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Anti-Cholinesterase Effect of *Emblica officinalis* Fruit Extract in Alzheimer's Induced Rat Brain - An Insight to Develop Novel Alzheimer's Therapeutics

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Abstract:

Alzheimer's Disease has been identified as proteopathic disease, caused by accumulation of abnormally folded A-beta, tau proteins in brain. As per Alzheimer's association, it is estimated that over 65 years and above age group one per person suffering in every eight from this devastating neurodegenerative disorder and may reach to 60 million by 2050 worldwide. The treatment of AD patient is also economically cost effective; nearly 80-100 billion dollars are spending AD health care in US only. It would be a great achievement that if any ideal compounds extracted from natural sources as potential therapeutics for Alzheimer's treatment Disease with low cost. In the present study an attempt was made to evaluate the *Emblica officinalis* fruit Methanolic (EoFM) extract effect on Alzheimer's Disease induced rats. The results of *invivo* studies against cholinergic neurotransmitters viz., ACh and AChE revealed that EoFM extract exerted anti-cholinesterase properties in AD-induced rats. The *inslico* results it was evident that bioactive compounds extracted from the *Emblica officinalis showed* ability to inhibit Human AChE (PDB ID: **4PQE).** Among the selected compounds, Ellagic acid (PI: 5281855), Lonicerin (PI: 5282152) and Phyllanemblinin-A (PI: 11135859) have effective AChE enzyme inhibitory activity. Finally, it may conclude that EoFM extract has potential neuroprtective activity to enhance cholinergic cognitive dysfunctions in Alzheimer's.

Key words: Alzheimer's Disease, Anti-Alzheimer's drugs, *Emblica officinalis* fruit extract, AD induced rat model, Cholinergic neurotransmitters, *Insilico* Analysis, Docking studies.

INTRODUCTION:

Alzheimer's Disease (AD) is the common neurodegenerative disease, identified as a protein misfolded proteopathy disease, caused by accumulation of abnormality folded A-beta, tau proteins in the brain. AD is characterized by notable memory loss, cognitive impairment and personality disorders accompanied by diffuse structural abnormalities in the brain of aged population (**Vasudevan, 2006**), worldwide nearly 50 millions of people and it has become a major medical and social burden globally and by 2050 nearly 152 million people will be affected (WHO, 2020). Clinically Alzheimer's disease is classified into (1) pre-clinical with mild memory loss (2) The mild

or early stage of AD, where patients with a loss of concentration and memory, disorientation of place and time, a change in the mood, and a development of depression (**Wattmo *et al.,* 2016**). (3) Moderate AD stage, an increased memory loss with trouble recognizing family and friends, difficulty in reading, writing, and speaking. (4) Severe AD or late-stage, which involves the spread of neuritic plaques and neurofibrillary tangles, resulting in a progressive functional and cognitive impairments, eventually leading to the patient's death due to these complications (**De-Paula *et al.,* 2012**).

Anatomically; four major alterations in brain structure are seen: Cortical atrophy, Degeneration of cholinergic and other neurons, Presence of Neurofibrillary tangles (NFTs) and accumulation of neuritic plaques (beta amyloid plaques). The main pathological hallmark of the AD is formation of extracellular Senile Plaques (SPs) and Intra neuronal Neurofibrillary tangles (NFTs) of the tau protein develop in the specific brain regions, leading to the death of neuronal cells. The sequential cleavage of Amyloid Precursor Protein (APP) by the beta- and gamma-secretases generates A-beta peptides; however, the alternate cleavage of APP by the beta- and gamma-secretases decreases A-beta production (**Stein TD *et al.,* 2002**). Oligomerization and accumulation of A-beta peptides are the key event in the pathogenesis of AD (**Albert *et al.,* 2011; Hussain *et al.*, 2018**).

The Cholinergic system in the brain, especially the basal forebrain projections to hippocampus and cortex, is responsible for memory and learning and known to be affected in AD. The shrinkage of the cerebral cortex and the medial temporal lobe is a typical trait of Alzheimer's Disease along with the enlargement of brain ventricles (**Brookmeyer *et al.,* 2007**). The diagnosis is rather difficult since the clinical features of AD overlap with the symptoms of various other neuropathological conditions. In addition, a definite conformation of AD is achieved only by morphological and histological examination of the brain at autopsy.

Currently approved Anti-AD drugs viz., Galantamine, Rivastigmine, Donepezil (AChE inhibitors), and Memantine (NMDA antagonist), which offer only symptomatic relief without preventing the progression of the disease (**Mangialasche *et al.,* 2010**) and having limited efficacy with side effects.

Herbal medicine causes least or no side effects with multiple actions and cost is also relatively low. Scientific studies suggest different lifestyle changes and use of appropriate herbal medicine use in the management of neurodegenerative disorders. Modern science has already, accepted the potential of the herbs as a source of new bio-active constituents. There are numerous plants derived drugs of unknown chemical structure that have been found clinically useful in different alternative system of medicine, including Ayurveda, Homeopathy and Unani system of medicine. The recent development of science of phyto-pharmaceuticals has generated new enthusiasm in herbal drug research to discover new medicines in various diseases. *Emblica officinalis* (Gaertn) offenly known as Amla or Indian Gooseberry), a member of the small genus of Emblica (Euphorbiaceae), grows in the tropical areas of India, China, Indonesia, and the Malay peninsula. The fruit of the plant is one of the most important medicinal ingredients used in Ayurveda, Siddha, Unani, Arabic, Tibetan, and various other folk systems for the management of multitude chronic ailments. *Emblica officinalis* fruits are reported to have potent antioxidant, analgesic, antipyretic, adaptogenic, immunomodulatory, and antiulcerogenic properties (**Baliga and Dsouza, 2011; Baliga et al., 2012; Thilakchand *et al.,* 2013**). Recent research finding on Anwalachurna, an Ayurvedic preparation from emblica, attenuates scopolamine induced memory deficits in rats by virtue of its antioxidant and anti-inflammatory properties (**Vasudevan and Parle, 2007**). Chyawanprash, another Ayurvedic preparation of *E. officinalis*, is used as a rejuvenative agent that nourishes the brain cells by supporting the nervous system and enhances memory and motor coordination (**Sharma et al., 1996**). Several reports indicated that the biological and therapeutic effects of *E. officinalis* attributed due to presence of vitamin-C (content ranging from 0.1 to 0.7% in fresh pericarp). But a study on

fresh juice and solvent extracts of *E. officinalis* indicated its antioxidant activity due to the presence of low molecular weight hydrolyzable tannoid compounds (Emblicanin A and B, Punigluconin, and Pedunculagin; content ranging from 10 to 12% in fresh pericarp) even in the complete absence of vitamin C (**Ghosal et al., 1996**). Tannins extracted from botanical origin reduced the size and incidence of skin tumor, lung tumors and gastrointestinal tumor in mice (**Huang et al., 1992; Chung et al., 1992; Kamei et al., 1999**). Its protective effects against esophageal, duodenal, pancreatic, hepatic, pulmonary, and mammary tumors were documented in animal models (Athar et al., 1989). But till now the effect of tannoid principles of *E. officinalis* (EoT) on AD in experimental animals was not reported. Therefore, this study has been undertaken to find out whether EoFM extract can offer neuroprotective action on AD-induced animals by employing various invivo and invitro studies, behavior tests (water maze) and biochemical assessments of AChE activity and expression of amyloidogenic proteins.

Currently available Anti-Alzheimer's drugs, in the market are cholinesterase inhibitors, these drugs acts on Acetyl cholinesterase (AChE) to reverse the enzyme activity or to restore acetylcholine levels in brain. However these drugs have side effects. So there is necessity to find a drug from plant products to make side effects fewer. Contribution of the traditional medicine to human health in the 21st Century is of paramount importance. It is hoped that the strong knowledge base on proteins involved in disease progression with combinatorial high-throughput screening techniques will improve drug discovery development process, thereby providing new functional leads for AD and other age-associated neurodegenerative diseases with fewer side effects.

Recent research findings suggested that, due to complex nature of the Alzheimer's Disease, it is dire need to find new, safe and economical cheaper drug compounds from natural resources which can modulate the neurotransmitters manifestation in Alzheimer’s Disease. Based on the beneficial medicinal effects of *Emblica officinalis* fruit an attempt was made in the present study to assess the effect of *Emblica officinalis* fruit extract on some selected parameters in A.D - induced rat model is an novel approach to development of anti-Alzheimer’s bioactive compounds from herbs.

