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In-Vitro Acetylcholine Esterase Enzyme Inhibition Potential of Siddha Formulation Vilvaver Chooranam: A Neuroprotective Assay

Research Article

Sureka A[1], Glara A F[1], Sabari Girija N[1*], Ramamurthy M[2], Christian GJ[3], Meenakumari R[4]

1. Resident Medical Officer, 2. Associate Professor, 3. Professor, Department of Noinaadal, 4. Director, National Institute of Siddha, Tambaram Sanatorium, Chennai, Tamil Nadu, India.

Abstract

Background and Aim: Alzheimer's disease (AD) is a progressive neurodegenerative condition evidenced by significant cognitive dysfunction. The state of cognitive impairment is made worse by increased levels of the enzyme acetylcholinesterase (AChE), which is crucial in the hydrolysis of the neurotransmitter acetylcholine (ACh). Siddha therapy gaining higher momentum in recent days due to its global acceptance considering its broad spectral safety and therapeutic window. Siddha originated from the southern geographic landscape of Asia now spreading its wings across the bounders in managing dreadful diseases like AD. The main objective of the present study is to evaluate AChE inhibition of the Siddha formulation *Vilvaver Chooranam* (VVC). Materials & Methods: *In-Vitro* Acetylcholine esterase enzyme inhibition Potential of Siddha formulation *Vilvaver Chooranam* by Ellman's method. Results:Results obtained from the study clearly demonstrate that the formulation VVC has shown promising acetylcholinesterase at stipulated concentration dose-dependently. Maximum percentage inhibition of about 54.53 ± 3.475 % was observed at 500µg/ml with the IC_{50} value of 411.9 ± 30.6 µg/ml when compared to that of the Physostigmine, a known AChE Inhibitor with a maximum inhibition 93.44 ± 4.434 % at the concentration of 40µg/ml with the IC_{50} value of 10.38± 5.29 µg/ml. Conclusion: These findings demonstrate the remarkable potential of these extracts as valuable sources of antioxidants with interesting acetylcholinesterase inhibitory activity.

Keywords: *Alzheimer's disease, Siddha, Vilvaver Chooranam,* Acetylcholinesterase, Ellman's method.

Introduction

Alzheimer's disease (AD) is the most common form of dementia and a neurodegenerative illness that affects roughly 30 million people all over the world (1). AD is a progressive clinical condition that causes a slow and steady decline of the central nervous system (CNS). 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). Clinical symptoms of AD include worsening of language function, dyspraxia, agnosia, and impairment in executive routine activities (3). Other symptoms include a reduced capacity to learn new knowledge and retain old information. Some of the neuropathological changes that can be seen include a reduction in the number of neurons, neurofibrillary tangles, senile neurotic plaques, and varied amyloid angiopathy. There is a significant drop in the levels of acetylcholine and a number of other neurotransmitters and neuromodulators as a result of the neurochemical alterations that take place (4). There have been a number of hypotheses put forward in an effort to explain what causes the disease, one of which is that it is caused by misfolded and aggregated proteins, such as amyloid beta and tau (5). However, the "cholinergic hypothesis" is the theory which is most widely accepted.

Acetylcholinesterase (AChE) is a critical enzyme in the cholinergic nervous system. The majority of treatments aimed at reversing the cholinergic deficit seen in AD are based on inhibitors of the enzyme AChE, which boost cholinergic transmission but have only limited and fleeting therapeutic effects. Several investigations have shown that cholinesterase inhibitors are capable of acting on multiple therapeutic targets, including inhibition of the beta-amyloid plaques, promotion of antioxidant activity, and modification of the processing of Amyloid Precursor Protein (APP) (6). Despite this, there is still a demand for new AChE inhibitor lead compounds that have a lower level of toxicity and a higher level of penetration into the CNS. Several different types of plant-derived natural compounds have been examined as prospective novel AChE inhibitors that could be effective for the treatment of AD (7).

In recent decades, there has been a trend toward selecting "back-to-nature" medicines, which has contributed to the rise in the popularity of traditional medicine. Traditional medicine, which is derived from a wide variety of plants used for therapeutic purposes, is

*** Corresponding Author:**
Sabari Girija N
Assistant Professor,
National Institute of Siddha,
Tambaram Sanatorium,
Chennai. 600047, Tamil Nadu, India.
Email Id: drgirijakrish@gmail.com

being practiced in many nations across the globe to treat a wide range of illnesses and disorders (8). According to a report by the WHO, nearly eighty percent of the world's population makes use of nutritional supplements and nutraceutical traditional medicine. This occurs primarily in developing countries due to the extraordinary pharmacological potential and low toxicity of nutritional supplements and nutraceutical traditional medicine, as well as the rarity of side effects. The practice of traditional medicine has led to the discovery of a large number of pharmaceutical substances; therefore, this field may be a suitable place to start in the search for new treatments (9,10).

