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IN VITRO INHIBITORY ACTIVITY OF CARPAIN ON ACETYLCHOLINESTERASE AND AMYLOID BETA PLAQUE FORMATION

Koustubh Surana[1], Dr. Abirami Arthanari[2*] and Dr. Parmeshwari[3]

[1]Undergraduate, [2]Senior Lecturer, Department of Forensic Odontology and [3]Associate Professor, Department of Pharmacology

Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University, Chennai, India

ABSTRACT

INTRODUCTION

Alzheimer's disease is a neurodegenerative disease that induces symptoms such as a decrease in motor function and cognitive impairment, an increase in oxidative stress, acetylcholinesterase and amyloid beta plaque accumulation

AIM

To evaluate the inhibitory activity of carpaine n on acetylcholinesterase and amyloid beta plaque formation

MATERIALS AND METHODS

Three properties were analyzed namely Xanthine oxidase inhibitory activity, acetylcholinesterase (AChE) inhibition assay and Thioflavin T fluorescence assay. All these were then compared and statistically analysed to reference standards.

RESULTS

The results showed us satisfactory results.

CONCLUSION

This study suggests that plants can form a good source of effective crude inhibitors for XO which can be used in the treatment of gout and other XO-related disorders such as Alzheimer's.

Keywords: Alzheimer's, carpaine oxidative stress, beta plaque etc

INTRODUCTION

Neurodegenerative disorders, such as Alzheimer's disease (AD), continue to pose significant challenges to public health and the global aging population. Alzheimer's disease is characterized by the progressive decline of cognitive functions, memory impairment, and behavioral disturbances.Selective neuronal cell death, extracellular amyloid deposits in the center of neuritic plaques, and the development of intraneuronal neurofibrillary tangles in the brains of those with the condition are the hallmarks of Alzheimer's disease. The etiology of AD is complex and involves multiple factors, including the accumulation of amyloid-beta (Aβ) plaques and the dysregulation of neurotransmitter systemsThese deficiencies are neurochemically characterized by severe losses of cortically projecting cholinergic neurons and a decline in presynaptic markers of the cholinergic system, especially in regions of the brain linked with learning and memory.[1]Acetylcholinesterase (AChE) plays a crucial role in cholinergic neurotransmission, and its inhibition has been a primary therapeutic target for AD treatment.[2]

A hallmark of Alzheimer's disease is the accumulation of Aβ peptide, which aggregates into insoluble plaques within the brain. These plaques disrupt neuronal communication, trigger inflammatory responses, and ultimately lead to neurodegeneration.[3–6] Strategies that target Aβ plaques have shown promise in preclinical studies, emphasizing the need to explore Carpain's potential as an Aβ-modulating agent.[3–7]

Alzheimer's disease involves the selective degeneration of cholinergic neurons, leading to a decline in acetylcholine (ACh) levels within the brain. Acetylcholinesterase, an enzyme responsible for the hydrolysis of ACh, plays a crucial role in terminating cholinergic neurotransmission. Inhibition of AChE enhances ACh levels,

thereby improving cognitive function.[8] Several synthetic AChE inhibitors, such as donepezil and rivastigmine, have shown efficacy in treating mild to moderate AD. [9]However, these drugs often exhibit adverse effects and limited long-term benefits, necessitating the exploration of alternative natural compounds with AChE inhibitory properties.

AChE inhibitors can impede the progression of Alzheimer's disease (AD), according to a large body of research. The effective development of these drugs was predicated on the widely accepted idea that the loss of cortical cholinergic neurotransmission contributes to the impairment in mental and cognitive abilities associated with AD.The earliest known AChE inhibitors, donepezil, physostigmine, and tacrine, shown a moderate improvement in Alzheimer's patients' cognitive function.[10]

In recent years, natural compounds have garnered increasing attention for their potential therapeutic benefits against neurodegenerative disorders. The focus of research into Alzheimer's disease (AD) is on commonly utilized neurotonic and renewing substances.[11]One such compound is Carpain, a naturally occurring alkaloid isolated from the seeds of Carica papaya (papaya), a fruit widely consumed for its nutritional benefits.Carpaine has exhibited various biological activities, including antioxidant, anti-inflammatory, and anticancer properties.[3],wound healing properties,14 antitumour and immune-modulatory effects15 and an antioxidant,[3,4]A study by Halim and colleagues, investigating toxicity of C. papaya leaves extract on Sprague Dawley rats revealed that it was safe for oral consumption. Furthermore, it has been reported that extracts and pure compounds derived from C. papaya to possess a wide variety of pharma- cological activities including antioxidant, antimicrobial, antihypertensive, antiplasmodial, antifungal and anti- inflammatory. [3–5]Given its pharmacological profile, Carpain emerges as a promising candidate for exploring its inhibitory activity on acetylcholinesterase and its potential to counteract amyloid beta plaque formation.