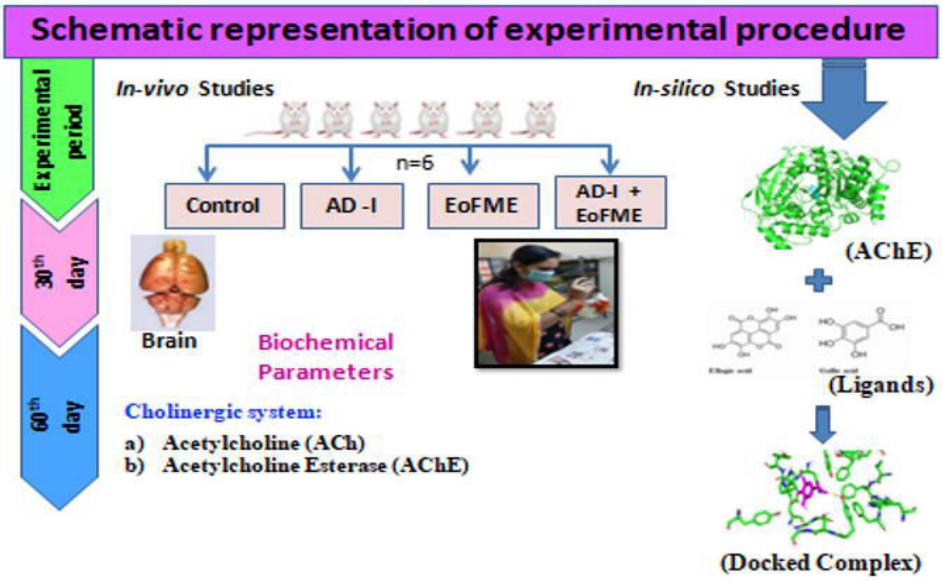
In view of multiple beneficial qualities of *Emblica officinalis*, future directions in research involve determining which compound affect which psychological and neurological disorders, with the goal being the development of specific Memory Enhancing compounds from the *Emblica officinalis* extract to treat such conditions. Since it is not known enough yet about which constitute compound *Emblica officinalis* will affect human neurophysiology. However, research has shown that crude Amlachurna may prove memory and recommended that useful remedy for the management of Alzheimer's Disease (AD) multifarious medicinal values but specific compounds were not identified to improve and reversal of memory deficits in AD.

MATERIALS AND METHODS

Preparation of *Emblica officinalis* fruit Methonolic (EoFM) extract:

Fresh *Emblica officinalis* fruits (Amla) were collected from Tirupati local market and authenticated by the Botanical Taxonomist from Department of Botany, Sri Ventakeswara University, Tirupati. Collected fruits were washed under running tap water and allowed to air-dry. After cleaning about 2kgs of fresh fruits were taken and seeds were removed and chopped into small pieces. The chopped fruits were complete air dried under shade. After that, dried fruit pieces were grinded to make fine powder. Methanolic extract was prepared using 500g of crude powder in to 2 liters of 80% methanol and socked for 24hrs; the filtrate was separated and taken for extracting preparation by using Soxhlet apparatus. The remaining residue was taken to repeat the same process for three times. The mixture was filtered to separate filtrate and residues. Total filtrate was concentrated by

Soxhlet and compete residues were collected and lyophilized (**Singh *et al.*, 2012**) to make powder. The extract was freeze dried and stored at -20^{0}C until further use. The yield of the extract was calculated as 30% w/w.



Procurement and Maintenance of Experimental Animals

About 3 months old disease free Male Albino (Wister Strain) rats weighing 150±25 grams were used as the experimental animals in the present investigation. The rats were purchased from the Sri Venkateswara Traders Pvt. Limited, Bangalore, maintained in the animal house of the department in polypropylene cages under laboratory conditions of 28±20C temperature with photoperiod of 12 hours light and 12 hours dark and 75% relative humidity. The rats were fed with standard pellet diet (Hindustan Lever Ltd., Mumbai) and water ad libitum. The rats were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA 438/01/a/cpcsea/dt.17.07.2001 in its resolution No.09/(i)/a/CPCSEA/IAEC/ SVU/ ZOO/ KY/ Dt.19-04-2012. Animals were allowed to acclimatize to the laboratory conditions for 10 days before the experimentation, the rats were randomly divided into four groupsof twelve each and were housed in separate cages. These different groups of rats except control were treated with D-Gal and EoFMextract as given below. All doses were given once in the morning hours in between 8 to 9 A.M, keeping in view the altered activity of rat during the nights compared to day time.

Grouping of Animals:

Group-I	Control Rat
Group-II **(AD-I)**	Rat, Intraperitoneally (IP) injected with D-Galactose (120 mg/kg body weight) up to end of the experiment (1st day to 60th day). **(Zhang *et al.*, 2005; Peera and Yellamma *et al.*, 2015).**
Group-IV **(AD-I+ EOE-T)**	Rat, intraperitoneally injected with **D-Galactose** (120 mg/kg body weight) once daily along with administered (oral) with EoFMextract(200 mg/kg body weight) up to 60th day.

Induction of Alzheimer's Disease to rat:

Until now, researchers developed several methods to induce AD in animal model but the application of D-Gal is considered to be quite successful method to inducing AD symptoms in animal models in the present work Induction of Alzheimer's disease to rat was done as per the

method of **Zhang *et al.*, 2005 modified by Peera and Yellamma, 2015.** Intraperitoneally injection of D-Gal induces the symptoms in animals, such as abnormal alterations in biochemical markers, retrograde changes in neural cells, and memory impairments quite similar to naturally accruing Alzheimer's Disease.

ISOLATION OF TISSUES:

For biochemical estimations, the rats were on sacrified selected time intervals i.e., on 30th day and 60th day of experimentation by cervical dislocation. The brain was isolated immediately and placed on a chilled glass plate. Different regions of brain such as, Cerebral Cortex (CC) and Hippocampus (HC) were separated by following standard anatomical marks **(Glowinski and Iverson, 1966)** and frozen in liquid nitrogen and then stored at -80^{o}C until further use. At the time of biochemical analysis, the tissues were thawed and used.

Estimation of Acetyl cholinesterase (E.C. 3.1.1.7) activity:

a. Acetylcholine (ACh) content: (Metcalf, 1957) as given by Augustinsson, 1957).

The Acetylcholine content was calculated using Hestrin's (1949) method, as described by Augustinson (1957). Each region of brain tissue was placed in a test tube and boiled for 10 minutes in a hot water bath to denature the AChE activity. The tissues were homogenised in 2.0 mL of distilled water. 1.0 ml of alkaline hydroxylamine hydrochloride was added to the homogenate, followed by 1.0 ml of 1:1 hydrochloric acid solution. The contents were properly combined and centrifuged. 0.5 ml of 0.37 M Ferric chloride was added to the supernatant, and the brown colour generated was measured at 540 nm against the reagent blank. Acetylcholine content was represented as µ moles of Acetylcholine/gm wet tissue weight.

b. Acetylcholinesterase (E.C. 3.1.1.7) activity: (Ellman *et al.*, 1961).

The method of **Ellman *et al.* (1961),** with a few changes, was used to measure the activity of Acetylcholinesterase (AChE, E.C. 3.1.1.7) in different parts of the brain. The reaction mixture had 270 µ moles of sodium phosphate buffer (pH 8.0), 10 µ moles of DTNB, 1.5 µl of Acetylthiocholine iodide (AtChI), and 100 µl of 2% brain homogenate. Before the substrate was added, a Hitachi U-2000 spectrophotometer read that the initial absorbance of the reaction mixture was 412 nm. Acetyl thiocholineiodide was put in to start the reaction. The yellow colour that appeared after 15 minutes of incubation at room temperature was measured to be 412 nm. To figure out how active an enzyme is, a molar extinction coefficient of 4.12x10-3 was used. By running parallel sets with a cholinesterase inhibitor (1:5-bis-[N-allyl-N-methyl-4-aminophenyl] pental-3-one dibromide (BW 284 C51 dibromide, Sigma Chemical Co., USA), the true cholinesterase activity could be estimated. The Acetylcholinesterase in the reaction mixture is strongly stopped by this. This set can be used to figure out the activity of pseudocholinesterase. To get the real cholinesterase activity, the pseudocholinesterase activity was taken away from the total cholinesterase activity. The activity of the enzyme was measured in µ moles of Acetyl thiocholine hydrolyzed/mg protein/hr. The results obtained were analyzed statistically.

