Indian J Anim Health (2024), 63(2): 303- 308
DOI: https://doi.org/10.36062/ijah.2024.10423

Research Article

In vitro therapeutic potential of selected flavonoids, polyphenols and vitamins against Alzheimer's disease

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Abstract

Alzheimer's disease is a progressive neurodegenerative disease that leads to impaired cognition. One of the important factors for neurodegeneration and memory impairment is depleted levels of acetylcholine, especially in the brain. Compounds that inhibit cholinesterase enzyme, which is involved in the metabolism of acetylcholine, will help in maintaining the optimum levels of acetylcholine to prevent degenerative changes in the neuron, especially in the brain. Natural compounds obtained from the plants possess various pharmacological properties that can be explored for their therapeutic potential in Alzheimer's disease. They target the cholinesterase inhibitory potential, thereby maintaining the optimum levels of acetylcholine. By keeping this in view, the present study was designed to determine the cholinesterase enzyme inhibition of selected flavonoids, polyphenols and vitamins. Four flavonoids, namely quercetin, morin, naringenin and chrysin; four vitamins, thiamine, pyridoxine, beta-carotene and ascorbic acid; and seven polyphenols, namely ellagic acid, tannic acid, cinnamic acid, quinic acid, syringic acid, ferulic acid and shikimic acid were used. The cholinesterase enzyme inhibition was measured as per Ellmans method using neostigmine as positive control in fish brain homogenate. The range of percentile inhibition of tested flavonoids was found to be 42% to 55%. While the percentage of ascorbic acid inhibition is higher among the vitamins screened. In the category of polyphenols, ferulic acid (66%), shikimic acid (66%) and syringic acid (65.58%) showed better cholinesterase enzyme inhibitory activity when compared to other compounds. From the above results, it is concluded that natural compounds like flavonoids, polyphenols and vitamins can be used as alternatives to synthetic cholinesterase inhibitors in Alzheimer's disease to prevent neuronal degeneration.

Keywords: Cholinesterase inhibitors, Flavonoids, Neostigmine, Polyphenols, Vitamins

Highlights

- Natural compounds such as flavonoids, polyphenols and vitamins possess antioxidant activity comparable to ascorbic acid.
- The cholinesterase enzyme inhibition activity of vitamins, polyphenolics and flavonoids was comparable to that of neostigmine.
- Compounds that possess antioxidant and anticholinesterase activity will serve as better therapeutic molecules to combat neuronal degeneration due to free radicals as well as decreased cholinergic transmission.

INTRODUCTION

Neurodegenerative disorders are increasing in recent times due to changes in lifestyle and dietary habits. Among various neurodegenerative disorders, Alzheimer's disease is of pivotal importance due to its impact on the cognitive ability of an individual. Alzheimer's disease is the fifth leading cause of death in old age people and stands as seventh leading cause of death in adults. The etiological factors for the development of Alzheimer's disease are many, like dietary factors, environmental factors, genetic predisposition, amyloid infiltration in the brain, and reduced cholinergic transmission in the brain. The two important factors responsible for Alzheimer's disease are amyloid accumulation and decreased acetylcholine levels in the brain (Breijyeh and Karaman, 2020).

Acetylcholine is a central nervous system neurotransmitter and plays a significant role in learning memory and cognitive abilities. The reduction in the cognitive ability of an individual is attributed to defective cholinergic transmission due to decreased levels of acetylcholine (Ferreira-Vieira *et al*., 2016). Research reports revealed that diminished levels of acetylcholine in the brain may cause some of the symptoms of Alzheimer's disease. Application of cholinesterase inhibitor, which produces inhibition of acetylcholinesterase enzyme that metabolises acetylcholine, increases the concentration of acetylcholine in the brain and may enhance memory and cognitive function (Grossberg, 2003). The