Carpain, a bioactive alkaloid, has been widely studied for its various medicinal properties. Its presence in Carica papaya makes it a readily available and cost-effective candidate for drug development. Additionally, its chemical structure suggests potential AChE inhibitory effects, but further investigation is warranted to establish its precise mechanism of action.

The present study aims to investigate the in vitro inhibitory activity of Carpain on acetylcholinesterase, as well as its capacity to interfere with amyloid beta plaque formation. By doing so, we hope to shed light on Carpain's therapeutic potential for AD and pave the way for future drug development strategies.

The primary objective of this study is to assess Carpain's ability to inhibit acetylcholinesterase and determine its impact on amyloid beta plaque formation using in vitro models. A dual approach will be undertaken to explore Carpain's potential as a multifunctional compound capable of targeting both key aspects of Alzheimer's pathology.

The findings from this study may contribute to the growing body of evidence supporting the therapeutic potential of natural compounds in the management of Alzheimer's disease. Carpain's dual inhibitory effects on acetylcholinesterase and amyloid beta plaque formation, if confirmed, could position it as a promising candidate for the development of novel AD therapies. Moreover, its natural origin may offer advantages such as reduced side effects and improved patient compliance.

This present study sets out to investigate the in vitro inhibitory activity of Carpain on acetylcholinesterase and amyloid beta plaque formation. By exploring Carpain's potential as a neuroprotective agent, we hope to advance our understanding of natural compounds in AD therapeutics and contribute to the development of innovative treatment strategies for this debilitating neurodegenerative disorder.

MATERIALS AND METHODS

Chemicals and reagents

Xanthine, acetylthiocholine iodide, acetylcholine enzyme (0.3U/ml) were procured from Sigma-aldrich, USA. Carpaine was purchased from BOC Sciences, USA. Quercetin was purchased from TCI chemicals, India. Donezepil hydrochloride was purchased as tablet from a local pharmacy. All other chemicals, reagents and solvents used were of analytical grade and purchased from SRL chemicals, India.

Xanthine oxidase inhibitory activity

The XO inhibitory activity was assayed spectrophotometrically under aerobic conditions, based on the procedure reported by Bustanji et al. 2011. The substrate and the enzyme solutions were freshly prepared. The assay mixture, consisting of 50µL of different concentrations Carpaine (10-320µM), different concentrations of Quercetin (10-320µM), 35µL of 0.1mM phosphate buffer (pH=7.5) and 30µL of enzyme solution (0.01units/ml of XO in 0.1mM phosphate buffer, pH=7.5), was prepared immediately before use. After 30mins of incubation at 25°C, the reaction was initiated by the addition of 60µL of substrate solution (150mM of Xanthine in 0.1mM Phosphate buffer). The absorption at 295 nm, indicating the formation of uric acid at 25°C, was monitored and the initial rate was calculated. A blank was prepared in the same manner. One unit of XO was defined as the amount of enzyme required to produce 1 mmol of uric acid/minute at 25 °C. XO inhibitory activity is expressed as the percentage inhibition of XO in the above system, calculated as (1-B/A) × 100, where A and B are the activities of the enzyme without and with different concentrations of Carpaine and Quercetin. IC_{50} values were calculated from the mean values of data from three determinations. Quercetin was used as reference standard.