***Insilico* Analysis:**

Protein – Ligand docking studies by Auto Dock Vina (PyRx). AChE proteinInformation and 3D crystal structure was reterived from PDB database with protein ID: **4PQE** and the bioactive compounds derived from EoFM extract were selected from literature (**Swetha and Krishna Mohan, 2014**) and the 3D structure of ligand molecules were downloaded from PubChem databases. The following Bioinformatics-Tools and Softwares applied for the present insilico studies:

Table-22: Tools and Software employed for determination of the selected parameters

S. No.	Name of the parameter	Tool / Software employed
1.	Retrival of Protein sequence and 3D structure	PDB Data bank
2.	Selection of Ligand	Pubchem Data base
3.	Energy Minimization	Aurgus lab
4.	Protein-Ligand docking	AutodockVina (PyRx)
5.	Interaction and Visualization	Pymol
6.	Biochemical test of lead molecule	PASS Prediction

Autodock Vina By Using PyRx:
PyRx is a Virtual Screening software for Computational Drug Discovery that can be used to screen libraries of compounds against potential drug targets. PyRx enables Medicinal Chemists to run Virtual Screening from any platform and helps users in every step of this process - from data preparation to job submission and analysis of the results. While it is true that there is no magic button in the drug discovery process, PyRx includes docking wizard with easy-to-use user interface which makes it a valuable tool for Computer-Aided Drug Design. The macromolecule and ligand preparation were done by **AUTO-DOCK Vina by using PyRx** with simple steps to prepare the ligand molecule in PyRx tool.
PyMol is widely used open-source molecular visualization program maintained by DeLano Scientific LLC as a user sponsored project. PyMol version 1.0 earned a reputation for its ease of use and numerous features. PYMOL uses object-oriented code hydrogen bond interactions, ionic interactions, identification of residues, atoms and their bond lengths were visualized graphically.

Bioactivity analysis of Selected Ligands were done by using Pass Prediction tool based on the structural properties of the ligands function was predicted.

Statistical Analysis:
Statistical analysis was performed by using SPSS 20.0. Values of the measured parameters were expressed as Mean ± SD. Repeated Measures of ANOVA were used to test the significance of difference among four different groups followed by Dunnet's Multiple Range Test (DMRT). The results were presented with the F-value and p-value.

RESULTS:
a) Acetylcholine (ACh) Content: (Table-1 and Graph-1)
From the results of the Acetylcholine (ACh) content, it was observed that the levels ACh were changed in both selected regions viz. Cerebral Cortex and Hippocampus of control and experimental rats. The outcomes were as follows:
On 30th Day: In control rats, maximum ACh content was found in the Hippocampal region (43.84 µmoles of Ach/gm) than the Cerebral Cortex (35.14 µmoles of Ach/gm) on the 30th day of experimentation. When compared to the control rats, the AD-induced rat showed decreased levels of ACh content in the HC region (-31.573%) followed by the CC region (-18.585). On the other hand, AD-induced rats, simultaneously treated with EoFM extract showed significant elevations in ACh content in both HC (19.85%) and CC (16.785%) brain regions compared to their respective controls. On the 30th day, the tendency of ACh to recover towards the control was also noticed.

On 60th Day: Similar to results on 30th day, the ACh content in control rats was highest in the HC region (45.08 moles of Ach/gm), followed by the CC (41.75 moles of Ach/gm) on 60th day of the experiment. In AD-Induced group, maximum depletion in ACh content was recorded in the HC region (-46.533%) rather than the CC region (-35.924%). Significant changes in ACh content were observed in AD-induced rats treated with EoFM extract when compared to the control and AD-

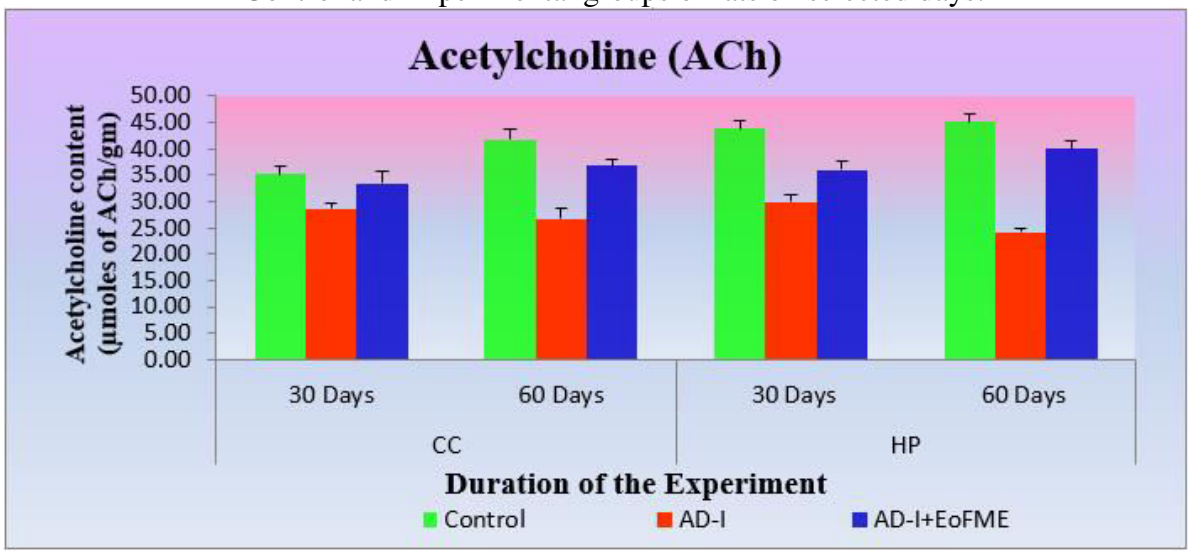
induced groups; the highest levels were observed in the HC (65.594%) than the CC (37.65%) brain regions with their respective controls.

According to the above findings, it was evident that when treated with EoFM extract, the ACh content levels began to rise from 30th Day on wards and reached their normal level by the end of the experiment i.e. on the 60th Day.

Table-1: Showing the changes in ACh content in selected regions of brain (Hippocampus and Cerebral Cortex) on selected Days.

Groups	Brain Region			
	Cerebral Cortex		Hippocampus	
	ACh 30th Day	ACh 60th Day	ACh 30th Day	ACh 60th Day
Control	35.14 ± 1.44369	41.75 ± 1.99863	43.84 ± 1.49091	45.08 ± 1.48738
AD-I	28.61 ± 1.00141 (-18.585*)	26.75 ± 1.86438 (-35.924*)	30.00 ± 1.37674 (-31.573*)	24.10 ± .92340 (-46.533*)
AD-I+ EoFME-T	33.41 ± 2.23164 (-4.92*) (16.785**)	36.83 ± 1.27137 (-11.80*) (37.655**)	35.95 ± 1.61973 (-17.989*) (19.851**)	39.92 ± 1.51334 (-11.462*) (65.594**)

Graph-1: Changes in Acetylcholine content in Hippocampus and Cerebral Cortex regions of Brain in Control and Experimental groups of rats on selected days.



ANOVA – ACh

Regions and Day	Group	Sum of Squares	df	Mean Square	F	Sig.
Cerebral Cortex 30th Day	Between Groups	137.371	2	68.685	25.542	.000
	Within Groups	40.336	15	2.689		
	Total	177.707	17			
Cerebral Cortex 60th Day	Between Groups	701.375	2	350.688	115.779	.000
	Within Groups	45.434	15	3.029		
	Total	746.809	17			
Hippocampus 30th Day	Between Groups	578.461	2	289.230	128.704	.000
	Within Groups	33.709	15	2.247		
	Total	612.170	17			
Hippocampus 60th Day	Between Groups	1433.608	2	716.804	401.557	.000
	Within Groups	26.776	15	1.785		
	Total	1460.384	17			

b) Acetylcholinesterase (AChE) activity:(Table-2 and Graph-2)
In contrast to the ACh content, the AChE activity levels showed exactly opposite trend in selective regions of rat brain on both selected days of experiments.

On 30th Day of experimentation: On the 30th Day, in control group, the CC region had higher (7.25 µ moles of AChE hydrolyzed per mg protein/h) AChE levels than the HC (7.20 µ moles of AChE hydrolyzed per mg protein/h) region. When compared with the control group, the AD induced rats administered with D-Gal were found to have increased AChE activity levels in both brain regions. The maximal increase was recorded in the HC region (10.21) with 39.873%, followed by the CC (9.06) region with 25.039%. Whereas, the AD-induced group on treatment with EoFM extract, AChE activity levels were decreased in the HC (-3.853%) and CC (-7.505%) brain regions.

