

IN VITRO SCREENING FOR ACETYLCHOLINESTERASE ENZYME INHIBITION POTENTIAL OF MUTHU PARPAM – THERAPEUTIC LEAD FOR ALZHEIMER'S DISEASESABARI GIRIJA N^{1*}, GUPTAJ S², SINEKHA MA³, SAKTHIMANIPRIYA L¹, SHANMUGAPRIYA P¹

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ABSTRACT

Objective: Siddha system is an ancient traditional system of medicine treats many chronic ailments and neurological disorders. Muthu parpam is one of the herbo marine Siddha drugs which have the indication for neurocognitive dysfunction. The main objective of this current study was to evaluate the acetylcholine esterase (AChE) inhibition of Muthu parpam.

Methods: AChE activity was evaluated using a modified 96-well microplate assay based on Ellman's method. Physostigmine (5, 10, 20, and 40 µg/ml) was used as the positive control.

Results: The result of this study clearly indicates that the test drug Muthu Parpam was effective in inhibiting AChE enzyme at the specified concentration dose dependently. Maximum percentage inhibition of about 71.68% was observed at 500 µg/ml when compared to that of the Physostigmine, a known AChE inhibitor with the maximum inhibition 84.87% at the concentration of 40 µg/ml.

Conclusion: Hence, this preliminary screening has proven the efficacy of Muthu parpam through AChE inhibition potential in the management of Alzheimer disease.

Keywords: Acetylcholine esterase, Muthu parpam, Alzheimer disease, Acetylcholine esterase inhibition, Cognitive function, Siddha.

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INTRODUCTION

Alzheimer's disease (AD) is characterized that occur during the formation of amyloid aggregates within the core of neuritic plaque, and therefore, the formation of intraneuronal neurofibrillary tangles within the brain of afflicted individuals [1]. In the human brain, there are two major forms of cholinesterases, namely, acetylcholine esterase (AChE) and butyrylcholinesterase [2]. The foremost remarkable biochemical change in AD patients may be reduced of ACh levels within the hippocampus and cortex of the brain [3]. ACh is the principal neurotransmitter which functions all told autonomic ganglia and is the only neurochemical that triggers motor division of the somatic systema nervosum. The deterioration of cholinergic neurons within the brain, and therefore, the loss of neurotransmission is the key causes of the decline in cognitive function in patients with AD [4]. In normal cholinergic activity consisting the sequence of release, binding and enzymatic deactivation of ACh by AChE, while abnormal cholinergic activity is characterized by a deficit or short-fall in cholinergic transmissions at synapses and has been attributed to reduced production of ACh or its excess deactivation/hydrolysis by AChE. Therefore, inhibition of AChE, the enzyme liable for the hydrolysis of ACh at the cholinergic synapse, is currently the foremost established approach to treating AD [5]. Impairment in signal transduction across synapses is the main etiology for cognitive impairment [6,7] and thus regulating the activities of AChE has become a crucial research focus [8].

Synthetic acetyl-cholinesterase inhibitors (AChEIs) include donepezil, rivastigmine, galantamine, and tacrine are commercially available drugs which influence the dynamics of ACh by inhibiting the activity of AChE. These actions taken place on cholinergic receptors of synaptic cells. Due to the side effects of synthetic AChEIs which include gastrointestinal disturbances, moderate effectiveness, high cost and short half-life of AChEIs, there always a need for exploring the character for newer potent and long-lasting AChE inhibitors with minimal side

effects [9-11]. There always a need for exploring the character for newer potent and long-lasting AChE inhibitors with minimal side effects. An outsized number of plant species from different parts of the globe are examined for cholinesterase inhibitory activity [8,12].

Traditional medicine is defined as the sum total of the knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illness [13]. Siddha system is one of the traditional systems of medicine mainly practiced in Southern India. This is a standard system of drugs mainly practiced within the southern a part of India. As per Siddha literature, Muthu Parpam is the herbo - marine Siddha formulation which has the indication for *Marutchi* (i.e., confusion and perplexity of mind). Muthu (Pearl) is one in all the nine gems possess the expectorant, stypitic, tonic, and spasmodic action [14,15]. Hence, this study was an attempt to find out the ACh esterase (AChE) inhibition potential of Muthu Parpam.

METHODS**The purification process of Muthu [14]**

Soak the pearl (35 g) in sour curd and should be kept under the sunlight for 3 days. Daily morning sour curd should be poured on the pearl. To stay dry, it should be kept under the daylight for 2 days without adding the curd. Repeat this process 2 times and then a pearl should be washed and dry it.