Siddha system of medicine offers considerable remedies for treating neurodegenerative disorders like Alzheimer's, the formulation in particular with single and polyherbal preparation of the indigenous *Siddha* system mediating excellent clinical improvement in patients with neurological disorders. One such potential *Siddha* formulation is *Vilvaver Chooranam* (VVC) as indicated in literature for its neuroprotective activity. Hence the main objective of the present research work is to investigate the acetylcholinesterase inhibitory activity of the *Siddha* drug VVC that could potentially be applied in the treatment of neurodegenerative disorders such as AD.

Materials and Methods

Ingredients of Vilvaver Chooranam

1. *Vilvaver* (Root of bael) – 100gm

Method of preparation

Purified root of *Vilvam* grinded and make it as the powder.

Indications: Strengthens the nerves.

In-vitro AChE enzyme Inhibition Assay

AChE enzyme inhibition activity of the *Siddha* formulation *Vilvaver chooranam* (VVC) was quantified and measured using a modified 96-well microplate assay that was based on Ellman's method (11). The enzyme hydrolyzes the substrate acetylthiocholine, producing thiocholine, which interacts with Ellman's reagent (DTNB) to create 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate, which can be detected at 412 nm. Throughout the experiment, a buffer of 50 mM Tris-HCl pH 8.0 was utilized. The AChE enzyme stock solution (518 U/ml) was stored at -80^{o}C, and the enzyme was diluted in 0.1% BSA in buffer. DTNB was dissolved in a solution of 0.1 M NaCl and 0.02 M $MgCl_2$. Deionized water was used to dissolve ATCI. At the 96-well plates, 100 µl of 3 mM DTNB, 20 µl of 0.26 U/ml AChE, and 40 µl of buffer (50 mM tris pH 8.0) were added to the wells, followed by 20 µl of test drug in various doses (25, 50, 100, 250, and 500 µg/ml) dissolved in buffer containing no more than 10% methanol. The dish was incubated for 15 minutes after being mixed (25^{o}C). The enzymatic process was started by adding 20 µl of 15 mM acetylthiocholine iodide, and the hydrolysis of acetylthiocholine was monitored by measuring the absorbance at 412 nm every 5 minutes for 20 minutes. As a positive control, physostigmine (5, 10, 20, and 40 µg/ml) was utilized. All reactions were carried out in triplicate (12).

Results

Effect of *Siddha* formulation VVC in AChE enzyme inhibition activity

The result obtained from the present study clearly indicates that the test drug VVC was effective in inhibiting the AChE enzyme at the stipulated concentration dose-dependently. Maximum percentage inhibition of about 54.53 ± 3.475 % was observed at 500 µg/ml with the IC 50 value of 411.9± 30.6 µg/ml. Results were depicted in table 1 and represented in figure 1.

Effect of physostigmine in AChE enzyme inhibition activity

Data from the current investigation has been compared with the standard reference physostigmine and the comparative investigation demonstrates that the drug physostigmine, a known AChE Inhibitor reveals maximum inhibition of 93.44 ± 4.434 % at the concentration of 40µg/ml with the IC 50 value of 10.38± 5.29 µg/ml. Results were depicted in table 2 and represented in figure 2.

Table 1: Effect of *Siddha* formulation VVC in AChE enzyme inhibition activity

Concentration of VVC in µg/ml	Percentage Inhibition of AChE Enzyme by VVC	IC 50 Value of VVC
VVC 25	3.366 ± 4.529	411.9± 30.6 µg/ml
VVC 50	13.53 ± 4.514	
VVC 100	23.13 ± 4.686	
VVC 250	41.61 ± 2.803	
VVC 500	54.53 ± 3.475	

Each value represents the mean ± SD. N=3

Table 2: Effect of standard physostigmine in AChE enzyme inhibition activity

Concentration of Physostigmine in µg/ml	Percentage Inhibition of AChE Enzyme by Std Drug	IC 50 Value of Std drug Physostigmine
5	33.74 ± 5.941	10.38± 5.29 µg/ml
10	50.41 ± 14.51	
20	74.47 ± 8.563	
40	93.44 ± 4.434	

