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ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF THANEERVITTAN NEI, A NEUROPROTECTIVE SIDDHA FORMULATION USING INVITRO TECHNIQUE

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ABSTRACT
Neurodegenerative disorders like Alzheimer's disease (AD) is a main cause of dementia, accounting for up to 75% of all dementia cases and has become a population aging-related concern for policymakers and public health systems around the world. Natural products have already proven to be promising sources of useful acetylcholinesterase (AChE) inhibitors. The currently approved drugs for AD, galantamine and rivastigmine are plant derived alkaloids which offer only symptomatic relief without preventing the progression of the disease. In search of identifying new AChE inhibitors preferably from siddha system of traditional medicine which is well tolerated and high efficacious on nervous system. The present study aimed at exploring the potential of siddha formulation ThaneervittanNei (TVN) for its acetylcholinesterase (AChE)enzyme inhibition property by usingEllman's method. The result obtained from the present clearly indicates that the test drug TVN was effective in inhibiting AChE enzyme at stipulated concentration dose dependently. Maximum percentage inhibition of about 47.25% was observed at 500 µg/ml with the IC 50 value of 503.4±49.17 µg/ml when compare to that of the standard drug physostigmine, a known AChE Inhibitor with the maximum inhibition 86.62% at the concentration of 40 µg/ml with the IC 50 value of 10.85 ± 1.381 µg/ml. It was concluded from the observation of the present study that formulation like TVN has a tendency to inhibit AChE enzyme and may tend to halt the progression of neurodegeneration in near future.

KEYWORDS: Siddha, ThaneervittanNei, Alzheimer's disease, acetylcholinesterase (AChE) inhibitor, Physostigmine.

1. INTRODUCTION
Alzheimer's disease (AD) is a progressive neuro-degenerative disorder associated with memory impairment and cognitive deficit. It is characterized by low levels of acetylcholine in the brain of AD patients. According to the cholinergic hypothesis, the inhibition of acetylcholinesterase (AChE), an enzyme that catalyzes acetylcholine hydrolysis, increases the levels of acetylcholine in the brain, thus improving cholinergic functions in AD patients.[1]

As of 2010, there were an estimated 35.6 million people with dementia worldwide. According to AD international report, this number will nearly double every 20 years to an estimated 65.7 million in 2030 and 115.4 million in 2050. As of 2011, the prevalence of the disease in India was said to be one in 20 for people over 60 years, and one in five for people over 80 years.[2] There are several strategies to ameliorate AD, although the one that has been most successful so far is the "cholinergic hypothesis". The drugs approved for the AD therapy act by counteracting the acetylcholine deficit, that is, they try to enhance the acetylcholine level in the brain. Inhibition of acetylcholinesterase (AChE) plays a key role not only in enhancing cholinergic transmission in the brain, but also in reducing the aggregation of amyloid-beta (Aβ) peptide and the formation of the neurotoxic fibrils in AD. Currently available AChE inhibitors such as tacrine, donepezil, rivastigmine, and galantamine, are found to be effective to treat mild to moderate AD only. Although around 40-70% patients benefit from AChE inhibitors.[3]

There is a considerable amount of drug research that is currently taking place to discover effective therapeutic treatments for AD. A range of molecules targeting amyloid beta (Aβ) aggregation, β, γ-secretases, which are involved in amyloid precursor protein (APP) processing, and modulators of tau phosphorylation are under various phases of clinical trials.[4] In spite of these clinical

strategies, cholinesterase inhibitors (ChEI's) are the first pharmacological treatments for AD to be approved by the US Food and Drug Administration (FDA). Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes play an important role in the pathophysiology of AD by hydrolyzing the neurotransmitter acetylcholine (ACh) thereby disrupting the synaptic transmission.[5]

Indian system of traditional medicine like siddha plays pioneering role in managing cognitive disabilities in mankind since several centuries. Thaneervittannei is novel siddha preparation which has been indicated for treating neural debilities as listed in the standard literature. Hence the main aim of the present study is to explore the AChE enzyme inhibition potential of the formulation TVN using in-vitro acetylcholinesterase (AChE) enzyme inhibition property by usingEllman's method.

2. MATERIALS AND METHODS[6,7]

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 mMTris–HCl pH 8.0 was used as a buffer throughout the experiment. AChE enzyme stock solution (518 U/ml) was kept at -80^{o}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 mMtris pH 8.0), to which 20 µl of test drug in various concentrations (25, 50, 100, 250 and 500 µg/ml) dispersed in buffer containing not more than 10% methanol were added to the wells. After mixing, the plate was incubated for 15 min (25^{o}C). The enzymatic reaction was initiated by the addition of 20 µl of 15 mMacetylthiocholine 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.

2.4. Statistical Method

The statistical analysis was carried by one-way analysis of variance ANOVA (GRAPH PAD PRISM 5 computer program). Results are expressed as ±SD.

3. RESULTS

3.1. Effect of Siddha Drug Tvn On Percentage Inhibition Of Ache Enzyme Activity

The result obtained from the present clearly indicates that the test drug TVN was significantly effective in inhibiting AChE enzyme at stipulated concentration dose dependently. Maximum percentage inhibition of about 47.25±3.63% at the concentration of 500 µg/ml with the IC 50 value of 503.4 ± 49.17 µg/ml. As shown in table 1 and 3.

Table 1: Percentage Inhibition of AChE Enzyme by Test Drug – TVN.

