



AN IN-VITRO STUDY TO SCREEN DOOSHIVISHARI AGADA FOR ITS BUTRYL CHOLENESTRASE ACTIVITY IN ALZHEIMER'S DISEASE

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ABSTRACT

Agadatantra is a specialized branch of Ayurveda which deals with the management of toxicity. The specialized branch has given the novel concept of Dooshivisha (cumulative poison) which is a transformable state of Visha (toxins) which can be attained by any type of poison. Dooshivisha (cumulative poison) is a concept which can be correlated with the etiological factors and pathogenesis of Alzheimer's disease in modern perspective. For the management of Dooshivisha various treatment procedures have been described, one among them is Dooshivishari. It is herbo-mineral formulation which is explained by Sushruta and Vagbhata. This is indicated in Dooshivisha (cumulative poison) and its complications, insect poisoning and other associated signs and symptoms. Recent understanding of Alzheimer's disease reveals the role of toxins as a potential etiological factors, where person is exposed to many toxins on daily basis like metals environmental pollutants, excessive use of fertilizers, cigarette smoking, Genetic and immunological factors leads to Alzheimer's disease^{1,2,3,4}. Many research works are carried out on the chemical constituents of the each ingredients of Dooshivishari Agada which supports Neuro-protective activity. Alzheimer's disease can be understood under the heading of Dooshivisha⁵. Alzheimer's disease is a neurodegenerative disorder that causes miss foldings in Amyloid plaques and cell death in the neurofibrillary tangles. This causes the memory loss that reduce a person's ability to do activity. Hence it is implicated that Dooshivishari Agada would possess Neuro-protective activity⁶, hence the present study has been taken.

KEY WORDS-Alzheimer's disease, Butryl cholenestrace Dooshivisha, Dooshivishari Agada, Gara visha, Visha

OBJECTIVES

- To screen the Inhibitory activity of Dooshivishari Agada against Butrylcholine esterase.

METHODOLOGY

- The Inhibition activity of Methanol extract Dooshivishari Agada was studied in vitro using BCHE. Acetylthiocholine iodide, Butrylcholinesterase, DTNB, Tacrin. Elman's reagent, was used for the evaluation of Inhibition activity.

RESULTS

In the present study, Dooshivishari Agada extract (Methanol) possess Inhibition activity in Butrylcholinestrace^{7,8}

INTERPRETATION AND CONCLUSION

The in-vitro study revealed that Dooshivishari Agada extract (Methanol) possess Inhibition activity in Butrylcholinestrace and thus Inhibitor Activity of Dooshivishari Agada on Butrylcholinestrace, In -Vitro Study is justified.

Introduction

Alzheimer's disease is the most common cause of degenerative dementias and accounts for 50%-60% of all cases of dementia. It is estimated that by the year 2020, approximately 70% of the world's population aged 60 and above will be living in developing countries, with 14.2% in India, In southern India prevalence of dementia including Alzheimer's Disease is about 4.86%⁹.

In modern science, Alzheimer's disease is treated with cholinergic & GSK 3 inhibitors, among cholinesterase inhibitors-Acetyl cholenestrace, is used, which may lack in beneficial effect in preventing disease progression based on clinical long term experience, as they increase tau phosphorylation. So there is a need for some modification in treating Alzheimer's disease, in



particularly, cognitive and memory dysfunctions. These inhibitors also result in side effects like falls syncope, elevated hepatic enzyme concentration, nausea, dizziness, headache, gastro-intestine symptoms, and rashes. *Dooshivishari Agada* is described for the treatment of *Dooshivisha*. It is found that all most all of the ingredients of *Dooshivishari Agada* are proven to possess activities like anti-stress activity, prevents loss of memory, and prevents hyperactive deep tendon reflexes⁶.

Dooshivishari Agada is described by *vagbhata* for the treatment of *Dooshivisha*. It is one of the formulations mentioned for the management of *Dooshi visha*. After reviewing the experimental study of herbs in *Dooshivishari Agada* it is found that all these herbs are useful in treating Alzheimer's disease

MATERIALS AND METHODS

Day Source

Ingredients of *Dooshivishari Agada*

Pippali (~ *Piper longum* Linn), Dhyamaka (~ *Cymbopogon martini* Wats), Jatamansi (~ *Nardostachys jatamansi* DC), Lodhra (~ *Symplocos racemosa* Roxb), Ela (~ *Elettariacardamomum* Maton), Suvarchika (~ *Tribulus terrestris* Linn), kutannata (~ *Oroxylum indicum* Linn), Nata (Tagara) (~ *Valeriana wallichii* DC), Kushta (~ *Saussurea lappa* CB Clarke), Yashti madhu (~ *Glycirhiza glabra* Linn), Chandana (~ *Santalum album* Linn) Gairika (~ Red ochre) are having Neuro protective activity.