Each value represents the mean ± SD. N=3

Figure 1: Percentage Inhibition of AChE Enzyme by the *siddha* formulation VVC

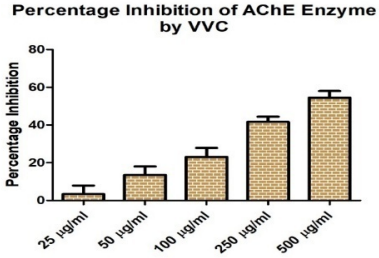
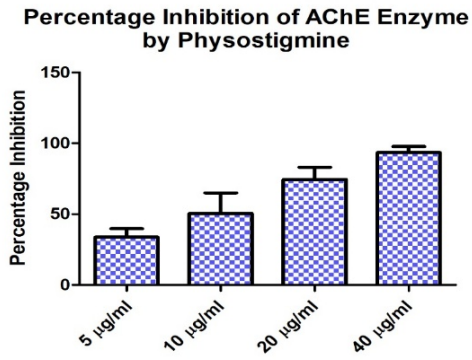


Figure 2: Percentage Inhibition of AChE Enzyme by the standard physostigmine



Discussion

The AChE enzyme is a promising target for the research and development of mechanism-based inhibitors, this could be due to hydrolysis of the neurotransmitter acetylcholine (13). AChE inhibitors like physostigmine, rivastigmine, donepezil, or galantamine are the most effective medications currently available to treat and counteract Alzheimer's type cognitive dysfunction (14). These drugs also have other putative therapeutic applications in the treatment of a wide range of neurodegenerative disorders other than AD.

Recent research on the prevention and treatment of AD gaining higher momentum towards naturally occurring AChE inhibitors from medicinal herbs, specifically compounds of polyphenolic and flavonoid origin revealing greater inhibitory capacity comparable to that of currently prescribed AChE inhibitors (15). The alkaloid galantamine is an excellent example of this type which is widely been utilized due to its safety and economic viability (16). Additionally, its antioxidant activity and strong metal chelator capability may also contribute to the decrease in oxidative stress that is associated with AD (17).

The formulation adopted for the present investigation was *Vilvaver Chooranam* made of dried root parts of the single potential herb called *Aegle marmelos (L.) Correa* also known as *vilvam* in Tamil, which is indicated for treating a considerable number of diseases listed in *Siddha* literature. The herb *Aegle marmelos (L.) Correa* is a woody tree, that belongs to the member of the Rutaceae family and may be found all over India, is more popularly referred to as the bael fruit tree. Traditional medicine employs it as a cure for a wide range of human conditions, including diarrhea, fever, diabetes, asthma, heart difficulties, ocular, hemorrhoids, and urinary disorders (18). The hypoglycemic impact of methanol extract and mucilage of *A. marmelos (L.) Correa* fruits was recently reported by (19), as were the chemical elements of the essential oil of *A. marmelos (L.) Correa*, which recorded potential antifungal and antibacterial activity. In addition, researchers identified two new cytotoxic alkaloids of the fluroquinolone class from the leaves of the *A. marmelos (L.) Correa* plant (20).

The secondary metabolites present in the plants produce are bioactive compounds that can be used to treat a variety of diseases. For instance, these compounds can be used to combat the damage that is brought on by reactive oxygen species, which can lead to a variety of human pathologies such as arthritis, cancer, inflammatory conditions, or heart disease (21). This oxidative stress is caused by a variety of factors, including chemical products, poisons, radiation, pollution, agricultural toxins, and food preservatives (22).

The bioactive components derived from phenols and flavonoids may have the ability to act as antioxidants by scavenging free radicals (23). Nowadays evaluation of naturally occurring antioxidants for use in pharmaceuticals (24) have become more focused on natural products and medications. The discovery of gallic acid and rutin from the herb *A. marmelos* (*vilvam*) bolstered the role of flavonoid and phenolic compounds in antioxidant activity (25). The result obtained from the present clearly indicates that the *Siddha* formulation VVC was effective in inhibiting the AChE enzyme at the stipulated concentration dose-dependently. Maximum percentage inhibition of about 54.53 ± 3.475 % was observed at 500µg/ml with the IC 50 value of 411.9± 30.6 µg/ml. To date there is no clear documentary evidence claiming the neuroprotective potential of root formulation made of *A. marmelos*. Hence, the outcome of the present investigation widens the scope of utilizing a formulation like VVC in the clinical management of AD in near future.

Conclusion

Alzheimer's disease (AD) is a neurological condition that progressively worsens over time and is the most common cause of dementia in elderly persons. Enhancers of the acetylcholine level in the brain, which is responsible for central cholinergic transmission, are the type of medications that are now utilized to treat Alzheimer's disease patients. The utilization of naturally occurring chemicals derived from plants as potential sources of AChE inhibitors becomes an appropriate method in the combating the adverse effects caused by conventional medications. The conclusion that can be drawn from the findings of the present investigation is that the *Siddha* formulation *vilvaver chooranam* was successful in suppressing the activity of the AChE enzyme dose dependently. Hence, neurotherapeutics with an herbal base promotes the promise of treating AD type dementia in near future.

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Conflict of Interest

The authors have declared conflicts of interest none.

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