ORIGINAL ARTICLE

An *in vitro* study to screen *Dooshivishari Agada* for its acetyl cholinesterase activity in Alzheimer's disease

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Abstract

Aim: The aim of this study was to screen the Inhibitory activity of *Dooshivishari Agada* against acetylcholine esterase. **Material and Methods**: The inhibition activity of methanol extract *Dooshivishari Agada* was studied *in vitro* using acetyl cholinesterase (ACHE). Acetylthiocholine iodide, ACHE, dithiobis nitro benzoate, donepezil, and Elman's reagent were used for the evaluation of inhibition activity. **Results and Discussion:** Test samples *Dooshivishari Agada* (MeOH) extract were tested for ACHE inhibitory activity using colorimetric method in 96-well plates. The results for donepezil and methanol extract of *Dooshivishari Agada* are – methanol extract has showed better activity with an IC_{50} value of 62.37 µg/mL followed by the sample aqueous extract with IC_{50} value of 106.8 µg/mL. Donepezil used as the standard ACHE inhibitor showed an IC_{50} of 1.41 µg/mL. **Conclusion**: The *in vitro* study revealed that *Dooshivishari Agada* extract (Methanol) possess inhibition activity in acetylcholinestrase and thus inhibition activity of *Dooshivishari Agada* on acetylcholinestrase. *In vitro study* is justified.

Key words: Acetyl cholinesterase, Alzheimer's disease, *Doosivishari*, *Dooshivishari Agada*, Gara visha, Visha

INTRODUCTION

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 such as metals environmental pollutants, excessive use of fertilizers, cigarette smoking, and Genetic and immunological factors which lead to Alzheimer's disease.[1-4] Many research works are carried out on the chemical constituents of the each ingredients of *Dooshivishari Agada* which supports neuroprotective activity. Alzheimer's disease can be understood under the heading of *Dooshivisha*. [5] 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 neuroprotective activity;[6] Objective of this study was to screen the inhibitory activity of *Dooshivishari Agada* against acetylcholine esterase Methanol extract of *Dooshivishari Agada* possess inhibition activity in acetylcholinestrase.[7,8] therfore, the present study has been taken.

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

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countries, with 14.2% in India, In Southern India, the prevalence of dementia including Alzheimer's disease is about 4.86%.[9]

In modern science, Alzheimer's disease is treated with cholinergic and GSK 3 inhibitors, among cholinesterase inhibitors-Acetyl cholinesterase, are used, which may lack in beneficial effect in preventing disease progression based on clinical long-term experience, as they increase tau phosphorylation. Hence, 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 such as falls syncope, elevated hepatic enzyme concentration nausea, dizziness, headache, gastrointestine 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 such as anti-stress activity, prevents loss of memory, and prevents hyperactive deep tendon reflexes.[6]

Dooshivishari Agada is described by *vagbhata* for the treatment of *Dooshivisha*. It is one of the formulations mentioned for the management of *Dooshivisha*. 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

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

Ingredients of *Dooshivishari Agada*

Pippali *(~Piper longumlinn*), Dhyamaka *(~Cymbopogon martini wats*), Jatamansi *(~Nardostachys jatamansi DC*), Lodhra *(~Symplocos racemose Roxb*), Ela *(~Elettaria cardamomum Maton*), Suvarchika *(~Tribulus terrestris* Linn.), Kutannata *(~Oroxylum indicum* Linn.), Nata (Tagara) *(~Valeriana wallichii DC*), Kushta *(~Saussurea lappa CB Clarke*), Yashtimadhu *(~Glycirhiza glabra Linn*), and Chandana *(~Santalum album Linn*) Gairika *(~Red ochre*) are having neuroprotective activity.

All the 12 drugs of *Dooshivishari Agada* are collected from local market, taken in equal parts (10 grams each) made into fine powder, and 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; and 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 20 g of dried sample powder and dissolved in 100 mL of methanol/water in 100 mL beaker with aluminium foil covered on it
- Then, the beaker was kept on hot water bath at 50°C for 4 h
- After incubation period, the extract was filtered with Whatmann filter paper and the filtrate was collected in 250 mL 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.

Principle

Acetyl cholinesterase hydrolyses acetylthiocholine to give thiocholine and acetate. The reaction between thiocholine and Dithiobisnitrobenzoate (DTNB) gives 2-nitro-5- mercaptobenzoate, a yellow compound which can be measured at 412 nm.

Cholinesterase

Acetylthiocholine + H_2O → Thiocholine + acetate

Thiocholine + DTNB → 2-nitro-5-mercaptobenzoate

Acetyl Cholinesterase Study[10]

- Acetylthiocholine iodide (CAS NO: 1866-15-5): store at 2-8°C, Acetyl cholinesterase (CAS NO: 9000-81-1): store at 2–8°C, Donepezil: store at 2-8°C, Elman's reagent: DTNB store at room temperature
- Sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$), disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$).