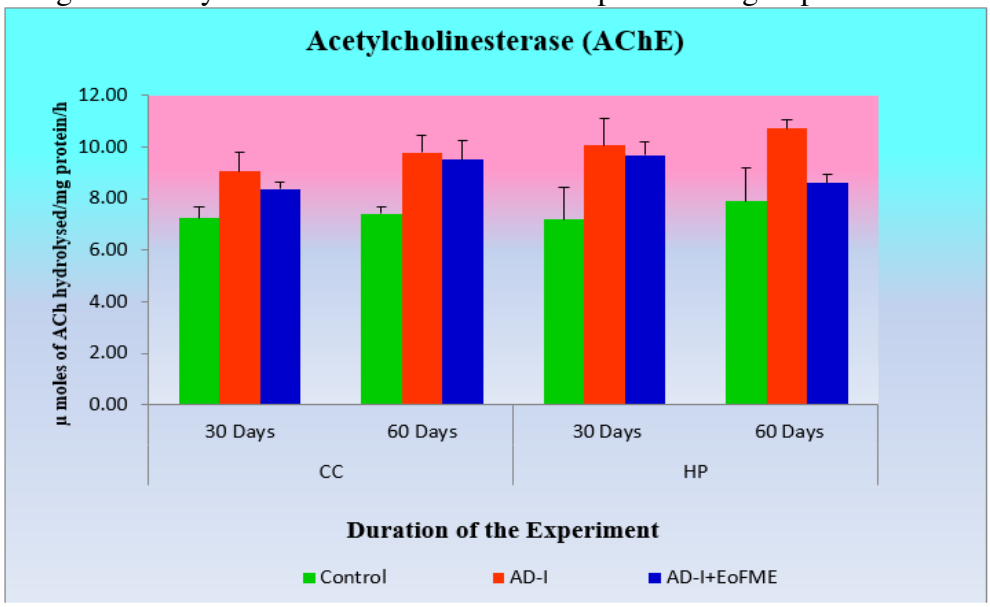
On 60th day of experimentation: In line with the result obtained on 30th day, quantified variations were noticed in the AChE level in all control and experimental rats during 60th day of experimentation, also with a similar trend. In comparison to controls, the HC region (10.73 µ moles of AChE hydrolyzed/mg protein/h) and the CC region (9.80 µ moles of AChE hydrolyzed/mg protein/h) showed the greatest increase in AChE levels in AD-induced rats. When compared to the AD control, a significant decrease in AChE levels was observed in rat brain regions subjected to EoFM extract; the maximum increase was recorded in the HC region (-19.79%) compared to the CC region (-2.918%).
The results of the AChE enzyme revealed that, when compared with control rats, a recovery tendency was recorded in AD induced rats treated with EoFM from the 30th to the 60th day of experimentation. The AChE levels were returned to normal levels in the treated group at the end of the experimentation.

Table-2: Showing the changes in AChE Leves in selected regions of brain (Hippocampus and Cerebral Cortex) on selected Days.

Groups	Brain Regions			
	CC		HP	
	30th Day	60th Day	30th Day	60th Day
Control	7.25±.45054	7.41±.27147	7.20± 1.23990	7.89±1.29183
AD-I	9.06±.75147 (25.039*)	9.80±.66644 (32.243*)	10.07±1.03153 (39.873*)	10.73±.34719 (35.870*)
AD-I+ EoFME-T	8.38 ± .26563 (15.653*) (-7.505**)	9.51± .72823 (28.383*) (-2.918**)	9.68± .50243 (34.483*) (-3.853**)	8.60± .32029 (8.981*) (-19.79**)

Values are Mean ± SEM of six observations each from tissues pooled from 6 rats.
Values in parentheses are percent changes from control.
*Values in parentheses are percent changes from Control rats.
**Values in parentheses are percent changes from AD-Induced rats.
Values are significantly different from control at $p < 0.05$

Graph-2: Changes in Acetylcholinesterase Levels and Experimental groups of rats on selected days



ANOVA - AChE

Regions and Day	Group	Sum of Squares	df	Mean Square	F	Sig.
Cerebral Cortex 30th Day	Between Groups	10.087	2	5.044	18.050	.000
	Within Groups	4.191	15	.279		
	Total	14.278	17			
Cerebral Cortex 60th Day	Between Groups	20.414	2	10.207	29.214	.000
	Within Groups	5.241	15	.349		
	Total	25.655	17			
Hippocampus 30th Day	Between Groups	29.079	2	14.540	15.284	.000
	Within Groups	14.269	15	.951		
	Total	43.348	17			
Hippocampus 60th Day	Between Groups	26.051	2	13.026	20.654	.000
	Within Groups	9.460	15	.631		
	Total	35.511	17			

Insilico studies:

Protein and Ligands Structural Information:

Based on the literature, the structure and sequences of the human AChE protein (PDB ID: 4PQE) were retrieved from the Protein Data Bank (PDB) and the protein properties were analysed to study the molecular interaction between Acetylcholinesterase (AChE) and selected bioactive compounds from *Emblica officinalis* retrived from Pubchem Data bases.

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Table-3: Showing FASTA format sequence of AChE protein & PDB 3D of 4PQE)

>4PQE_1|Chain A|Acetylcholinesterase|Homo sapiens (9606)
EGREDAELLVTVRGGRLRGIRLKTPGGPVSAFLGIPFAEPPMGPRRFLPPEPKQP
WSGVVDATTFQSVCYQYVDTLYPGFEGTEMWNPNRELSEDCLYLNVWTPYPR
PTSPTPVLVWIYGGGFYSGASSLDVYDGRFLVQAERTVLVSMNYRVGAFGFLA
LPGSREAPGNVGLLDQRLALQWVQENVAAFGGDPTSVTLFGESAGAASVGMH
LLSPPSRGLFHRAVLQSGAPNGPWATVGMGEARRRATQLAHLVGCPPGGTGGN
DTELVACLRTRPAQVLVNHEWHVLPQESVFRFSFVPVVDGDFLSDTPEALINAG
DFHGLQVLVGVVKDEGSYFLVYGAPGFSKDNESLISRAEFLAGVRVGVPQVSDL
AAEAVVLHYTDWLHPEDPARLREALSDVVGDHNVVCPVAQLAGRLAAQGAR
VYAYVFEHRASTLSWPLWMGVPHGYEIEFIFGIPLDPSRNYTAEEKIFAQRLMRY
WANFARTGDPNEPRDPKAPQWPPYTAGAQQYVSLDLRPLEVRRGLRAQACAF
WNRFLPKLLSAT

3D Structural details of the Protein 4PQE	
PDB ID	**4PQE**
Name	**Acetylcholinesterase (Human)**
No. Amino acids	**543**
Molecular Weight	**59.58 kDa**

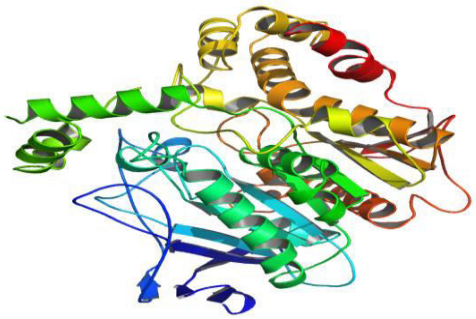
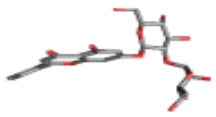
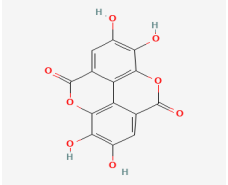
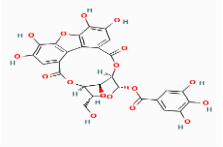
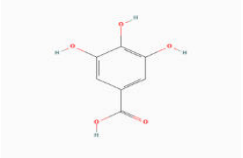
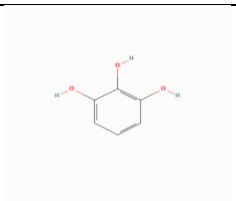
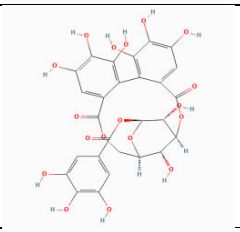
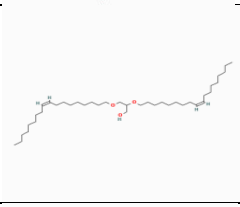
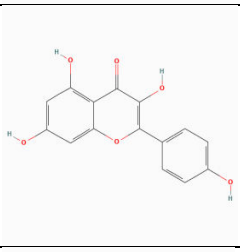
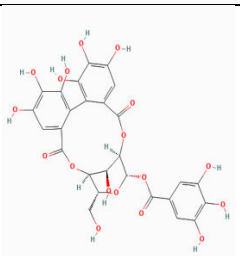
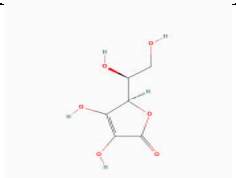


Table-4: Structures of 10 Ligands selected from *Emblica officinalis* Fruit Extract.