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neurodegeneration is the primary change in the development of Alzheimer's disease. The degeneration in the neuron may be due to reduced cholinergic stimulus or due to direct damage due to reactive oxygen species. The nerve degeneration can be prevented by improving the acetylcholine levels by inhibiting cholinesterase and reducing the oxidative damage produced by reactive oxygen species with antioxidant supplements (Melo *et al*., 2011). Natural products obtained from plants serve as an important therapeutic strategy in the treatment of Alzheimer's disease due to their vivid pharmacological properties like cholinesterase inhibition and antioxidant potential (Murray *et al*., 2013). By keeping this in view, the present study was designed to screen some of the selected flavonoids, polyphenols and vitamins for their antioxidant and acetylcholinesterase inhibitory potential. Taherdehi *et al*. (2016) reported that vitamin C supplementation in rats exposed to malathion improved the cholinesterase activity. The proper function of the central and peripheral nervous system depends largely on vitamin B6. It offered protection against glutamate-induced neurotoxicity and ischaemia in mice (Dakshinamurti and Dakshinamurti, 2015). Thiamine is essential for brain function because of the coenzyme role of thiamine diphosphate (ThDP) in glucose and energy metabolism. Thiamine showed neuroprotective effects against traumatic brain injury-induced neuroinflammation in albino mice (Husn *et al*., 2023). Beta carotene at the dose of 1.02 and 2.05 mg/kg offered protection against intra cerebro ventricular injected streptozotocin induced memory impairment in mice (Hira *et al*., 2019). The treatment of rats with chrysin-loaded magnetic PEGylated silica nanospheres has attenuated amyloid beta induced memory impairment, possibly through the reduction of hippocampal lipid peroxidation levels and the elevation of antioxidant molecules (GSH, GPX, catalase, SOD, GSH), enabling neuroprotection (Vedagiri and Thangarajan, 2016). According to the findings of Hong *et al*. (2022), morin has the potential to be used as a functional food in the treatment of Parkinsonism due to its efficient reduction in glial activations and other neurodegenerative conditions linked to neuroinflammation. Perusal of available literature suggests that no reports are available on cholinesterase enzyme inhibitory activity on brain cholinesterase using fish brain homogenate as *in vitro* model. The present study was designed to determine the cholinesterase enzyme inhibitory potential of selected flavonoids namely quercetin, morin, naringenin and chrysin, vitamins thiamine, pyridoxine, beta-carotene and ascorbic acid and seven polyphenols namely ellagic acid, tannic acid, cinnamic acid, quinic acid, syringic acid, ferulic acid and shikimic acid.

MATERIALS AND METHODS

Drugs and chemicals: The chemicals of analytical grade procured for the estimation of cholinesterase activity were S-acetyl thiocholine iodide, 5-5-dithiobis 2-nitrobenzoic acid (DTNB), sodium bicarbonate and magnesium chloride. S-acetylthiocholine iodide (0.075M) used as substrate was prepared by dissolving 216.7 mg in 10 mL of 1M PBS (PH7.0). DTNB 0.01 M was prepared by dissolving 39.6 mg in 10 mL PBS (PH7.0) and 15 mg of sodium bicarbonate was added. All the chemicals were obtained from Himedia laboratories, Mumbai.

Enzyme: Fish brain tissue was used as a source of cholinesterase enzyme in the present study. Fish for food purposes were used in the current experiment. The live fishes were brought from the local fish market to the lab, and brain tissue was collected immediately after sacrifice in chilled PBS and washed thrice with ice cold PBS.10% brain homogenate was prepared by taking 10 g of tissue in 100 mL of 1% triton X. The mixture was centrifuged at 12000 rpm for 20 min at 4°C. The supernatant collected was used as a source of cholinesterase enzyme. Rajaretinam *et al*. (2012) studied the cholinesterase inhibitory activity of *Tephrosia purpurea* using Zebra fish brain homogenate as source of acetylcholinesterase. The experimental protocol was approved by the Institutional Animal Ethics Committee of NTR College of Veterinary Science, Gannavaram (Approval No.1/ IAEC/NTRCVSc/23 dt. 06.05.2023).

Preparation of test compounds: All test compounds used in the study were prepared as 10 mg/mL stock solution using DMSO. Flavonoids used in the study were used @1 mg/mL. Some of the selected flavonnoids are not soluble in water, all the test compounds were prepared in DMSO.

Assay of cholinesterase enzyme inhibition: The enzyme inhibition was measured by employing modified Ellmans method using fish brain extract. To the 2 mL of PBS 0.1M, 0.2 mL of 13mM magnesium chloride was added, followed by the addition of 20 µL of acetylthiocholine iodide and 100 µL of DTNB. 25 µL of test compound was added before adding the enzyme. Finally, 10 µL of the enzyme was added to the reaction mixture and incubated at 37°C for 30 minutes, preferably in the dark. The formation of the

yellow-coloured complex was measured at 412nm using a Thermomultiscan microplate reader (Thermo Fischer Scientific, Waltham, Massachusetts, USA). Neostigmine was used as a positive control to compare the percentile inhibition of test compounds (Ellman *et al*., 1961).

