Exploration of Neuroprotective Potential of Siddha Herbal Formulation Brammiyathi Bhavanai Chooranam Using In-Vitro Acetylcholinesterase Enzyme Inhibition Assay

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder that gets worse over time. It is characterised by the selective loss of cholinergic neurons in the basal forebrain, followed by cognitive and behavioural impairments that progressively disrupt daily activities, resulting in impaired memory and behaviour, as well as loss of intellectual, social, and eventually death. In 2010, 35.6 million people worldwide were estimated to have dementia. As per AD International analysis, the cases will roughly quadruple in 20 years once, reaching 65.7 million and 115.4 million in 2030 and 2050 respectively. Acetylcholinesterase (AChE) is a crucial enzyme which aggravates the deposition of amyloid protein and subsequent inflammation. AChE inhibition has been one of the common strategies for treating AD. The drugs available in the market are effective only against mild to moderate cases and have considerable side effects. Hence, the search for new options with more effectiveness and selective enzyme inhibitors with lesser side effects is necessary. Identifying structurally varied phytotherapeutics has emerged as one of the most current themes in novel medication discovery. This strategy clearly reduces the time and money required to produce a novel medicinal entity. Siddha system of medicine holds promising therapeutic potential with the availability of numerous viable formulations, but still, the majority of the preparation is not much explored for managing neurodegenerative disorders. The aim of this current research work is to explore the potential of the Siddha herbal drug Bhavani Chooranam (BBC) in AChE enzyme inhibition by in-vitro Ellman's screening technique. Based on the result obtained, it is obvious that BBC successfully suppresses the AChE enzyme dose-dependently. The inhibition level at a concentration of 500 µg/ml was very much effective and the value was approximately 57.33±4.44 %, the corresponding IC50 value was 397.3±39.18µg/ml in comparison with the standard drug Physostigmine. It was concluded from the overall outcomes of the present investigation that formulations like Brammiyathi Bhavani Chooranam may be an ideal drug of choice for managing neurodegenerative diseases like AD.

Keywords: Acetylcholinesterase, Alzheimer's disease, Brammiyathi Bhavanai Chooranam, Neurodegeneration, Siddha

1. Introduction

A well-known cause of dementia in a geriatric group of people is Alzheimer's disease (AD), a progressive neurodegenerative disorder. It was estimated that there are over 50 million people living with dementia all over the world in 2019 [1,2]. AD is one of several neurological disorders. The "cholinergic hypothesis" postulates that the neurodegenerative process is

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caused by a decrease in the amount of the neurotransmitter acetylcholine present in the brain. This is a theory that has not been proven to be correct. Enhancers of the acetylcholine level in the brain, which is important for central cholinergic transmission [3]; these are the medications that are now being utilised to treat AD patients. Acetylcholine, once it has been transported across neural synapses, is subjected to hydrolysis by acetylcholinesterase, which results in the formation of choline and an acetyl group [4]. Because of this, acetylcholine is not hydrolysed when inhibitors of acetylcholinesterase are used [5]. This allows acetylcholine to keep its function as a neurotransmitter. However, some of the existing drugs for the treatment of AD symptoms show hepatotoxicity [6], a higher risk of urinary incontinence [7], an increased risk of bradycardia and other cardiovascular adverse effects [8], pulmonary disorders, and involuntary weight loss [9]. Despite this, the search for new drugs that are more effective in brain penetration, less harmful, and with a high bioavailability continues.

The World Health Organization suggests using traditional items made from plants to treat a number of illnesses. Numerous factors, including many concerned organisations in the development and research of herbal-based medications, have contributed to the rise in the usage of traditional medicines [10,11]. Earlier research on the therapeutic benefits of herbal remedies significantly supports the therapeutic effectiveness of the phytocomponents in treating disorders by controlling cellular physiology.

The use of herbal remedies has been embraced in many developing countries for thousands of years as their indigenous system of medicine. Indian traditional medicine, which may be alternative medicine, records numerous rudimentary medicinal concoctions for the treatment of numerous ailments. These medications' compositions have a long history of using botanical extracts [12]. Different plant species have been employed as therapeutic options for a number of terrible ailments and have been a component of folk medicine in various civilisations [13].