Concentration of TVN in µg/ml	Percentage Inhibition of AChE Enzyme by Test Drug
TVN 25	11.57 ± 2.604
TVN 50	19.05 ± 1.359
TVN 100	26.91 ± 2.959
TVN 250	37.07 ± 1.932
TVN 500	47.25 ± 3.63

Each value represents the mean ± SD. N=3

3.1. Effect of Standard Drug Physostigmine on Percentage Inhibition of AChE Enzyme activity

The result obtained from the present clearly indicates that the standard drug physostigmine was effective in inhibiting AChE enzyme at stipulated concentration dose dependently. Maximum percentage inhibition of about 86.62 ± 4.448% at the concentration of 40 µg/ml with the IC 50 value of 10.85 ± 1.381µg/ml. As shown in table 2 and 3.

Table 2: Percentage Inhibition of AChE Enzyme by Standard Drug Physostigmine.

Concentration of Physostigmine in µg/ml	Percentage Inhibition of AChE Enzyme by Std Drug
5	39.91 ± 2.261
10	46.95 ± 1.341
20	69.3 ± 4.343
40	86.62 ± 4.448

Each value represents the mean ± SD. N=3

Table 3: IC 50 Value of test and Standard Drug.

IC 50 Value of test drug TVN	503.4 ± 49.17 µg/ml
IC 50 Value of Std drug Physostigmine	10.85 ± 1.381µg/ml

4. DISCUSSION

A world-wide survey carried by Alzheimer's disease international (ADI) which is a worldwide federation of Alzheimer disease, came to the conclusion that 24.3 million people had dementia, in which 4.6 million new cases of dementia occur every year. The epidemiologists

forecast expected that the number of people affected would double every 20 years and will be around 81 million by the year 2040. The group noted that most people with dementia live in developing countries (60 per cent in 2001, rising up to 71 per cent by 2040). Rates of increase are not uniform; numbers in developed countries are considered to increase by several fold between the years 2001 to 2040, but by more than three-fold in India, China and their south Asian and western Pacific neighbor's.[9,10]

Oxidative stress is the result of an imbalance between pro-oxidant and antioxidant homeostasis that leads to the generation of toxic reactive oxygen species (ROS).[11] Compared to other parts of our body, the central nervous system (CNS) is more sensitive to oxidative stress due to its high oxygen consumption and lipid content. Increased oxidative stress in the CNS will further lead to lipid peroxidation, DNA and protein damage.[12] Oxidative stress in the CNS has been demonstrated to involve excitotoxicity and apoptosis, the two main causes of neuronal death. Furthermore, oxidative stress has also been implicated the progression of Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS) and other neurodegenerative diseases.[13,14] Antioxidants may have a positive effect in the CNS and seem to be a promising approach of neuroprotection therapy, as they can protect the CNS against free radical mediated oxidative damage.[15]

AChE is an enzyme splitting the neurotransmitter acetylcholine in cholinergic synapsis into choline and acetic acid.[16] Sensitivity of AChE to neurotoxic compounds (anti-Alzheimer's drugs, pesticides, and nerve agents) can be use in their measurement.[17] Commonly used method is based on reaction of thiocholine, formed from acetylthiocholine during enzymatic hydrolysis, with Ellman's reagent producing yellow 5-thio-2-nitrobenzoate measurable by spectrophotometry in 412 nm.[18] One of the best approach in devastating AD is based on accelerating decomposition of available of acetylcholine which mainly improves the pathological symptom.[19,20] Inhibition of AChE is a promising strategy to develop novel and causal therapeutics in AD treatment.[21,22] AChE inhibitors are one of the most intensively probed categories of compounds in seeking an effective treatment of AD.[23,24]

Some of the AChE inhibitor molecules currently being evaluated in clinical studies, such as memoquin targets different additional pathways of AD by exhibiting antioxidant functions, acting as β-secretase inhibitor, preventing Aβ aggregation and influencing tau hyperphosphorylation.[25] Natural drug candidates with antiamyloidogenic and antioxidant properties in addition to cholinesterase inhibitory activity could be regarded as especially desirable.

Herbal preparations with medicinal values are the resource for simple to complex secondary metabolites with impending therapeutic applications. These metabolites are effectively known to prevent several chronic diseases including AD, through different mechanisms like prevention of oxidative stress, inhibition or modulation of enzymes and receptor, interfering with the cellular signals, and so on. The result obtained from the present study clearly indicates that the test drug TVN was effective in inhibiting AChE enzyme at stipulated concentration dose dependently. Maximum percentage inhibition of about 47.25% was observed at 500 µg/ml with the IC50 value of 503.4±49.17 µg/ml when compare to that of the standard drug physostigmine, a known AChE Inhibitor with the maximum inhibition 86.62% at the concentration of 40 µg/ml with the IC 50 value of 10.85 ± 1.381 µg/ml.

CONCLUSION

The primary therapeutic stratagem against AD till date engrosses the use of cholinesterase inhibitors (ChEIs) which increase residual cholinergic activity, improve cognition and global performance, and reduce behavioral disturbances. The result obtained from the present clearly indicates that the test drug TVN was effective in inhibiting AChE enzyme at stipulated concentration dose dependently. Maximum percentage inhibition of about 47.25% was observed at 500 µg/ml with the IC50 value of 503.4±49.17 µg/ml. The potency of the formulation might be due to presence of bioactive therapeutics with powerful antioxidant property which need to explored at molecular level in near future.

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