Percent inhibition = 1-Sample OD/Blank OD x100

Assay of total antioxidant activity: The antioxidant activity of the test compounds was determined using phosphomolybdate assay as described by Prieto *et al*. (1999). The test compound and phosphomolybdate reagent were mixed in the ratio of 1:10 and incubated for 90 min at 95°C. Absorbance was recorded at 695 nm using UV-Visible spectrophotometer (Thermo Fischer).

Statistical analysis: The percentage of cholinesterase inhibition of test compounds was compared by using non parametric statistical approach following Kruskal Wallis test. Different variables were expressed as mean with SEM. The mean ranks of different variables were taken as criteria to compare between the tests compounds (Kruskal and Wallis, 1952).

RESULTS

The results obtained from the above study revealed that the antioxidant potential of the selected polyphenolic substances follows the order tannic acid > ferulic acid > ellagic acid > syringic acid > shikimic acid and cinnamic acid in comparison to ascorbic acid. The order of antioxidant potential of flavonoids follows morin > quercetin > naringenin > chrysin. The order of total antioxidant activity follows ascorbic acid > thiamine > pyridoxine > beta-carotene, which are presented in Table 1.

Table 1. Total antioxidant activity of selected flavonoids, polyphenols and vitamins

Sl. No.	Test compound	Ascorbic acid equivalent (mg)
1	Tannic acid	1.07
2	Ferulic acid	0.703
3	Cinnamic acid	0.098
4	Ellagic acid	0.581
5	Quinic acid	0.111
6	Shikimic acid	0.128
7	Quercitin	1.098
8	Morin	1.98
9	Naringenin	0.87
10	Chrysin	0.67
11	Beta carotene	0.65
12	Thiamine	0.82
13	Pyridoxine	0.73

In our study, we determined the cholinesterase enzyme inhibitory activity of selected flavonoids, polyphenols and vitamins using neostigmine as standard. The dose-response curve was drawn for various doses of neostigmine, and the IC_{50} value was 0.006 mg/mL (Fig. 1). The percentage inhibition of various test compounds was calculated and compared with neostigmine. The cholinesterase enzyme inhibitory potential was better in ascorbic acid (66%), followed by pyridoxine (65%) and thiamine (63%) among the tested vitamins (Fig. 2, Table 2). In the case of flavonoids, chrysin (55%) showed better inhibition, followed by naringenin (49%) and quercetin (47%), which are presented in Table 3. The cholinesterase inhibition was higher in ferulic, shikimic acid and syringic acid among tested polyphenols, as presented in Table 4. Ellagic acid inhibited the cholinesterase enzyme below 50%.

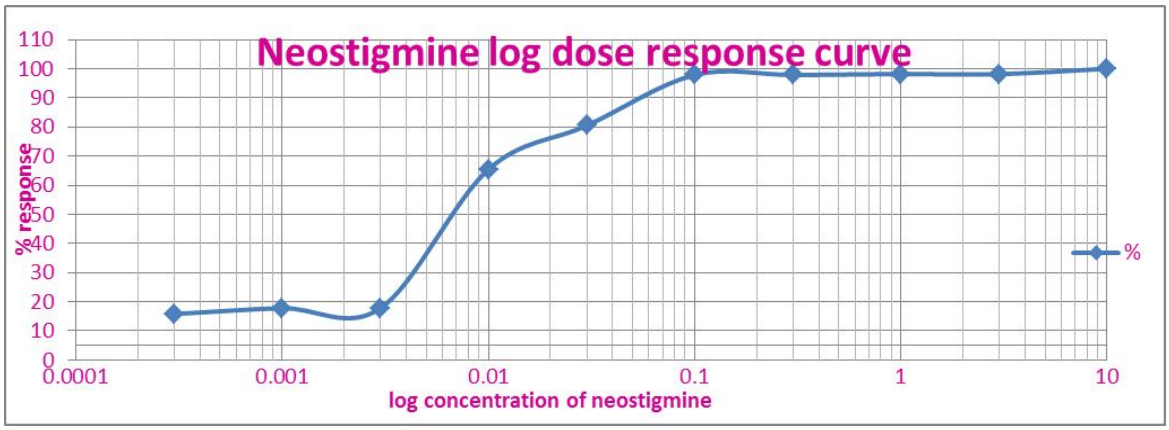

Fig. 1. Log-doses of neostigmine vs percentile response of neostigmine on cholinesterase inhibition