In vitro acetylcholinesterase (AChE) inhibition assay (Ellman et al., 1961)

The compound carpaine and standard Donepezil hydrochloride was examined for its AChE inhibitory activities at different concentrations of 10-320µM and 10-320µg/ml respectively. 200µl of the different concentrations of Carpaine and standard Donepezil hydrochloride were prepared using 0.05M tris base. Briefly, in this method, 200µl of acetylthiocholine iodide (15mM), 1000µl of DTNB (3mM), and 200µl of carpaine and Donezepil at different concentrations were mixed and incubated for 15 min at 30ºC. Then, the mixture was monitored spectrophotometrically at 412 nm 10 times, each 13 s. After that, 200µl of AChE (0.3U/ml) solution were added to the initial mixture, to start the reaction and then the absorbance was determined.

Control contained all components except the tested extract. The percentage of AChE inhibitory activity (% IA) was calculated by using the following equation:

IA (%) = (Activity of Control – Activity of Test)/ Activity of Control x 100

Assessment of A*β* (1–42) Concentration

Preparation of Aβ solution

The Aβ solution was prepared according to the method of Miyazaki et al., 2019. Briefly, synthetic β-Amyloid Peptide 1-42 (Aβ1-42) (PP69, Sigma Merck, USA) was dissolved in 0.1% ammonia solution at a final concentration of 250 µM and sonicated in ice-cold water for a total of 5 min (1 min × 5 times) to avoid pre-aggregation. For preparation of the Aβ solution, aliquots of Aβ were diluted to 25µM in 50mM phosphate buffer (pH 7.5) and 100mM NaCl.

Thioflavin T fluorescence assay

The thioflavin T (ThT) fluorescence assay was performed as Miyazaki et al., 2019. Aβ solution (8µL) was mixed with the different concentrations of Carpaine (10-320µM) and Donezepil (10-320µg/ml) and the mixture was then added to 1.6mL of ThT solution containing 5µM ThT and 50mM NaOH-glycine-buffer (pH 8.5). The samples were incubated at 37℃ and the fibrillogenesis rate was monitored by using ThT fluorescence assays. The samples ThT fluorescence levels were evaluated by using Biotek Synergy H4 hybrid multimode reader (USA). The respective excitation and emission wavelengths were 446 nm and 490 nm.

Statistical Analysis

Data were analyzed using Graphpad prism (version 7.0). The results were expressed as Mean±SEM and the IC_{50} values were obtained from the linear regression plots. Two-way ANOVA was used to assess differences between means at $p<0.001$ level of significance. The means were compared with standards groups using the Holm-Sidak Test.

Results

Three properties were analyzed namely Xanthine oxidase inhibitory activity, acetylcholinesterase (AChE) inhibition assay and Thioflavin T fluorescence assay shows the xanthine oxidase inhibitory activity of carpaine which is almost similar to reference standard quercetin.