S.No.	Compound ID and Ligand name	Properties					Structure
		Molecular weight (g/mol)	molecular formula	H - Donor Count	H- Bond Acceptor Count	XLog P3 -AA	
1	5282152 (Lonicerin)	594.5	$C_{27}H_{30}O_{15}$	9	15	-0.6	
2	5281855 (Ellagic acid)	302.19	$C_{14}H_6O_8$	4	08	1.1	
3	11135859 (Phyllanemblinin A)	616.4	$C_{27}H_{20}O_{17}$	9	17	0.9	
4	370 (Gallic acid)	170.12	$C_7H_6O_5$ or $C_6H_2(OH)_3COOH$	4	5	0.7	

5	**1057 (Pyrogallol)**	**126.11**	**$C_6H_6O_3$ Or $C_6H_3(OH)_3$**	**3**	**3**	**0.5**	
6	**73568 (Corilagin)**	**634.5**	**$C_{27}H_{22}O_{18}$**	**11**	**18**	**0.1**	
7	**5280343 (1,2-Dioleylglycerol)**	**593.0**	**$C_{39}H_{76}O_3$**	**1**	**3**	**15.1**	
8	**5280863**	**286.24**	**$C_{15}H_{10}O_6$**	**4**	**6**	**1.9**	
9	**10941235**	**634.5**	**$C_{27}H_{22}O_{18}$**	**11**	**18**	**0.1**	
10	**54670067**	**176.12**	**$C_6H_8O_6$ Or $HC_6H_7O_6$**	**4**	**6**	**-1.6**	

Results on *In silico* studies: (Table-5&6)

Docking studies aid in understanding how Ligands engage with AChE speripheral sites, alter its secondary structure, and finally increase its activity. Docking of Ligands selected from the fruit of *Emblica officinalis* was performed using an advanced molecular docking tool, AutoDock Vina (1.1.2) to estimate the binding affinities to the protein active site. The Ligands rotational bonds were viewed as flexible, but the protein's were treated as rigid. Grid boxes were placed around the protein's active site. For searching, a Genetic Algorithm (GA) was utilised; the score functions for the interactions were constructed; and docking evaluation was performed using pymol visuvalization software.

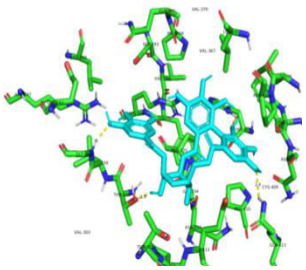
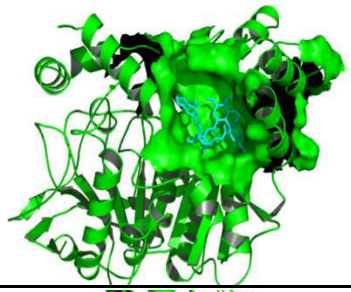
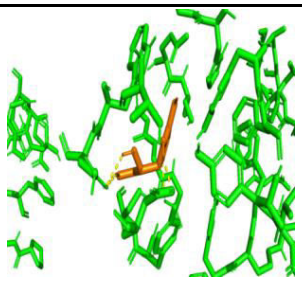
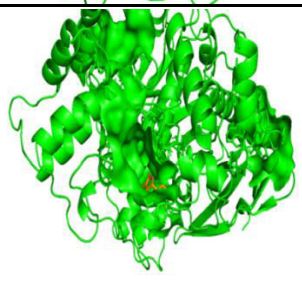
Table-5: Showing the interaction studies between AChE (4PQE) Protein and selected 10 Ligands by AutodockVina.

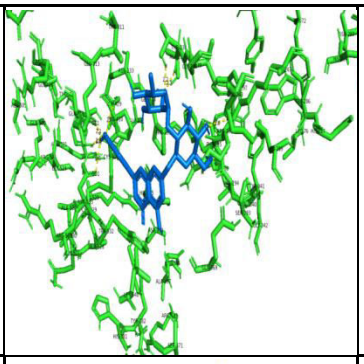
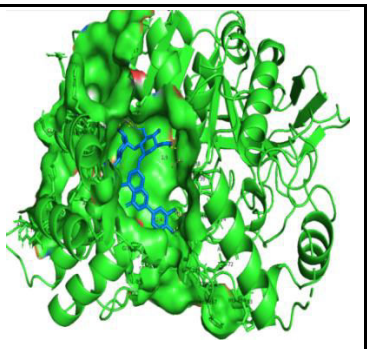
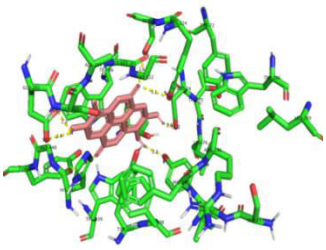
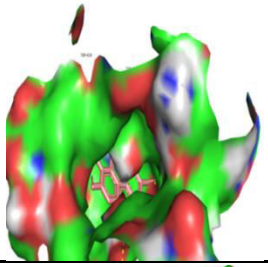
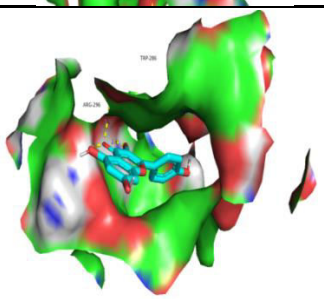
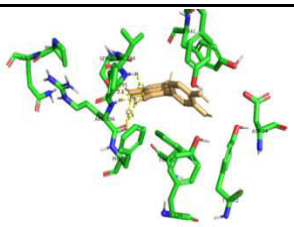
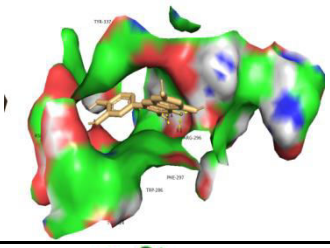
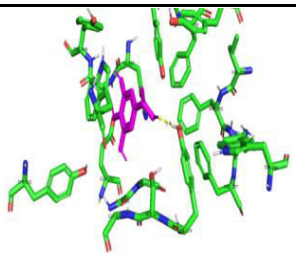
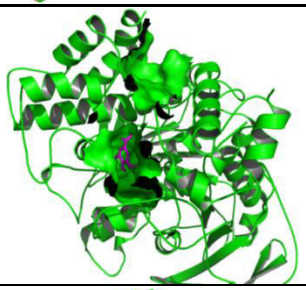
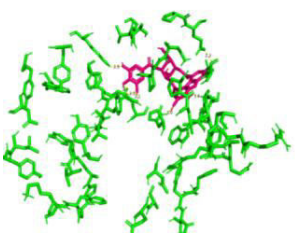
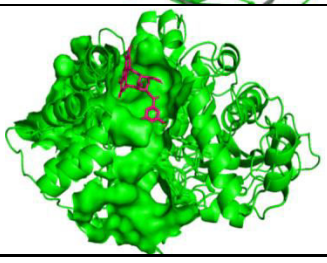
S.No.	Ligand	Binding affinity Kcal/mol	No. bonds formed	Bond formation between	Bond length A^0	RMSD
1	4pqe_EM_73568_uff_E=749.04	-9	04	THR-238 – OG1---H ASN-233 – O --- H GLN-413 – O --- H HIS-405 – H --- O	1.9 2.2 2.4 2.8	0
2	4pqe_EM_54670067_uff_E=200.65	-6	04	ASN-87 – OD1 --- H ASN-87 – OD1 --- H ASP-74 – OD2 --- H TYR-124 – H --- O	2.1 2.3 2.1 2.5	0
3	**4pqe_EM_5282152_uff_E=649.48**	**-9.7**	**10**	**ARG-296 –H --- O ARG-247 –H --- O THR-238 –H --- O THR-238 –H --- O GLY-234 –O--- H ASN-233 –H --- O GLN-413 –H --- O GLN-413 –H --- O ASN-533 –OD1--- H GLU-313 –OE1---H**	**2.4 2.8 2.2 2.3 1.9 2.7 2.8 2.0 2.5 2.1**	**0**
4	**4pqe_EM_5281855_uff_E=227.58**	**-9.7**	**07**	**TYR-341 – OH--- H ASP-74 – OD2--- H TYR-124 – OH--- O SER-125 -OG ---O HIS-447 – O --- O SER-203 -OE1 ---H SER-203 -OE2 ---H**	**2.2 2.0 3.1 2.6 3.4 2.3 2.4**	**0**
5	4pqe_EM_5280863_uff_E=362.50	-8.5	05	PHE-295 – H --- O ARG-296 –O --- O ARG-296 –H --- O TYR-337 –OH --- H SER-293 -OG ---O	2.7 3.1 2.4 2.4 2.7	0
6	4pqe_EM_5280343_uff_E=380.43	-8.5	05	ARG-296 –H --- O ARG-296 –O --- H ARG-296 –O --- O PHE-295 –H --- O SER-293 –OG --- O	2.4 2.4 3.1 2.7 2.7	0
7	4pqe_EM_**370**_uff_E=77.82	-6.2	01	TYR-124 – OH --- H	2.4 A	0
8	4pqe_EM_**11135859**_uff_E=1056.48	**-9.3**	08	ARG-296 – H --- O ARG-296 – H --- O ARG-296 – H --- O HIS-405 – H --- O ASN-533 – OD1 --- H GLN-413 – H --- O GLU-313 – OE2 --- H ARG-247 – H --- O	2.1 2.3 2.4 1.9 1.6 1.9 2.3 2.5	0
9	4pqe_EM_**10941235**_uff_E=900.57	-8.9	08	GLN-413 – H --- O GLN-313 – H --- O HIS-405 – H --- O	1.8 2.2 2.7	0