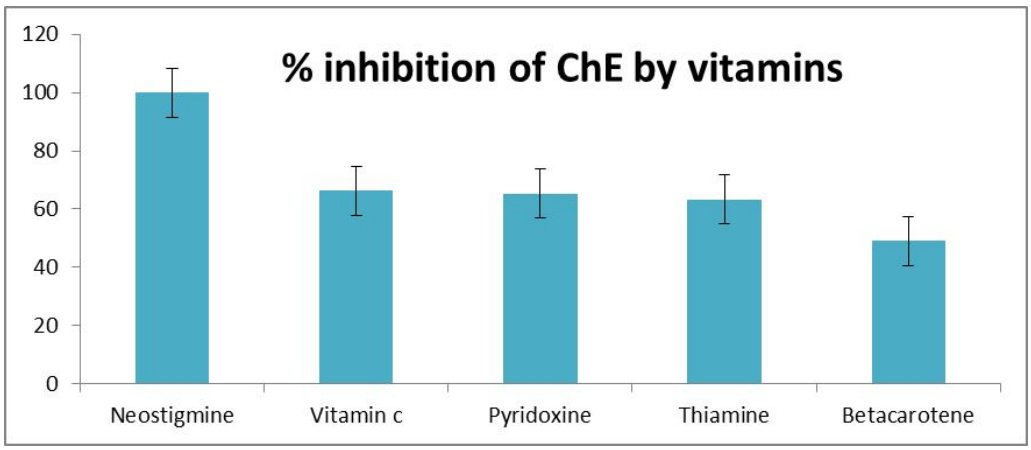

Fig. 2. Percentile inhibition of cholinesterase enzyme by selected vitamins in fish brain homogenate

Table 2. Percentage inhibition of ChE by selected vitamins in fish brain homogenate

Sl. No.	Name of the test compound	% inhibition of ChE
1	Beta carotene (vit A)	49.03±3.63
2	Thiamine (vit B1)	63.21±2.04
3	Pyridoxine (vit B6)	65.28±4.41
4	Ascorbic acid (vit. C)	66.24±3.77

Table 3. Percentage inhibition of ChE by selected flavonoids in fish brain homogenate

Sl. No.	Name of the test compound	% inhibition of ChE
1	Morin	42.6±1.20
2	Quercetin	47.1±1.37
3	Naringenin	49.63±2.15
4	Chrysin	55.3±5.5

Table 4. Percentage inhibition of ChE by selected polyphenols in fish brain homogenate

Sl. No.	Name of the test compound	% inhibition of ChE
1	Ellagic acid	48.2±0.98
2	Tannic acid	58.42±1.51
3	Cinnamic acid	58.74±7.17
4	Quinic acid	64.32±0.38
5	Syringic acid	65.58±1.01
6	Ferulic acid	66.04±1.34
7	Shikimic acid	66.06±2.32

DISCUSSION

Neurodegenerative diseases are becoming more prevalent and increasing causes of mortality and morbidity globally (Erkkinen *et al*., 2018) due to changes in lifestyle, environmental conditions and dietary habits. According to an epidemiological estimate, 24 million people worldwide suffer from neurological disorders (Mayeux and Stern, 2012). Among the neurodegenerative disorders, Alzheimer's disease has a significant impact on the cognitive abilities of the individual. Several factors are responsible for the onset of Alzheimer's disease, among which depleted levels of acetylcholine play a role in the onset of symptoms of Alzheimer's. Acetylcholine affects learning, memory, and other cognitive functions. Cholinesterase inhibitors enhance central cholinergic function by inhibiting the enzymes that degrade acetylcholine, thereby increasing the availability of acetylcholine to the brain (Marucci *et al*., 2021). Acetylcholinesterase inhibitors, such as donepezil, rivastigmine and galantamine, act by increasing the level of ACh and this has resulted in better improvement in cognition (Chopra *et al*., 2011).

Hence, cholinesterase inhibitors serve as a standard approach for the symptomatic treatment of Alzheimer's disease. In addition to depleted levels of acetylcholine, oxidative damage to the neurons also leads to neuronal degeneration and impaired brain function. Compounds with cholinesterase inhibitory activity and antioxidant activity may become a better option to overcome the symptoms of Alzheimer's disease.