Preparation of Working Solution

Phosphate buffer (50 mM) pH 7.7

(A) Sodium dihydrogen phosphate – 0.78 g in 100 mL of de-ionized water.
(B) Disodium hydrogen phosphate – 0.89 g in 100 mL of de-ionized water.

Mi×11 mL of solution A with 89 mL of solution B and make up to 200 mL with de-ionized water.

Elman's Reagents

3.95 mg DTNB dissolved in 50 mL phosphate buffer pH 7.7 (0.25 mM).

Donepezil Stock

1.4 mg/mL.

Equipment's

Incubator, plate reader.

Procedure

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

Calculations

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

RESULTS

After crude extraction of methanol and aqueous *Dooshivishari Agada*, yield summary are shows in Table 1 and extracts were tested for ache inhibitory activity using colorimetric method in 96-well plates. The results are shows in Tables 2 and 3 as well as Graphs 1 and 2 for *Donepezil* and test samples, respectively.

Table 1 : Yield summary after crude extraction

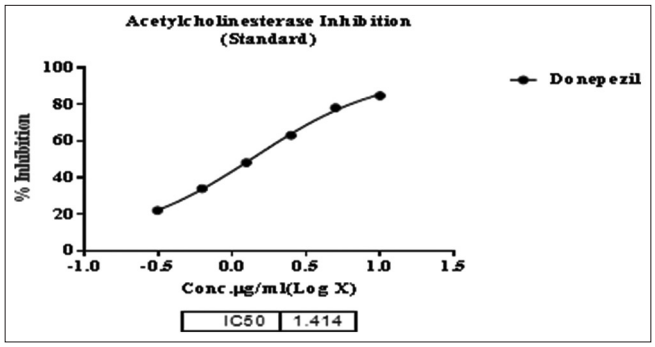
Sample	Sample taken for extraction	Solubility	Yield
Dooshivishari Agada	20 g	Methanol	1967.5 mg
	20 g	Aqueous	1870.2 mg

Table 2: Acetyl cholinesterase inhibition by standard (*Donepezil*)

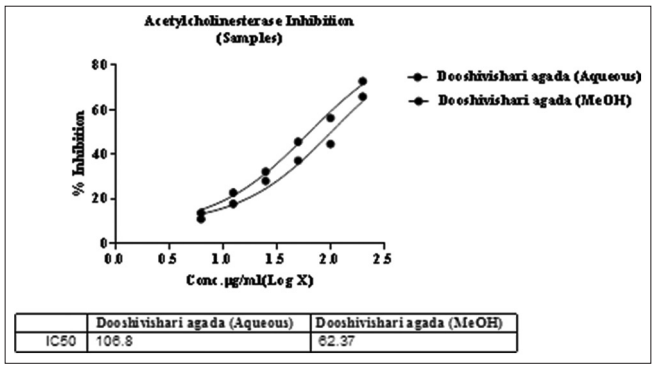
Samples	Concentration µg/ml	Absorbance at 412 nm	% Inhibition	IC_{50} value in µg/mL
Control	0	0.833	0.00	
Donepezil	0.3125	0.649	22.00	1.41
	0.625	0.550	33.98	
	1.25	0.432	48.14	
	2.5	0.308	63.07	
	5	0.182	78.12	
	10	0.127	84.75	

Table 3: Acetyl cholinesterase inhibition by samples

Samples	Conc. µg/mL	Abs at 412 nm	% Inhibition	IC_{50} value in µg/mL
Control	0	0.833	0	
Dooshivishari Agada (Aqueous)	6.25	0.742	10.87	106.8
	12.5	0.685	17.74	
	25	0.599	28.04	
	50	0.523	37.17	
	100	0.461	44.61	
	200	0.284	65.90	
Dooshivishari Agada (Methanol)	6.25	0.719	13.70	62.37
	12.5	0.643	22.75	
	25	0.564	32.22	
	50	0.453	45.62	
	100	0.364	56.31	
	200	0.226	72.84	



Graph 1: Graph of acetyl cholinesterase inhibition by standard (*Donepezil*)



Graph 2: Graph of acetyl cholinesterase inhibition by samples

The Methanol extract has showed better activity with an IC_{50} value of 62.37 µg/mL followed by the sample aqueous extract with IC_{50} value of 106.8 µg/mL.

Donepezil used as the standard ache inhibitor showed an IC_{50} of 1.41 µg/mL.

DISCUSSION

The cause of Alzheimer's disease is not known; however, several factors are thought to be included in this disease – neurochemical factors such as acetylcholine, nor-epinephrine, environmental factors such as metals, environmental pollution, and excessive use of fertilizers, and hazardous toxic chemicals during the production of food materials cigarette smoking.[11] Hence, Alzheimer's disease can be included under *Dooshivisha*. *Dooshivishari Agada* helps in the management of *Dooshivisha* and 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 Acetyl cholinesterase Ellman's method for Alzheimer's disease – 106.8 IC_{50} value in µg/mL in aqueous extract and 62.37 IC_{50} Value in µg/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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