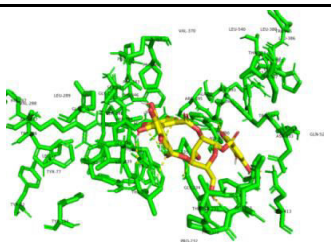
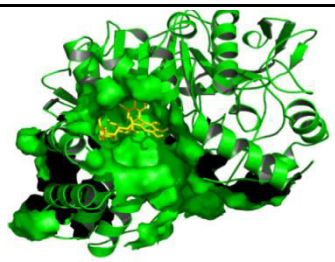
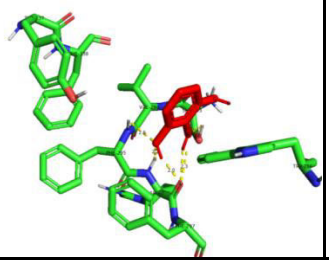
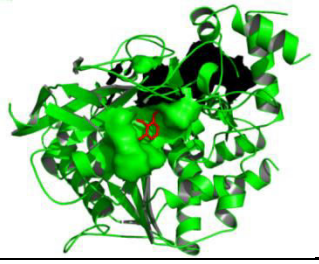
				GLY-234 – O --- H PRO-235 – O --- H PRO-235 – O --- H THR-238 – H --- O THR-238 – O --- H	2.3 2.6 2.4 2.1 2.7	
10	4pqe_EM_**1057**_uff_E=62.72	-5.5	05	PHE-295 – H --- O ARG-296 – H --- O ARG-296 – O --- H ARG-296 – O --- H SER-293 – H --- O	2.6 2.2 2.0 2.3 2.4	0

The results generated by AutodockVina, revealed that all selected compounds showed binding affinity with AChE (4PQE). Three compounds were found to have a high binding affinity. The ligand, **Lonicerin (ID: 5282152)** showed the highest binding affinity score (-9.7 kcal/mol) against 4PQE with the following amino acids: ARG-296, ARG-247, THR-238, THR-238, GLY-234, ASN-233, GLN-413, ASN-533, and GLU-313 ofthe AChE binding project. In total, 9 hydrogen donor and 15 hydrogen acceptor atoms were involved in forming 10 bonds between the Ligand and protein. The second ligand, **Ellagic acid (ID: 5281855),** also formed the highest binding affinity **(Score-9.7**kcal/mol) on par with Lonicerin except for the number of Hydorgen receptors-4 and Donars-8 involved to form 07 inhibitory bonds with the following amino acids: TYR-341, ASP-74, TYR-124, SER-125, HIS-447, SER-203 respetively. When compare with **Lonicerin and Ellagic Acid, Phyllanemblinin-A (ID: 111358)** and **Phyllanemblinin-B (ID: 10941235)** also showed good binding affinity as -9.3 and -8.9 kcal/mol and formed "8" bonds each.

Table-6: Pymol Visualization of Protein- ligand interaction:

S.No.	**Ligand docking pose**	**Docking**	**Ligand binding at active site of the protein**
1	4pqe_EM_73568_uff_E=749.04		
2	4pqe_EM_54670067_uff_E=200.65		

3	4pqe_EM_5282152_uff_E=649.48		
4	4pqe_EM_5281855_uff_E=227.58		
5	4pqe_EM_5280863_uff_E=362.50		
6	4pqe_EM_5280343_uff_E=380.43		
7	4pqe_EM_370_uff_E=77.82		
8	4pqe_EM_11135859_uff_E=1056.48		

9	4pqe_EM_10941235_uff_E= 900.57		
10	4pqe_EM_1057_uff_E=62.72		

From the results on Docking interpretation it was obvious that the bioactive components from *Emblica officinalis* Fruit Extract have significant affinity for Acetylcholinesterase (AChE enzyme). Further, as per the docking scores they have been proven to be very competent Acetylcholinesterase inhibitors (AChE-I). The selected Ligands have already been identified as therapeutic candidates for the treatment of different disorders and the details were already presented in the literature.

Bioactivity Analysis of Selected Ligands by Pass Prediction: (Table-7,8 & 9)

The pass prediction results generated based on the structures of the selected Ligands viz., attributed AChE inhibitory activities for these three bioactive compounds, viz., Ellagic acid, Lonicerin and Phyllanemblin which confer neuronal protection, under acute neurological disease conditions. The therapeutic aspects for each of the 3 Ligands were given separately in Tables: 27, 28 and 29.

Table-27: Pass Prediction output for Lonicerin (5282152):

S.No.	Pa	Pi	Activity
1	0,037	0,020	Dopamine precursors
2	0,060	0,047	(acetyl-CoA carboxylase) kinase inhibitor
3	0,065	0,054	N-acetylglucosamine kinase inhibitor
4	0,110	0,094	Nicotinamidephosphoribosyltransferase inhibitor
5	0,121	0,059	Glutamate release inhibitor
6	0,195	0,037	Glutathione S-transferase substrate
7	0,288	0,038	Antineoplastic (colorectal cancer)
8	0,300	0,025	Antineoplastic (brain cancer)
9	0,332	0,199	Acute neurologic disorders treatment
10	0,430	0,004	Beta-glucosidase inhibitor
11	0,436	0,004	Neurotrophic factor enhancer
12	0,436	0,005	Beta-amylase inhibitor
13	0,463	0,004	Vascular dementia treatment
14	0,598	0,013	Antidiabetic
15	0,625	0,004	Dementia treatment
16	0,799	0,008	Apoptosis agonist
17	0,800	0,006	Oxidoreductase inhibitor
18	0,814	0,001	Glutathione-disulfide reductase inhibitor
19	0,846	0,003	Antioxidant
20	0,938	0,002	Lipid peroxidase inhibitor

21	0,974	0,001	Free radical scavenger

Table-28: Pass Prediction output for Ellagic acid (5281855):

S.No.	Pa	Pi	Activity
1.	0,745	0,011	UDP-N-acetylglucosamine 4-epimerase inhibitor
2.	0,659	0,005	Neurotransmitter antagonist
3.	0,612	0,012	Steroid N-acetylglucosaminyltransferase inhibitor
4.	0,620	0,020	L-glutamate oxidase inhibitor
5.	0,596	0,006	Free radical scavenger
6.	0,587	0,023	Methylamine-glutamate N-methyltransferase inhibitor
7.	0,512	0,004	Neurotrophic factor enhancer
8.	0,530	0,028	N-acetylneuraminate 7-O(or 9-O)-acetyltransferase inhibitor
9.	0,534	0,054	Acetylcholine neuromuscular blocking agent
10.	0,487	0,024	Acetylgalactosaminyl-O-glycosyl-glycoprotein beta-1,3-N-acetylglucosaminyltransferase inhibitor
11.	0,491	0,034	Acetylesterase inhibitor
12.	0,424	0,012	N-acetylneuraminate synthase inhibitor
13.	0,347	0,019	Dopamine release stimulant
14.	0,379	0,069	Neuropeptide Y4 antagonist
15.	0,348	0,039	Glutamate-tRNA ligase inhibitor
16.	0,247	0,037	Glutamate 5-kinase inhibitor
17.	0,225	0,020	Glutamate-1-semialdehyde 2,1-aminomutase inhibitor
18.	0,217	0,027	Glutamate (mGluR5) agonist
19.	0,204	0,019	Chloramphenicol O-acetyltransferase inhibitor
20.	0,319	0,150	Glutamate-5-semialdehyde dehydrogenase inhibitor
21.	0,193	0,034	Glutamate synthase (ferredoxin) inhibitor
22.	0,172	0,021	Glutamate decarboxylase inhibitor
23.	0,159	0,029	Glutamate N-acetyltransferase inhibitor
24.	0,170	0,040	Phosphate acetyltransferase inhibitor
25.	0,181	0,063	Protein-glutamate methylesterase inhibitor
26.	0,157	0,048	Acetyl-CoA C-acyltransferase inhibitor
27.	0,114	0,020	Glutamate (mGluR8) agonist
28.	0,118	0,030	Glutamate dehydrogenase (NADP+) inhibitor
29.	0,109	0,052	Glutamate (mGluR6) antagonist
30.	0,084	0,029	(acetyl-CoA carboxylase) kinase inhibitor
31.	0,071	0,018	Glutamate uptake inhibitor
32.	0,119	0,067	Neuropsin inhibitor
	0,041	0,014	Dopamine precursors
	0,059	0,041	Glutamate formimidoyltransferase inhibitor