Hence, in the present study, the total antioxidant activity and cholinesterase inhibitory potential of selected flavonoids, polyphenols and vitamins was assessed. The selected compounds possess antioxidant activity comparable with ascorbic acid. Tannic acid among the polyphenols, morin among the selected flavonoids and ascorbic acid among the vitamins possess maximum antioxidant potential (Table 1)

The cholinesterase enzyme inhibitory activity was estimated in fish brain homogenate using neostigmine as standard using Ellmans method. In the selected

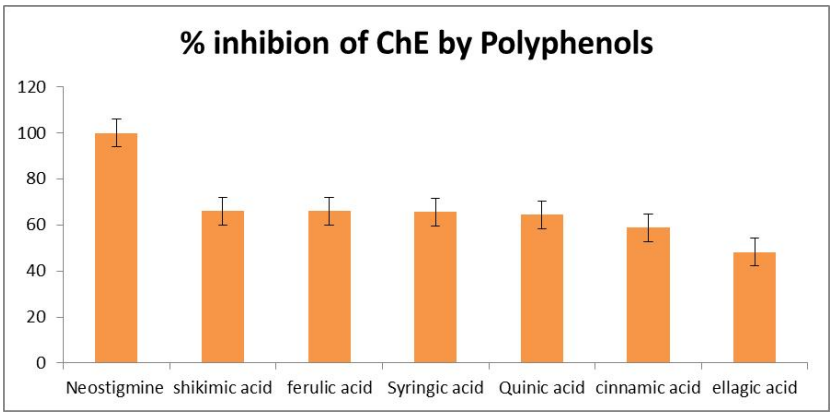

Fig. 3. Percentile inhibition of cholinesterase enzyme by selected polyphenolics in fish brain homogenate

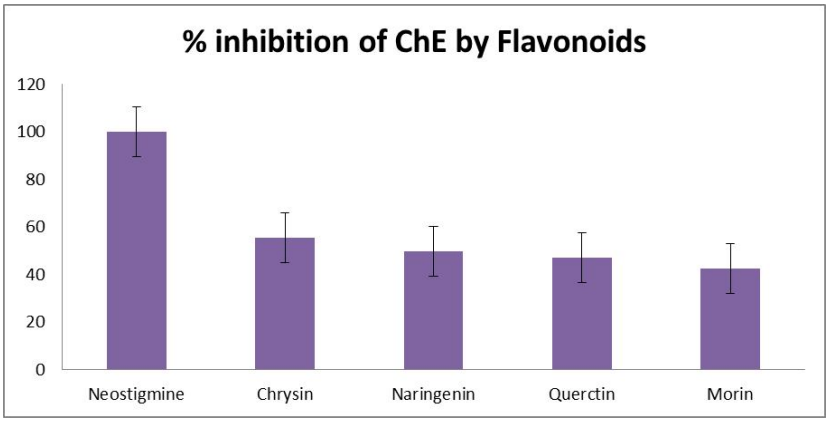

Fig. 4. Percentile inhibition of cholinesterase enzyme by selected flavonoids in fish brain homogenate

vitamins, ascorbic offered significantly high percentage of inhibition, followed by thiamine and pyridoxine. There is no significant difference between thiamine and pyridoxine in their percentile inhibition of cholinesterase (Fig. 2). Beta-carotene showed significantly low level of inhibition ($p<0.05$) when compared with neostigmine. The cholinesterase enzyme inhibition percentage of ferulic acid is higher among the selected polyphenolic acids (Fig. 3). Ellagic acid showed a significantly low level of inhibition, i.e. below 50% among the tested polyphenols. Tannic acid and cinnamic acid showed 58% of cholinesterase enzyme inhibition at 5% level of significance (Table 4). Among the screened flavonoids, chrysin produced more than 50% inhibition, quercetin, morin and naringenin offered less than 50% inhibition cholinesterase in fish brain homogenate (Fig. 4). Polyphenols, flavonoids and vitamins can be used as dietary supplements that serve as natural antioxidants and alternative to synthetic cholinesterase inhibitors which can be used in the conditions of Alzheimer's disease. Further studies have to be conducted on the specific receptor binding and safety aspects of selected phytochemicals for developing them as therapeutic molecules in the treatment of Alzheimer's disease.

Conflict of interest: Authors declare that they have no conflict of interest in the present study.

Author's contribution: GS, GSR: Conception and design; GS, PA, GSP: Acquisition of data; GS, PA, GSP: Performed statistical analysis of data; GS: Performed interpretation of the results.

Data availability statement: All details of the obtained results were available with the corresponding author.

Ethical statement: The experimental protocol was approved by the Institutional Animal Ethics Committee of NTR College of Veterinary Science, Gannavaram (Approval No.1/IAEC/NTRCVSc/23, dated 06.05.2023).

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Received - 24.11.2023, *Accepted* - 24.06.2024, *Published - 04.07.2024 (Online), 01.12.2024 (Print)*
Section Editor: Dr. I. Samanta, Associate Editor