The traditional system of medicine imposes tremendous medicinal benefits with viable therapeutic preparations since centuries together. Phytochemicals occupy considerable space in the management of dreadful diseases. Polyherbal formulations excel in the art of reversing physiology through a multidimensional approach. Siddha system of medicine has numerous formulations with the potential of managing neurodegenerative disease as documented in the literature. There are limited therapeutic options available for the clinical management of the neurodegenerative disease. The medications that are available on the market for treating symptoms come with a number of disadvantages, including adverse effects, low bioavailability and high cost [14]. The aim of this current study is to explore the inhibition potential of the Siddha herbal formulation Brammiyathi Bhavanai Chooranam (BBC) against the AChE enzyme by in-vitro screening technique.

2. Materials and Methods

In-vitro Assay on AChE enzyme Inhibition

The AChE enzyme inhibition activity of Brammiyathi Bhavani Chooranam (BBC), a Siddha formulation, was quantified and assessed using a modified 96-well microplate assay based on Ellman's approach [15]. AchE hydrolyzes acetylthiocholine to produce thiocholine, which, along with Ellman's reagent (DTNB), brings out the production of 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate, which may be detected at 412 nm. A buffer of 50 mM Tris-HCl with pH 8.0 was used throughout the experiment. The stock solution of AChE enzyme (518 U/ml) was stored at -80oC, and the enzyme was diluted in a buffer with 0.1% BSA. DTNB was dissolved in 0.1 M NaCl and 0.02 M MgCl2 solution. ATCI was suspended in deionized water. The wells were filled with 100 µl of 3 mM DTNB, 20 l of 0.26 U/ml AChE, and 40 l of buffer (50 mM tris pH 8.0), followed by 20 µl of test drug at different doses (25, 50, 100, 250, and 500 µg/l) dissolved in a buffer containing 10% methanol. After being combined, the dish was incubated for 15 minutes (25oC). The enzymatic activity was initiated by adding 20 µl of 15 mM acetylthiocholine iodide, and acetylthiocholine hydrolysis was observed by measuring absorbance at 412 nm every 5 minutes for 20 minutes. Physostigmine (5, 10, 20, and 40 µg/ml) was used as a positive control. All responses were performed in triplicate [16,17].

3. Results

Effect of *Brammiyathi Bhavanai Chooranam* in AChE enzyme inhibition activity

According to the result of the present study, it is abundantly obvious that BBC, the test drug was successful in suppressing the AChE enzyme dose-dependently. The inhibition level at a concentration of 500 µg/ml was very much effective and the value was approximately 57.33±4.44 %, the corresponding IC50 value was 397.3±39.18µg/ml. The findings are reported in table 1, and they are illustrated in figure 1.

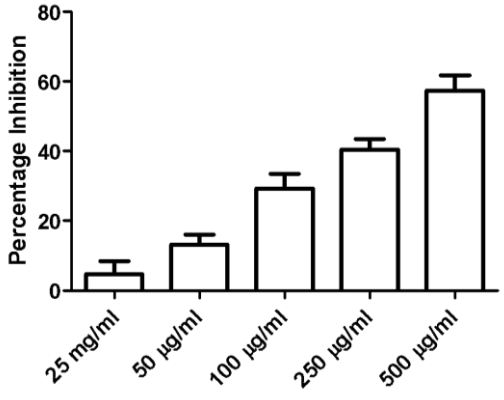

Figure 1: Percentage Inhibition of AChE Enzyme by the

siddha formulation BBC

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 utmost inhibition of 93.44 ± 6.487% at the concentration of 40 µg/ml with the IC 50 value of 12.5 ± 2.804 µg/ml. Results are depicted in table 2 and represented in figure 2.

