



Original Research Article

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Evaluation of acetylcholinesterase inhibitory activity of *Brahmi nei*, a *Siddha* formulation using *in - vitro* techniques.

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Abstract

Acetylcholine is the chief organic chemical responsible for the function of brain and various other activities of human body. It plays a major role in neurotransmission and performs its function in both Central nervous system and peripheral nervous system. Alzheimer's disease, Myasthenia gravis, Dementia are some of the worst illness caused due to the disturbances in the activity of acetylcholine. In our *Siddha* text many formulations have been mentioned for the treatment of neurological disorders. *Brahmi nei* (medicated ghee) is one among them and being used from ancient times to cure neurological disorders. The chief ingredient of this formulation is *Bacopamonnieri*. The prevalence of brain disorders are highly increasing nowadays and it seems as a worsen thing in society. In order to overcome these disorders a study was conducted in *Brahminei* to evaluate its acetylcholinesterase inhibitory activity using *in vitro* techniques. The results are confirming that *Brahminei* is having strong acetylcholinesterase inhibitory activity. This study creates the platform for the therapeutic use of *Brahmi Nei* to treat brain disorders in future.

Keywords: *Brahmi*, Herbal, Disorder, Medicated Ghee, Acetylcholine.

Introduction

Alzheimer's disease is a chronic neurodegenerative disease that destroys memory and other important mental functions. It is the most common cause of dementia that affects 60% to 70% of the people with dementia. Alzheimer's disease is characterised by loss of neurons and synapses in the cerebral cortex and certain subcortical regions. This loss results in gross atrophy of the affected regions, including

degeneration in the temporal lobe and parietal lobe, and parts of the frontal cortex and cingulate gyrus. Degeneration is also present in brainstem nuclei like the locus coeruleus. Alzheimer's is a progressive disease, in its early stages, memory loss is mild, as Alzheimer's advances through the brain it leads to increasingly severe symptoms, including disorientation, mood and behaviour changes; deepening confusion about events, time

and place; unfounded suspicions about family, friends and professional caregivers; more serious memory loss and behaviour changes and difficulty speaking, swallowing and walking. Although the speed of progression can vary, the average life expectancy following diagnosis is three to nine years.

Siddha system is one of the holistic system of medicine. Herbal sources of medicine in siddha system were not only effective for treating Alzheimer's disease, but also effective in the treatment of other neurological disorders such as myasthenia gravis, Down's syndrome etc. Recent studies suggested that cholinesterase inhibitors were effective drugs for treating mild to moderate AD. The present study was aimed to give effective Ach Einhibitor used by siddha system of medicine. The purpose of this study was to determine the in-vitro activity of *Brahminei* for AchE inhibition.

Materials and Methods

In-Vitro AChE enzyme Inhibition Assay

AChE activity was measured using a modified 96-well microplate assay based on Ellman's method enzyme hydrolyses the substrate acetylthiocholine 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 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 kept at -80°C and the enzyme-dilution was done in 0.1% BSA in buffer. DTNB was dissolved in the buffer containing 0.1 M NaCl and 0.02 M MgCl₂. ATCI was dissolved in deionized water. In the 96-well plates, 100 µl of 3 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 buffer containing not more than 10% methanol were 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 m Macetylthiocholine iodide and 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 as positive control. All the reactions were performed in triplicate.

Statistical analysis

Results are expressed as Mean ± SD.