Table-29: Pass Prediction output for Phyllanemblinin A (11135859):

S.No.	Pa	Pi	Activity
1	0,895	0,002	Free radical scavenger
2	0,860	0,003	Antioxidant
3	0,810	0,006	Antiinflammatory
4	0,714	0,014	Apoptosis agonist
5	0,633	0,007	Lipid peroxidase inhibitor
6	0,617	0,008	Antibacterial
7	0,373	0,006	Antineoplastic (renal cancer)
8	0,393	0,033	Antineoplastic (breast cancer)
9	0,357	0,070	GABA aminotransferase inhibitor
10	0,386	0,103	Glutamate-5-semialdehyde dehydrogenase inhibitor

11	0,274	0,046	Antineoplastic (lung cancer)
12	0,236	0,038	Neurotrophic factor enhancer
13	0,205	0,018	Radical formation agonist
14	0,134	0,015	Glycine receptor antagonist
15	0,284	0,165	Dementia treatment
16	0,209	0,106	Apoptosis antagonist
17	0,126	0,023	N-acetylglucosamine-6-phosphate deacetylase inhibitor
18	0,162	0,063	Glucosyltransferase inhibitor
19	0,125	0,033	Nucleotide diphosphatase inhibitor
20	0,153	0,062	Antiviral (HIV)
21	0,130	0,039	Phosphofructokinase-1 inhibitor
22	0,097	0,041	Galactosylgalactosylglucosylceramidase inhibitor
23	0,091	0,035	3-Oxoacyl-(acyl-carrier-protein) synthase inhibitor
24	0,141	0,085	3'-Nucleotidase inhibitor
25	0,107	0,054	Ca2+-transporting ATPase inhibitor
26	0,087	0,040	Estrogen agonist
27	0,079	0,032	NMN nucleosidase inhibitor
28	0,185	0,166	Vascular dementia treatment
29	0,133	0,127	Glutathione peroxidase inhibitor
30	0,031	0,030	Dopamine precursors
31	0,013	0,013	N-acetylgalactosamine-4-sulfatase inhibitor
32	0,058	0,057	Glucosamine 6-phosphate N-acetyltransferase inhibitor

From the above data, it was evident that several Ligands from different sourced also exhibited strong binding affinity along with high docking score which was some times equal or less efficient than the 10 Ligands selected in the preset study.

DISCUSSION

From the result, it was obvious that on both selected days of experiment, in D-Galactose (D-Gal) treated rats, the levels of ACh content were significantly inhibited while the levels of AChE activity were elevated. However, oral administration of *Emblica officinalis* fruit Methanolic (EoFM) extract significantly increased the levels of ACh and decreased the levels of AChE activity there by restoring near control levels, there by demonstrating that EoFM extract may inhibit AChE and thus acts as an Anticholinesterase. This effect may be attributed to the potential chemicals in EoFM extract it was discovered that Tannins, Terpenoids, Phenols, Comarins and other active compounds, by virtue of having this quality was extensively used in a number of memory-related neuronal diseases. It is also used as an antidepressant to treat mild depressive disorders that do not cause any side effects. Long-term administration of *Emblica officinalis* fruit extract had no effect. Non-analgesic herbal preparations are safe to use in the treatment of opiate addiction and other adverse neurological conditions. It is a safe nonanalgesic preparation that can be used to treat opiate addiction. It is included in the formulation of syrup with shankhapushpi, which is used to treat a variety of neurodisorders in conjunction with other Ayurvedic preparations.

The cholinergic system is important in memory biochemically. Scopolamine and other anticholinergic drugs impair memory. Choline acetyl transferase (the enzyme responsible for the formation of Acetylcholine) and nicotinic cholinergic receptors are both known to be deficient in Alzheimer's patients. There is substantial evidence that blocking musarinic receptors with drugs like scopolamine causes disruptions in behavioural inhibition, working (short-term) memory, retrieval from reference (long-term memory), attention and decisional processes, movement and strategy selection, and altered sensory processing **(Nahata *et al.*, 2010).**

Alzheimer's Disease (AD) is characterised by progressive loss of Memory, Thinking ability, Comprehension, Speech, Behavioural abnormalities, Mutism, Akinesia, Ideational and Ideomotor Apraxia, Visuospatial Disorientation and sign language Aphasia **(Verdon *et al.,* 2007).** Currently, the FDA has approved just a few medications for the symptomatic treatment of mild-to-moderate Alzheimer's Disease, including ChE inhibitors such as Tacrine, Donepezil, and Rivastigmine. These medicines were linked to side effects such as liver damage, aggressiveness and depression, as well as GI, Cardio-respiratory, Extrapyramidal, Genitourinary and Musculoskeletal abnormalities. Weekly blood tests and the expensive expense of these medications add to their limitations **(Thompson *et al.,* 2004).** All of these limits emphasise the crucial need for novel lead compounds derived from natural sources, such as plants having strong ChE inhibitory properties **(Mukherjee *et al.,* 2007).**

Other factors that contribute to pathogenesis of Alzheimer's Disease are neuronal malfunction and cell death due to an imbalance between free radical generation and antioxidant defenses**.** Advanced glycation end products, on the other hand, are formed as a result of posttranslational protein changes and may have a function in AD that is linked to oxidative alterations of Aβ peptides and Tau **(Gella and Durany, 2009**). Furthermore, the brain is mostly constituted of readily oxidised lipids, has a high oxygen consumption rate, a high transitional metal metabolic rate and lacks strong antioxidant defences, making it extremely prone to oxidative damage **(Gandhi and Abramov, 2012).** According to recent research, brain tissue in people with Alzheimer's Disease is subjected to protein oxidation, DNA oxidation, lipid oxidation, glycoxidation and other processes along the course of the disease **(Perry *et al.,* 2002).**

The results in the present study were well justified with the chelation properties of Emblicanin-A and Emblicanin-B in *Emblica officinalis* fruit juice. Several previous in vitro studies revealed that the Fruit of *Emblica officinalis* Methanol extract inhibited the Acetylcholinesterase (AChE) enzyme (IC50 100 g/mL), which is a major cause of cholinergic dysfunction in Alzheimer's Disease. **Biswas *et al.,* (2017)** found that FPE crude methanol extract inhibited AChE (IC50 53.88 g/mL) and BuChE (IC50 65.12 g/mL). According to **Thenmozhi *et al.,* (2016b),** *Phyllanthus emblica* tannin compounds also reversed changes in the concentrations of Aluminum, Acetylcholine esterase activity, and amyloid-beta synthesis-related molecules in the brain. A gradual decrease in AChE activity and improved performance in neurobehavioral tests were observed in rodents treated with *Phyllanthus emblica*, indicating its potential role in the treatment of Alzheimer's Disease. Oral administration of *Phyllanthus emblica* at a dose of 100 mg/kg (b.w.) for 60 days protected rats from aluminium chloride-induced toxicity and cognitive deficit. There was a reduction in oxidative stress, inhibition of apoptosis markers such as Bax, caspases-3, caspase-9, cytosolic cytochrome-c, and pTau, and increased expression of GSK-3 and pAkt genes **(Bharati and Thenmozhi, 2018).**
Further, **Husain *et al.,* (2018)** discovered that tannin-enriched fractions of *Phyllanthus emblica* protect rats from cognitive impairment caused by a high-salt, high-cholesterol diet. The treatment of rats with hydroalcoholic extract of FPE for 7 days in different moderate-to-high doses, namely 300, 500 and 700 mg/kg i.p. in pentylenetetrazole (PTZ) and kainic acid-induced epilepsy, alleviated the generalised tonic seizure and status epilepticus, respectively **(Golechha *et al.,* 2010).**

The following research findings lend strong support to present results. **Xie *et al.,* (2012)** investigated the epileptic potential of epigallocatechin-3-gallate and the polyphenol component of *Phyllanthus emblica* in PTZ-induced epilepsy in male Sprague-Dawley rats. A study in mice using traditional ayurvedic preparations of *Phyllanthus emblica* called Anwalachurna on exteroceptive (plus-maze and Hebb-Williams maze) as well as hypothetical behaviours revealed significant memory improvement and amnesia reversal with scopolamine and diazepam **(Vasudevan and Parle, 2007a,b). Golechha *et al.,* (2012)** also reported the efficacy of *Phyllanthus emblica*

hydroalcoholic extract against dementia caused by cholinergic dysfunction. **Wankhar *et al.,* (2014)** investigated the anti-stressor potential of *Phyllanthus emblica* against noise-induced stress in Wistar albino rats. All these reports provided substantial evidences that neuroprotective medications play an important role in a variety of brain disorders, including neurodegenerative diseases. In the traditional Indian medical system, *Phyllanthus emblica* has neuroprotective properties. **Reddy *et al.,* (2011)** reported that FPE had a protective effect, as evidenced by the reversal of altered levels of NO and protein carbonyls, as well as improved activity of the endogenous antioxidant system and cytochrome-c oxidase, which resulted in the attenuation of alcohol (20%)-induced brain mitochondrial dysfunction in male Wistar rats**.** Another study found that giving rats dried powder from *Phyllanthus emblica* (100 mg/day) reduced fluoride-induced neurotoxicity **(Shalini and Sharma, 2015). Rajalakshmi *et al.,* (2022)** recently reported the neuroprotective effect of FPE on human neural cell lines (PC12) against glutamate-induced cellular inhibition. Further, research into the effects of the FPE is required to reveal the molecular mechanisms and gene regulation underlying these effects.