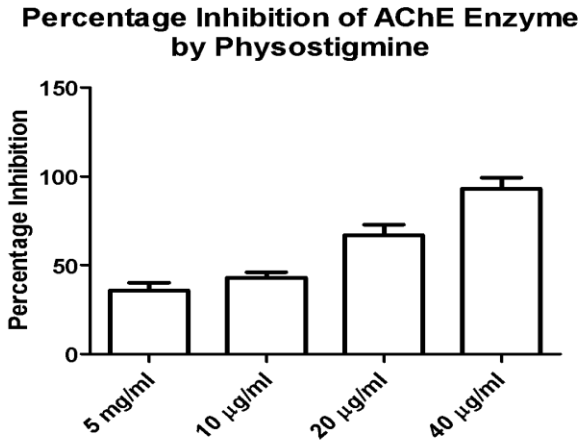

Figure 2: Percentage Inhibition of AChE Enzyme by the standard physostigmine

4. Discussion

Alzheimer's Disease (AD) is a neurological condition that affects the brain cells and impairs reasoning, memory, and behaviour [18]. Rarely is a suitable drug available that could return the brain's natural functions [19]. There is currently no acknowledged specialised management strategy for AD [20]. Serine hydrolases called acetylcholine esterase (AChE) are found in a number of bodily tissues. By hydrolyzing acetylcholine into choline and acetate, AChE stops signal transmission at cholinergic synapses [21]. In the tissues, AChE exhibits a sophisticated molecular diversity of quaternary structure. The catalytic activity of the various forms is comparable, but they vary in hydrophobic interactions and ionic or hydrodynamic characteristics [22].

Based on in-vitro studies, AChE induces the development of amyloid fibrils, which results in the formation of an extremely toxic complex to brain cells namely acetylcholinesterase-beta-amyloid peptide complex [23]. AD is caused by the deposition of these complexes as amyloid plaques in the brain [24]. Amyloid plaques make good therapeutic targets for Alzheimer's disease. Acetylcholinesterase (AChE) is widely accepted as a suitable target for methodical advancement in the treatment of AD. AChE inhibitor therapies are helpful in improving cognitive dysfunction and are most successful in improving perceptual responses [25].

The quest is on for novel compounds with anti-AChE activity since acetylcholinesterase (AChE) inhibitors are a crucial part of AD treatment. According to the cholinergic hypothesis, the enzyme AChE increases the levels of acetylcholine in the brain, enhancing cholinergic functions in people with AD and alleviating the symptoms. Because naturally-occurring compounds from plants are thought to be an excellent new source of inhibitors, numerous secondary metabolites of plant extracts with this ability have been discovered [26].

The traditional practice of siddha was used to restore a person's health and welfare. Despite the country's long history of using herbs, new technological advancements have explored the drug's exact mechanism of action. Secondary metabolites, which are biologically active medicines found in herbal remedies, have the potential to slow the spread of a variety of illnesses [27]. According to the result of the present study, it is abundantly obvious that the test drug BBC was successful in suppressing the AChE enzyme dose-dependently. At a concentration of 500 µg/ml, the level of inhibition that was found to be most effective was approximately 57.33 ± 4.44 %, while the IC 50 value was 397.3 ± 39.18µg/ml. The phytoconstituents isolated from medicinal plants have been proven to show evidence of a broad range of pharmacological activities. When it comes to the treatment of AD, the bioactive components that have an effect on the cholinergic function of the nervous system are extremely important.

5. Conclusion

Alzheimer's disease (AD) is a neurodegenerative disorder that gets worse over time. It is characterised by the selective loss of cholinergic neurons in the basal forebrain, followed by cognitive and behavioural impairments that progressively disrupt daily activities, resulting in impaired memory and behaviour, as well as loss of intellectual, social, and eventually death. The inhibition of AChE enzyme, which catalyses the breakdown of acetylcholine neurotransmitters, may be one of the most pragmatic approaches to the symptomatic treatment of AD. The conclusion that can be drawn from the findings of the present investigation is that the Siddha formulation *Brammiyathi Bhavanai Chooranam* was successful in inhibiting the activity of the AChE enzyme dose-dependently. Hence, neurotherapeutics from traditional siddha therapy extrapolate the promise of treating AD-type dementia in near future with the prior preclinical investigation.

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Table 1: Effect Of Siddha Formulation Bbc In Ache Enzyme Inhibition Activity		
Concentration of BBC in µg/ml	Percentage Inhibition of AChE Enzyme by BBC	IC 50 Value of BBC
BBC 25	4.742 ± 3.769	397.3±39.18µg/ml
BBC 50	13.14 ± 3.012	
BBC 100	23.91 ± 4.229	
BBC 250	40.44 ± 3.061	
BBC 500	57.33 ± 4.448	
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 Standard Drug	IC 50 Value of Std drug Physostigmine
5	36.5 ± 4.927	12.5 ± 2.804 µg/ml
10	43.95 ± 3.899	
20	67.44 ± 5.999	
40	93.44 ± 6.487	
Each value represents the mean ± SD. N=3		