Results

Absorbance of 96 well plate contains Control and Test drug BN reaction mixture

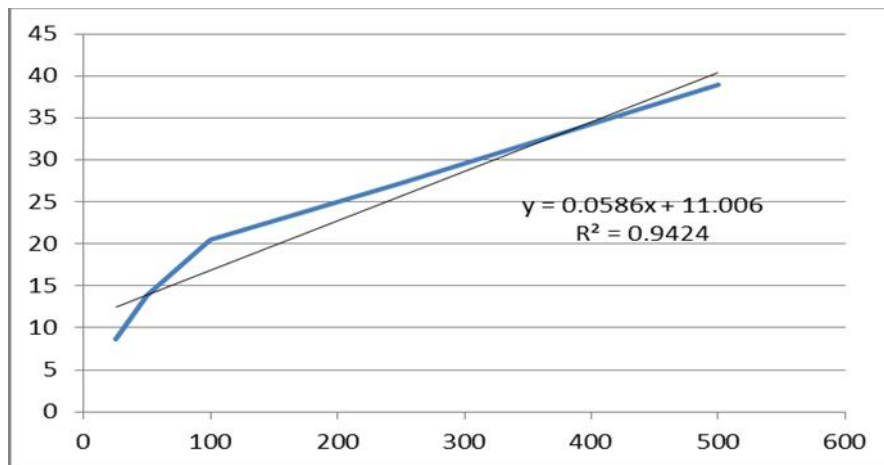


Absorbance of 96 well plate contains Standard (Physostigmine) - reaction mixture

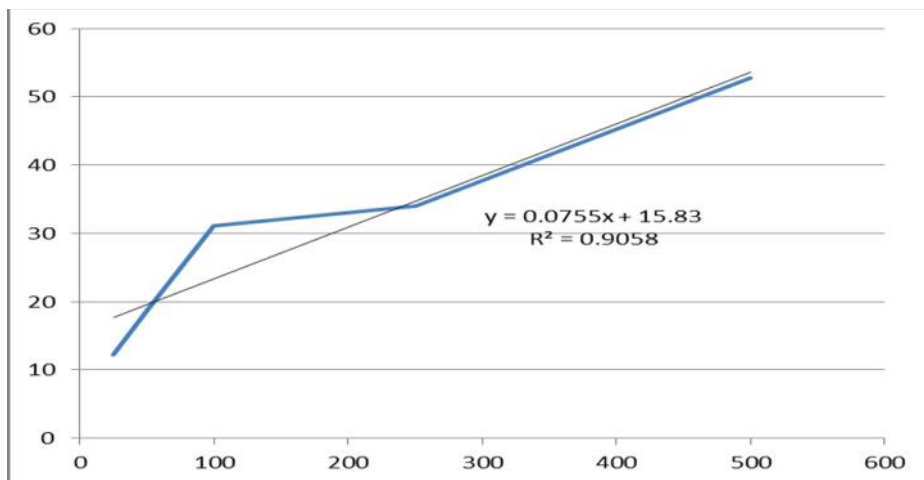


Percentage inhibition of BN on AChE Enzyme Inhibition assay

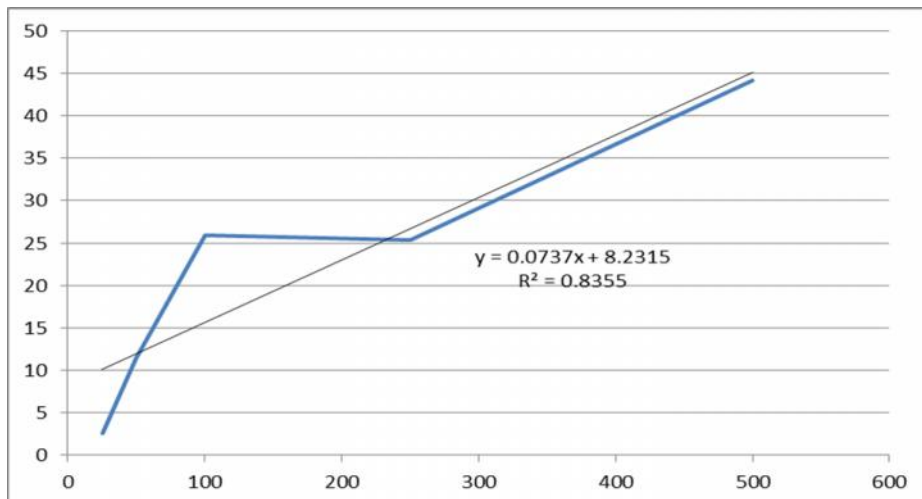
Triplicate – 1



Triplicate – 2

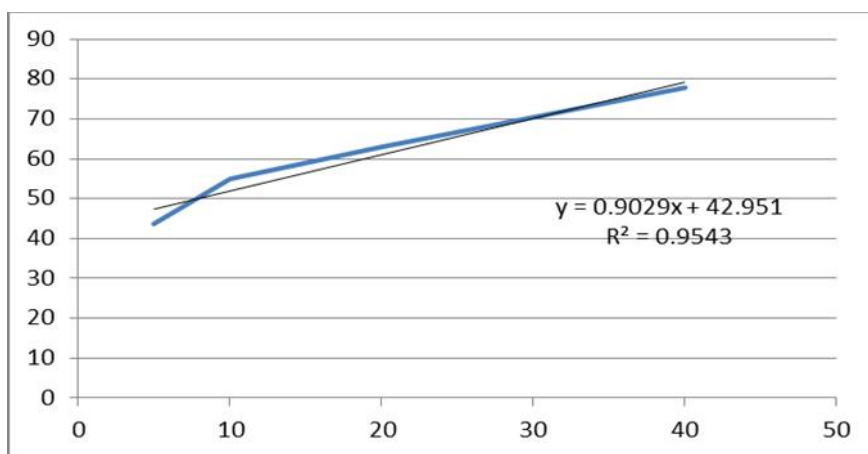


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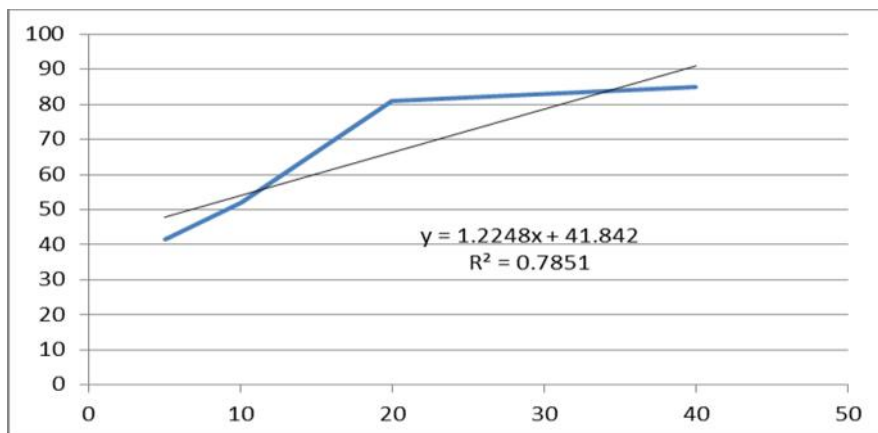


Percentage inhibition of Standard - Physostigmineon AChE Enzyme Inhibition assay

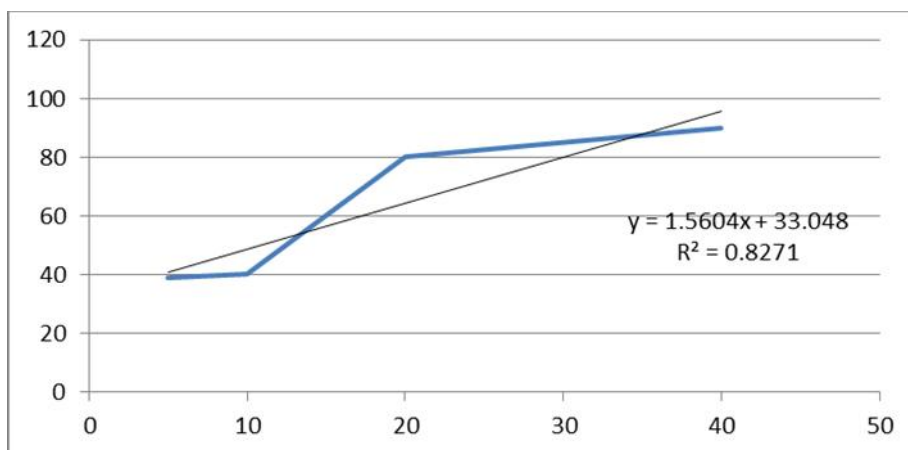
Triplicate – 1



Triplicate – 2



Triplicate – 3



Final Result

Percentage Inhibition of AChE Enzyme by Test Drug

Concentration of BN in µg/ml	Percentage Inhibition of AChE Enzyme by Test Drug
BN 25	7.81 ± 4.90
BN 50	14.74 ± 3.78
BN 100	25.85 ± 5.29
BN 250	28.83 ± 4.55
BN 500	45.28 ± 6.94

Each value represents the mean ± SD. N=3

Percentage Inhibition of AChE Enzyme by Standard Drug

Concentration of Physostigmine in µg/ml	Percentage Inhibition of AChE Enzyme by Std Drug
5	41.38 ± 2.44
10	49.04 ± 7.49
20	74.74 ± 10.2
40	84.16 ± 6.02

Each value represents the mean ± SD. N=3

Discussion

The results indicate that *Brahmi nei* at the concentration of 500 µg/ml produced maximum inhibition (45.28 %) when compared to that of the Physostigmine (known AChE Inhibitor) with showed maximum inhibition of 84.16 % at the concentration of 40 µg/ml. Already some research

findings explained that BM has some memory enhancing capacity. Thus it is clear that *Brahmi nei* has immense potential in the amelioration of cognitive disorders, as well as prophylactic reduction of oxidative damage, NT modulation and cognitive enhancement in healthy people.

Conclusion

From the results of the study it was concluded that the test drug *Brahmi Nei* possess convincing AChE enzyme inhibition property.

Acknowledgements

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