Ingredients [14]

- Purified Muthu (*Pinctada margaritifera*) – 1 palam (35 g)
- Notchi (*Vitex negundo*) – 2 palam (70 g)
- Nilappanai kizhangu (*Curculigo orchoides*) – 2 palam (70 g).

Method of preparation

Thirty-five grams of purified Muthu (pearl) should be soaked in 70 g of notchi juice for 2 days and then grind it with the notchi juice for 2 days. Then, it should be made into villai (pellets) and dry it in the sunlight. The pellets should be placed in an earthen vessel which should be closed with another vessel and the margin of vessels sealed with seven layered clay cloths and the set up should be dried in sunlight for 1 day. After that, it should be placed in a deep pit and pudam process (calcination process) should be done with 40 cow dung cakes. Then, this same procedure should be repeated with the juice of nilappanai kizhangu and then make it as a fine powder. The final product, the parpam should be stored in the airtight glass container.

Indication and adjuvants [16]

- Nirvidam (*Asparagus racemosus*) [15] – Nithiraiyil undaagum marutchi (Confusion and perplexity of mind during the sleep)
- Water – Maalaikan noi (Night blindness)
- Milk – Pithathaal undaagum veppam (Heat due to pitham)
- Nilathulasi juice – valippu (epilepsy)
- Nilavilaa juice – pitha diseases
- Devadhaaru decoction – Vatha diseases
- Butter – moolam (Piles)
- Vilvam leaf juice – Adhisaaram (diarrhoea)
- Brown sugar – vaanthi (Vomit).

Test drug for analysis

The test drug Muthu Parpam prepared with the above manner was procured from the GMP certified pharmaceutical (Earth India Naturals) company and given for the analysis.

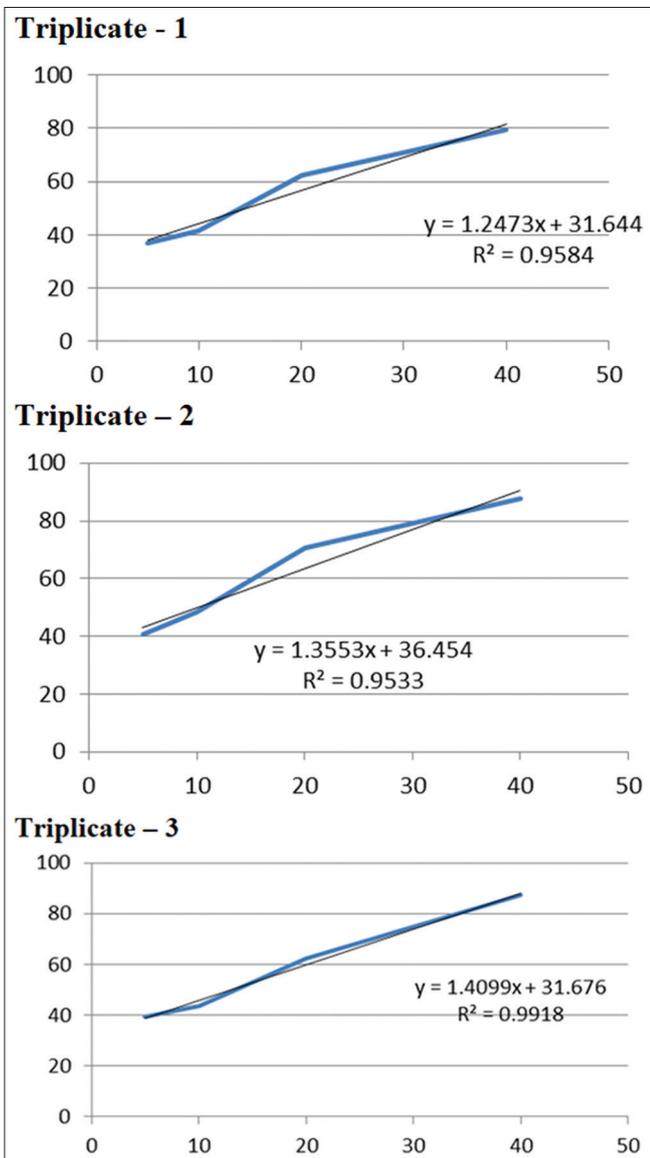
In vitro AChE enzyme inhibition assay – methodology [17]