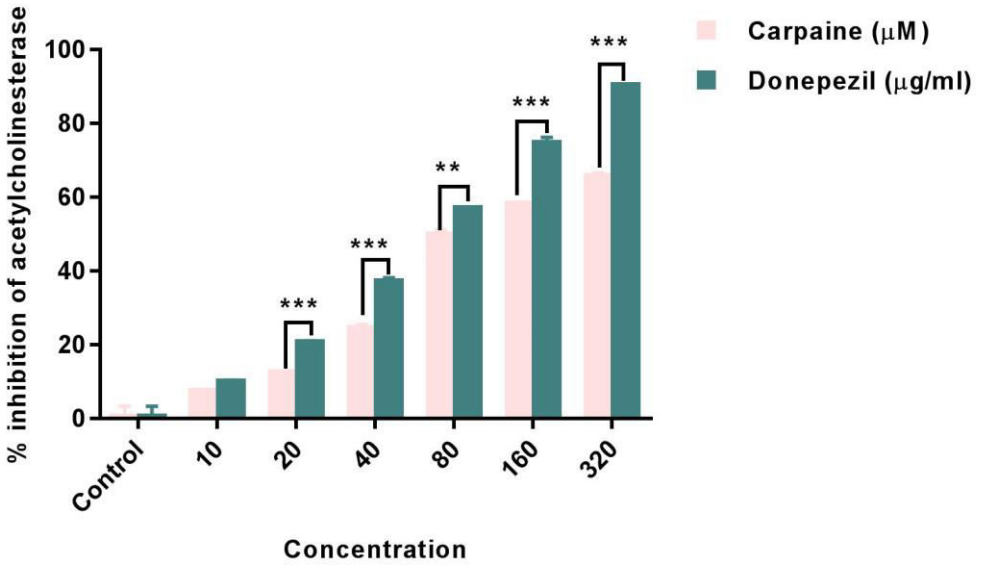


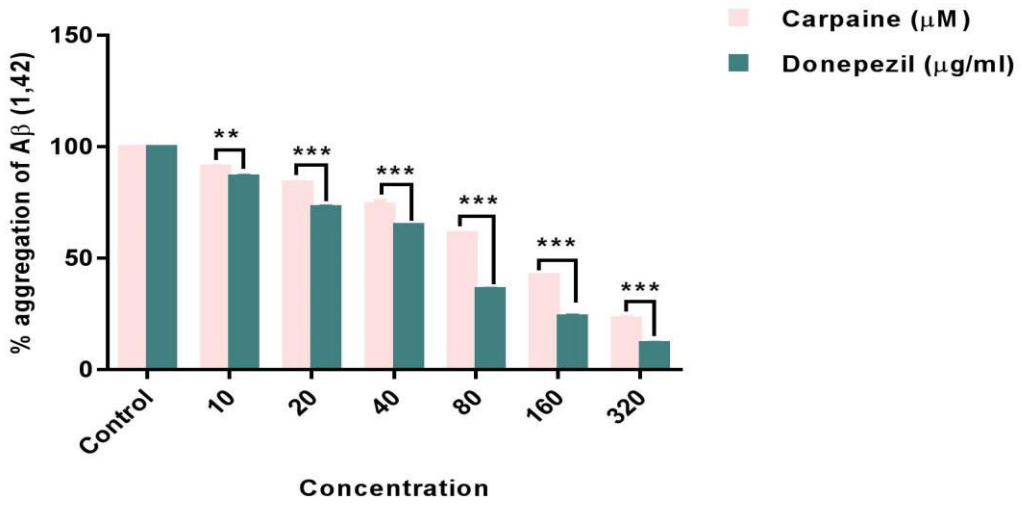
Fig 1-

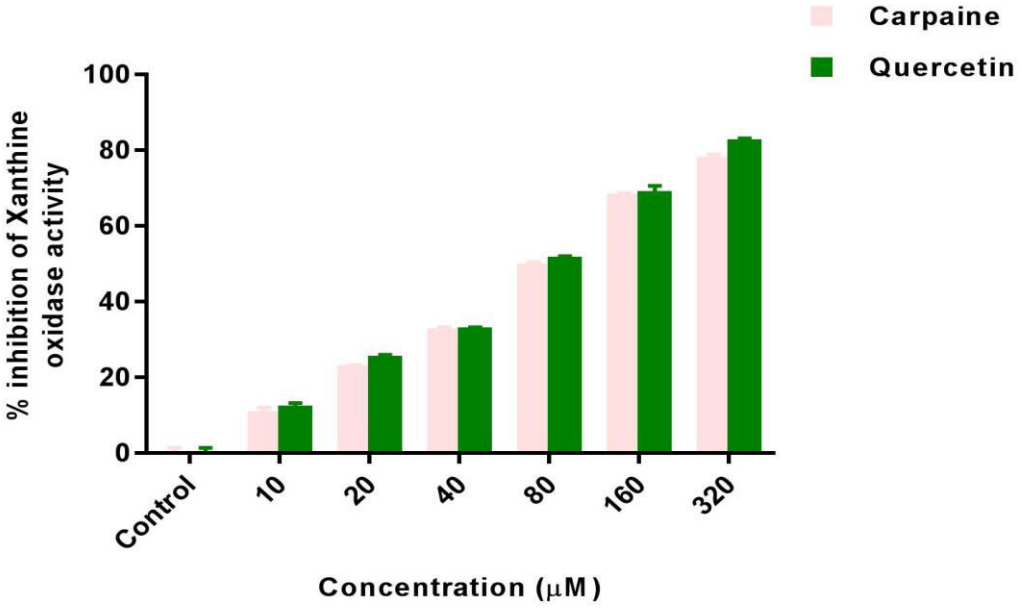
The results have been showcased in the figure below

Fig 1 - Shows the in-vitro acetylcholinesterase (AChE) inhibition assay of carpaine to standard cholinesterase inhibitor.