All the 12 drugs of *Dooshivishari Agada* are collected from local market, taken in equal parts (10grams each) made into fine powder & formed homogeneous mixture.

Extraction – It is the first step to separate the desired natural products from the raw materials. Extraction methods include solvent extraction, distillation method, pressing and sublimation according to the extraction principle. Solvent extraction is the most widely used method. The extraction of natural products progresses through the following stages: the solvent penetrates into the solid matrix; the solute dissolves in the solvents; the solute is diffused out of the solid matrix; the extracted solutes are collected. Any factor enhancing the diffusivity and solubility in the above steps will facilitate the extraction. The properties of the extraction solvent, the particle size of the raw materials, the solvent-to-solid ration, the extraction temperature and the extraction duration will affect the extraction efficiency.

➤ Preparation of Extract

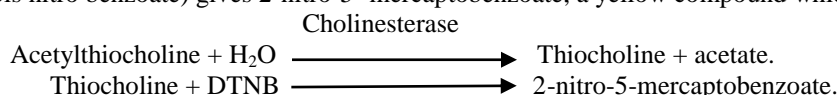
- Weighed 20g of dried Sample powder and dissolved in 100ml of Methanol / Water in 100ml beaker with aluminium foil covered on it.
- Then the beaker was kept on hot water bath at 50° C for 4 hours.
- After incubation period the extract was filtered with Whatmann filter paper and the filtrate was collected in 250ml beaker. Residue present over the filter paper was discarded and filtrate was taken for further use.
- Then the filtrate was kept at 50°C for few hours until the extract got completely dried and turned into semisolid form.
- This semi solid sample was weighed and the yield was noted.

Table 1: Yield summary after crude extraction

sample	Sample taken for Extraction	Solubility	Yield
<i>Dooshivishari Agada</i>	20g	Methanol	1967.5mg
	20g	Aqueous	1870.2mg

Principle

Butyryl cholinesterase is a hydrolase related to Acetyl cholinesterase that catalyses the hydrolysis of esters of choline including acetylcholine, butyrylcholine and succinylcholine as well as the hydrolysis of cocaine and acetylsalicylic acid. Butyryl cholinesterase hydrolyses acetylthiocholine to give Thiocholine and acetate. The reaction between Thiocholine and DTNB (Dithiobis nitro benzoate) gives 2-nitro-5-mercaptobenzoate, a yellow compound which can be measured at 412 nm.



Butryl cholenestrace study¹⁰:

1. Acetylthiocholine iodide (CAS NO: 1866-15-5): store at 2 - 8°C, Butyrylcholinesterase (CAS NO: 9001-08-5): store at 2 - 8°C, Tacrine: store at 2-8°C, Elman's reagent: DTNB (Dithiobis nitro benzoate) store at room temperature.
2. Sodium dihydrogen phosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$), Disodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$)

**Preparation of working solution****Phosphate buffer (50 mM) pH 7.7**

(A) Sodium dihydrogen phosphate – 0.78g in 100mL of de-ionized water.

(B) Disodium hydrogen phosphate – 0.89g in 100mL of de-ionized water.

Mix 11 mL of solution A with 89 mL of solution B and make up to 200 mL with de-ionised water.

Elman's reagents: 3.95 mg DTNB dissolved in 50mL phosphate buffer pH7.7 (0.25 mM)

Tacrine stock: 20mg/ml

Equipment's: Incubator, Plate Reader

Procedure

The Bche enzyme was incubated with various concentrations of test compounds in Microtiter well and incubated for 5 min. Then 100µM Acetylthiocholine iodide was added to each Microtiter well. The contents were further incubated for 5 mins. After incubation, 180µl of DTNB reagent from the stock of 10mg/ml was added. The absorbance was measured at 412nm.

Calculations:

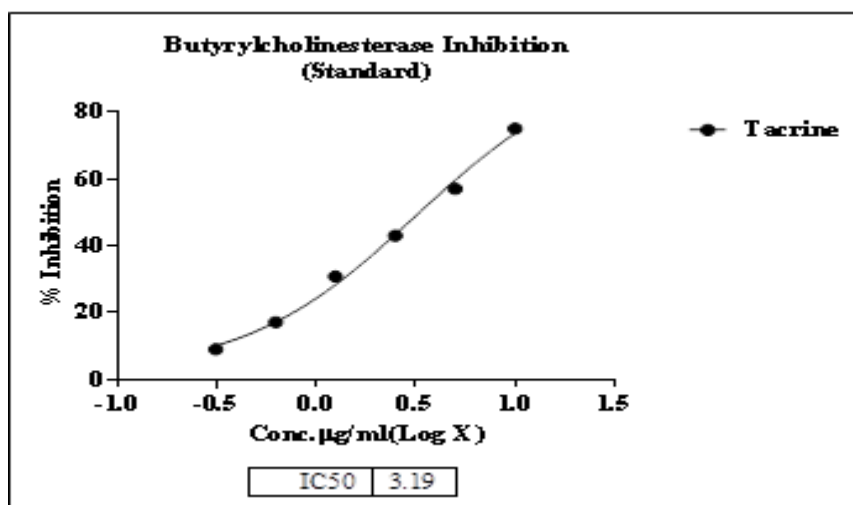
% Inhibition = ((Control O.D- Sample O.D)/ Control O.D) *100