AChE activity was measured employing a modified 96-well microplate assay supported Ellman’s method. Enzyme hydrolyses the substrate acetylthiocholine leading to the merchandise thiocholine which reacts with Ellman’s reagent (DTNB) to provide 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which might be detected at 412 nm. 50 mM Tris-HCl pH 8.0 was used as a buffer throughout the experiment. AChE enzyme stock solution (518 U/ml) was stored at -80°C and therefore, the enzyme-dilution was done in 0.1% BSA within the buffer. DTNB was dissolved within the buffer containing 0.1 M NaCl and 0.02 M MgCl2. ATCI was dissolved in deionized water. Within the 96-well plates, 100 µl of three mM DTNB, 20 µl of 0.26 U/ml of AChE, and 40 µl of buffer (50 mM tris pH 8.0), to which 20 µl of test drug in various concentrations (25, 50, 100, 250, and 500 µg/ml) dissolved in a buffer containing no more than 10% methanol was added to the wells. After mixing, the plate was incubated for 15 min (25°C). The enzymatic reaction was initiated by the addition of 20 µl of 15 mM acetylthiocholine iodide and therefore the hydrolysis of acetylthiocholine was monitored by reading the absorbance every 5 min for 20 min at 412 nm. Physostigmine (5, 10, 20, and 40 µg/ml) was used because of the positive control. All the reactions were performed in triplicate.

RESULTS AND DISCUSSION

AChE activity has been shown to be increased within and around amyloid plaques to develop the assembly of amyloid beta-peptides into fibrils and to extend the cytotoxicity of those peptides. The results obtained from the four extracts of Muthu Parpam against AChE enzyme inhibition activity and also the percentage inhibition was evaluated and tabulated (Tables 1 and 2). Muthu parpam showed very potent inhibition (71.68 ± 2.38%) at the concentration of 500 µg/ml in comparison with physostigmine (84.87 ± 4.78%) at the concentration of 40 µg/ml (Graph 1).

Physostigmine was first isolated from Calabar beans (*Physostigma venenosum*) in 1864 [18] and is an AChE inhibitor [19]. Although physostigmine can cross the blood brain barrier, this drug contains a narrow therapeutic index due to its short half-life and various side effects. The common side effects of physostigmine are hypersalivation, nausea, vomiting, and arrhythmia in arterial blood vessel patient and bradycardia [20]. Some studies have suggested that the AChE inhibitor, rivastigmine could also be fatal [21], while cholinesterase inhibitors



Graph 1: Percentage inhibition of standard - physostigmine on AChE enzyme inhibition assay

Table 1: Percentage inhibition of AChE enzyme by test drug - MP

The concentration of MP in µg/ml	Percentage inhibition of AChE enzyme by test drug
MP 25	14.71±4.24
MP 50	28.75±3.92
MP 100	36±3.78
MP 250	48.63±2.19
MP 500	71.68±2.38

Each value represents the mean±SD. n=3. MP: Muthu Parpam

Table 2: Percentage inhibition of AChE enzyme by standard drug

The concentration of physostigmine in µg/ml	Percentage inhibition of AChE enzyme by std drug
5	38.87±1.99
10	44.48±3.53
20	65.12±4.64
40	84.87±4.78

Each value represents the mean±SD. n=3

remain to be a vital therapeutic tool against AD. AChE inhibitors in contemporary medicines should be undertaken for the treatment to prevent the side effects.

This study revealed that the Muthu Parpam significantly inhibited AChE in a very dose-dependent manner. Notchi (*V. negundo*) juice employed in the preparation of Muthu Parpam has the pharmacological activity of anxiolytic and anti-amnesic [22,23]. Some pharmacological studies are reported that the Nilappanai kizhangu (*C. orchioides*) have the anticonvulsant activity and alleviate the cerebral ischemic injury in *in vivo* and *in vitro* experimental models [23,24]. Hence, adding these juices additionally enhance the cognitive function of the brain.

CONCLUSION

Aging is a natural and normal process; it is decaying the body continuously. Inappropriate aging may be preventable through principles suggested by ancient sages (Siddhars) which are called as *Noianugavidhi* (prevention of diseases). Promoting mental health state is that the need of the hour. Effectiveness of Muthu Parpam in the treatment of AD was proven through this study on the inhibition potential of acetylcholine choline esterase. It had been a preliminary screening for evaluating the efficacy. Hence, further clinical studies on Muthu Parpam should be necessary to position the footprints within the neurocognitive field.

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AUTHORS' CONTRIBUTION

The authors, Dr. N. Sabari Girija, Dr. S. Gupta, and Dr. M. A. Sinekha conceived of the presented idea. The corresponding author prepared the manuscript with the help of Dr. L. Sakthimanipriya. Experimental and manuscript work was supervised by Dr. P. Shanmugapriya. All the authors have equally contributed in preparing this manuscript.

CONFLICTS OF INTEREST

The authors have declared that they have no conflicts of interest in this study.

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