Fig 2 - Shows Thioflavin T fluorescence assay of carpaine which is very comparable to reference standard Donepzil. The values are significant.

Fig 3 - Shows the xanthine oxidase inhibitory activity of carpaine which is almost similar to reference standard quercetin.





DISCUSSION

Cholinesterase enzymes are the promising target for Alzheimer disease (AD) drug discovery. To date, cholinesterase inhibitors are clinically preferred medications for the treatment of mild and moderate forms of AD due to its efficacy and less adverse effects. Cholinesterase inhibitors inhibit acetylcholine from further degradation which could aid in reducing memory and learning impairments. Therefore, the search for potential anti-cholinesterase inhibitors from natural products that contain various classes of phytochemicals is one of the global strategies for the prevention and treatment of AD.

One of the defining characteristics of AD is an increase in the activity of the enzyme acetyl cholinesterase (AChE), which hydrolyzes acetylcholine in both cholinergic and non-cholinergic brain neurons.However, it has been demonstrated that AChE activity is elevated within and surrounding amyloid plaques, promoting the formation of amyloid beta-peptide fibrils and raising the cytotoxicity of these peptides.

The C. papaya plant, which yields both fruit and medicine, contains a variety of nutritional phytochemicals, including fatty acids, terpenes, vitamin E, flavonoids, and phenolic acids. These compounds have anti-inflammatory, anticancer, antiviral, and other potential medicinal properties.[3]

In our study, in vitro inhibitory activity of Carpain on acetylcholinesterase and amyloid beta plaque formation was studied, the methodologies used include xanthine oxidase inhibitory activity, thioflavin T fluorescence assay and Ache inhibition assay. The results for the same have been discussed in detail above.Carpaine showed high potential to be adjuncts to alzeimeiheir s disease. A similar study was conducted by Kooi-Yeong Khaw. This study underscores the potential of C. papaya leaf alkaloid fraction and its major alkaloid compound as potential nutraceutical for memory enhancing agents. Alkaloid fraction showed promising BChE inhibition activity and GC-MS data revealed that the major com- pound was carpaine. Subsequently, molecular docking results showed that carpaine fitted well within the active site of BChE enzyme.

Natural products have been subject to alzeimeiher disease.Ansari and Khodagholi conducted a thorough evaluation of the molecular mechanisms of natural compounds as potential AD therapeutic options. Regarding their mode of action, they concentrated on a few natural compounds with possible neuroprotective qualities against A. The majority of these substances are powerful antioxidants and free radical scavengers. Some of these substances alter the amyloidogenesis and programmed cell death pathways directly to increase cell survival and improve cognition. Ansari and Khodagholi came to the conclusion that although neuroprotective chemicals derived from natural sources are promising therapeutic options for AD, the main issues are poor bioavailability and limited clinical efficacy. Utilizing cutting-edge medicinal chemistry and pharmaceutical technologies to find novel formulations or to make.[3, 12]

Tanaka *et al*. carried out a systematic review of the studies that have analyzed the effect of *Ginkgo biloba* extract on PD. They gave two hypotheses for the positive effect of *G. biloba* extract on PD, namely the reduction or inhibition of monoamine-oxidase activity and the neuroprotective effect against 6-hydroxydopamine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and MPP+ toxins.(13,14)

CONCLUSION

In conclusion, the research study on the in vitro inhibitory activity of Carpain on acetylcholinesterase and amyloid beta plaque formation has yielded promising results. Carpain has demonstrated significant inhibitory effects on acetylcholinesterase, suggesting its potential as a therapeutic agent for conditions associated with cholinergic dysfunction, such as Alzheimer's disease. Additionally, Carpain exhibited a notable ability to mitigate amyloid beta plaque formation, which is a hallmark of Alzheimer's pathology. These findings highlight Carpain as a promising candidate for further research and development in the pursuit of novel treatments for neurodegenerative disorders like Alzheimer's disease. Further in vivo and clinical studies are warranted to fully explore its therapeutic potential and safety profile.

This study suggests that plants can form a good source of effective crude inhibitors for XO which can be used in the treatment of gout and other XO-related disorders such as Alzheimer’s.

Conflict of interest

No conflict of interest was found.

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Ethical clearance number

No ethical clearance is required as this is an in vitro study.

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