RESULTS

Test samples *Dooshivishari Agada* (Methanol) and (Aqueous) extracts were tested for Bche inhibitory activity using colorimetric method in 96 well plates. The results are show in Table 1 and Table 2 for Tacrine and test samples respectively. The Methanol extract has showed better activity with an IC50 value of 96.09µg/ml followed by the sample Aqueous extract with IC50 value of 124.4µg/ml. Tacrine used as the standard Bche inhibitor showed an IC50 of 3.19µg/ml.

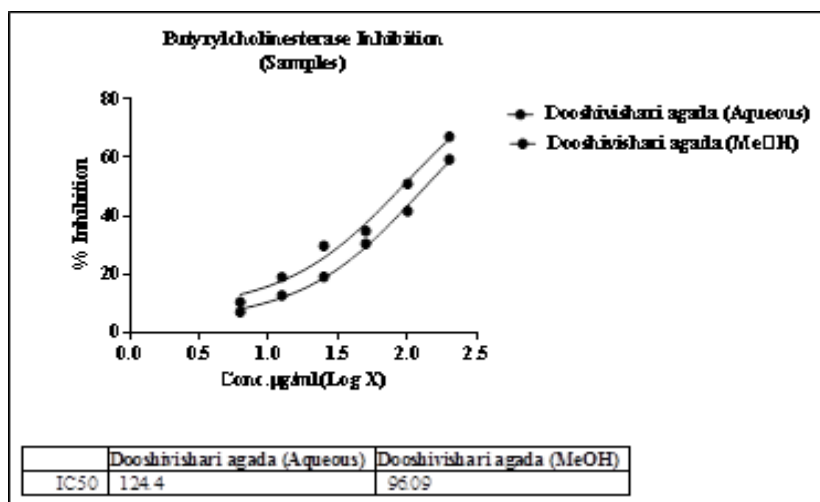
Table 1: Butyryl cholinesterase Inhibition by Standard (Tacrine)

Samples	Conc µg/ml	Abs at 412nm	% Inhibition	IC50 Value in µg/ml
<i>Control</i>	0	0.765	0.00	
<i>Tacrine</i>	0.3125	0.703	8.21	3.19
	0.625	0.634	17.17	
	1.25	0.529	30.84	
	2.5	0.406	46.99	
	5	0.329	57.06	
	10	0.228	70.21	

**Graph 1: Graph of Butyryl cholinesterase Inhibition by Standard (Tacrine)**

**Table 2: Butyryl cholinesterase Inhibition by Samples**

Samples	Conc $\mu\text{g/ml}$	Abs at 412nm	% Inhibition	IC50 Value in $\mu\text{g/ml}$
Control	0	0.765	0	
<i>Dooshivishari Agada (Aqueous)</i>	6.25	0.710	7.19	124.4
	12.5	0.667	12.82	
	25	0.619	19.14	
	50	0.532	30.52	
	100	0.447	41.66	
	200	0.312	59.27	
<i>Dooshivishari Agada (Methanol)</i>	6.25	0.684	10.58	96.09
	12.5	0.619	19.13	
	25	0.538	29.74	
	50	0.499	34.85	
	100	0.375	50.97	
	200	0.252	67.02	

**Graph 2: Graph of Butyryl cholinesterase Inhibition by Samples**

DISCUSSION

The cause of Alzheimer's disease is not known, however several factors are thought to be included in this disease-Neuro chemical factors like Butryl-choline, nor-epinephrine,-Environmental factors like metals,environmental pollution and excessive use of fertilizers and hazardous toxic chemicals during the production of food materials cigarette smoking¹¹. Hence Alzheimer's disease can be included under *Dooshivisha*. *Dooshivishari Agada* helps in the management of *Dooshivisha*. *Dooshivisha* & also having immune modulatory effect. Absorption of drugs occurs quickly in a detoxified body so the use of *Dooshivishari Agada* can do their work effectively. It is found that there is inhibitory activity of *Dooshivishari Agada* in Butryl cholenestrse Ellman's method for Alzheimer's disease- 124.4 IC50 Value in $\mu\text{g/ml}$ in Aqueous extract & 96.09 IC50 Value in $\mu\text{g/ml}$ in Methanol extract.

CONCLUSION

It is proved from Ellman's method that there is inhibitory activity of *Dooshivishari Agada* in Alzheimer's disease, Hence this Disease may be considered to treated with *Dooshivishari Agada* also.

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