifera* were checked for Acetylcholinesterase inhibition on the acetylcholinesterase from brain homogenate of *Danio rerio*. The plant extract showed the inhibition of Acetylcholinesterase whose activity is elevated during disorders like Alzheimer's disease, Parkinson's disease, and Huntington's disease. *Moringa olifera* is therefore, a potential source of Acetylcholinesterase inhibitors that can be used for the treatment of neurodegenerative disorders. Further studies are required to identify the specific compounds/molecules that act as acetylcholine esterase inhibitors.

REFERENCES

Anwar F, Latif S, Ashraf M, Gilani AH (2007) *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytother Res.*, 21: 17-25.

Dennis J Selkoe (2002) Alzheimer's disease is a Synaptic Failure. *Science*; 298: 789-791.

Dennis J Selkoe (2001) Alzheimer's disease: Genes, Proteins and Therapy. *Physiological Reviews.* : 81

Clark MD, Howe K, Torroja CF, Torrance J et al. (2013) The zebrafish reference genome sequence and its relationship to the human genome. *Nature*, 496 (7446): 498-503.

Jayanth A and Guruprasad R (2014) Assay of Acetylcholine Esterase Enzyme Activity By Titrimetric Method. *International Journal of Pharma and Bio Sciences*, 5(4): 643-647

Jayanth A (2014) Muralidhar.S.Talkad. Neuroprotective Activity of *Stachytarpheta indica* on Rotenone induced Parkinson's disease in Zebrafish. *World Journal of Pharmacy and Pharmaceutical Sciences*, 3(7): 940 - 955.

Mark Holden and Cornelius Kelly (2002) Use of Cholinesterase inhibitors in Dementia. *Advances in psychiatric treatments*. APT; 8:89-96.

Mehta Mona, Abdu Adem, and Marwan Sabbagh (2012) New Acetylcholinesterase Inhibitors for Alzheimer's Disease, *International Journal of Alzheimer's Disease*, (2012) Article ID 728983, 8 pages?

Morton JF (1991) The horseradish tree, *Moringa pterigosperma* (Moringaceae). A boon to arid lands. *Econ Bot.* 45: 318-333.

Orosco LA, Priyadarshini M, Panula PJ (2013) Oxidative stress and regulation of Pink1 in Zebrafish (*Danio rerio*). *PLoS One.* 26; 8(11):e81851. doi: 10.1371/journal.pone.0081851. eCollection 2013. PubMed PMID: 24324558; PubMed Central PMCID: PMC385007.

Rao VA, Devi PU, Kamath R (2001) *In vivo* radioprotective effect of *Moringa oleifera* leaves. *Indian J Exp Biol.* 2001;39: 858-863.

Somali MA, Bajnedi MA, Al-Faimani SS(1984) Chemical composition and characteristics of *Moringa peregrina* seeds and seed oil. *J Am Oil Chem Soc.*, 61: 85-86.

Rogers SL, Farlow MR, Doody RS, Mohs R, Friedhoff LT and Donepezil(1998) A 24-week, double blind, placebo-controlled trial of donepezil in patients with Alzheimer's disease. *Neurology*, 50: 136-145.

Leuzinger Walo and Baker AL (1967) Acetylcholinesterase, I. Large-Scale Purification, Homogeneity, and Amino acid Analysis. *Proc Natl Acad Sci, U S A.* ; 157 (2): 446-451.

Leuzinger W., Goldberg M, Elsa Cauvin (1969) Molecular properties of acetylcholinesterase. *Journal of Molecular Biology.*40(2): 217-225

Nair V Priya, Jennifer M Hunter (2004) Anticholinesterases and anticholinergic drugs. *Oxford Journals*4(5); 164-168.

Sreelatha S, Padma PR (2009) Antioxidant Activity and Total Phenolic Content of *Moringa oleifera* Leaves in Two Stages of Maturity. *Plant Foods Hum Nutr.*64: 303-311.

Tsimogiannis DI, Oreopoulou V (2006) The contribution of flavonoid C-ring on the DPPH free radical scavenging efficiency. A kinetic approach for the 3', 4'- hydroxyl substituted members. *Innovative food sci emerg technol.*, 7: 140-146.