Previous research evidence suggests that the enzyme AChE plays a role in the development of hazardous senile plaques by increasing Aβ deposition and aggregation. It has been demonstrated that AChE forms a stable compound with senile plaque components via its peripheral anionic site, which may be implicated in speeding Aβ fibril formation **(De Ferrari *et al.,* 2001).** Because of the diverse variety of chemicals found in medicinal plants, all of which act in distinct ways, mixed-type inhibition kinetics is widespread **(Mills and Bone, 2000).** The AChE inhibition kinetics in this work suggest a possible method through which the aqueous extract may have a novel therapeutic potential for AD, as these chemicals may be able to alleviate cognitive impairment by decreasing Aβ aggregation. Due to their capacity to bind to the peripheral anionic site, mixed-type or non-competitive inhibitors have been proposed as model possibilities for blocking AChE-induced Aβ aggregation **(Bartolini *et al.,* 2003).** Other investigations have suggested that mixed or non-competitive inhibitors can inhibit AChE aggregation during the early stages of AD **(Choudhary *et al.,* 2005).**

Further, damage to the brain's cholinergic (acetylcholine-producing) system has been linked to the memory problems associated with Alzheimer's Disease. Some aspects of learning and plasticity in the cortex appear to be Acetylcholine-dependent. **Bear *et al.,* (1986)** discovered that when cholinergic projections to the striate cortex are depleted, the usual synaptic remapping that occurs during monocular deprivation is diminished. A kinetic investigation of dual-cholinesterase inhibition by an aqueous extract of *Emblica officinalis* fruit and its method of inhibition reduced the degenerative process by blocking both AChE and BuChE enzymes, boosting ACh concentration, or prolonging synaptic residence time after ACh is released from cholinergic nerve terminals.

One of the primary advantages of employing a natural substance such as *Embelica officinalis* is the vast variety of therapeutic characteristics reported for central nervous system-related activities. *Emblica officinalis* also demonstrated having positive effects on cholinergic functioning in rats which had scopolamine-induced amnesia. It can thus be inferred that *Emblica officinalis*, in addition to carrying memory-enhancing, antiaging, antistress, antioxidant and also possesses anti-AChE/BuChE activities and so it could potentially provide innovative approaches for treating the symptoms associated with AD **(Golechha *et al.,* 2012).**

Since decades, ample evidences are available to demonstrate a close link between the functional cholinergic system to age-related diseases such as Alzheimer's. The activity of the enzyme responsible for Acetylcholine synthesis, Choline Acetyltransferase, a reliable marker of Cholinergic neurons and synapses was shown to be significantly reducedinthe pathological samples from the

cortex and hippocampus of Alzheimer's patients. Depolarization-induced Acetylcholine release and choline absorption in nerve terminals to replenish the Acetylcholine synthetic machine were both diminished in the same tissues. Chronic D-Gal injection increased the Actetylcholinesterase (AChE) enzyme, one of the particular cholinergic indicators in ageing animals. Previously, **Zhong *et al.,* (2009)** found that AChE activity, which is responsible for the breakdown of acetylcholine in synaptic clefts, rose dramatically in D-Gal treated mice**.** Thus, Cholinergic neurotransmission may be a specific target for β-amyloid because it has been shown *in vitro* to impair both choline absorption and ACh release.
Since, Acetylcholinesterase (AChE) is a critical enzyme in the cholinergic nerve system, many Therapies, aimed at reversing the cholinergic deficit in AD are largely based on AChE inhibitors, which improve cholinergic transmission with limited and temporary therapeutic effects. Several investigations have indicated that cholinesterase inhibitors can work on a variety of therapeutic targets, including the prevention of β-amyloid plaque formation, antioxidant action and modulation of APP processing **(Bolognesi, 2009).** However, there is still a need for new AChE inhibitor lead compounds with reduced toxicity and more Central Nervous System (CNS) penetration. Many plants have been studied using bioassay-guided approaches to identify new AChE inhibitors **(Mehta *et al.,* 2012; Murray *et al.,* 2013)** and different classes of plant-derived natural products have been considered as potential AChE inhibitors useful for AD treatment **(Ferreira *et al.,* 2006).**

The majority of cortical AChE activity in the AD brain is related with the amyloid core of senile plaques rather than the neuritic component present in the periphery. AChE, a major enzyme in the breakdown of the neurotransmitter Acetylcholine found in cholinergic terminals, increases aggregation by promoting the development of a stable complex with the enzyme. In fact, the compound causes neuronal cell death and astrocyte hypertrophy when injected into the rat hippocampus **(Reyes *et al.,* 2004).** Changes in the molecular form of AChE in cerebrospinal fluid (CSF) also reflect changes in the brain **(Sáez-Valero *et al.,* 2000a).** The most numerous light-chain constituents in plasma are likewise becoming more widespread in AD plasma **(Garca-Ayllón *et al.,* 2010).** Experiments utilising different approaches to boost acetylcholine output or damage cholinergic neurons **(Burk *et al.,* 2002)** have revealed that aged brain cholinergic neurons function pretty normally until stressed.

Similarly, memory function is subject to a wide range of pathologic events, including neurodegenerative illnesses, strokes, tumours, head trauma, hypoxia, heart surgery, starvation, attention deficit disorder, depression, anxiety, drug side effects, and normal ageing. Learning and memory are produced through a central nervous system alteration that is experience-dependent and long-lasting. They entail the activation of receptor-linked enzymes that are responsible for the creation of intercellular messengers by neurotransmitters such as Acetylcholine, Dopamine, and Serotonin. Cholinergic neurons originating in the nucleus basalis and the medial septum appear to be much more sensitive than neostriatal cholinergic neurons **(Jhamandas *et al.,* 2001).**

Numerous studies have revealed that the cholinergic system is vital in learning and memory. Furthermore, loss of cholinergic neurons and decreased choline-acetyltransferase activity in the cerebral cortex and hippocampus are constantly associated with Alzheimer's Disease. It is worth mentioning that changes in the brain of Alzheimer's Disease are centred on pyramidal neurons, which are lost in the disease, vulnerable to tangle formation, a primary source of APP, and are regulated by a neurotransmitter, ACh that is impacted early in AD. Lesions in animals that disrupt cholinergic input from the basal forebrain to the neocortex or hippocampus (e.g., nucleus basalis medial septum/diagonal band) impair illness.

***Insilico* analysis inference:**

As per the data presented in *insilico* studies, it was obvous that

1. The phytochemical constituents of *Emblica officinalis* fruit contains a variety of active pharmacological principles components such as Polyphenols, Flavonoids, Tannin Components and hydrolysable enzymes.
2. These preliminary *Inslico* studies demonstrated that the above bioactive Ligands may act as efficient AChE inhibitors in treatment of Alzheimer's Disease.
3. This *insilico* approach can be expanded to develop more effective and useful medications using ligand-based drug design approaches. It may also be extended to develop analogues for Ellagic acid, Lonicerin and Phyllanemblinin from *Emblica officinalis* in order to generate novel compounds with fewer side effects to treat neurodegenerative illnesses in general and including Alzheimer's Disease in particular.

Further the selected phytochemicals viz. Lonicerin, Ellagic acid, Phyllanemblinin A & B, Gallic acid, Pyrogallol, Corilagin, 1,2-Dioleylglycerol, Kaempherol and Ascorbic Acid mostly shared an single biding site. In that binding groove, the most common amino acids such as TYR-124, ASN-233, THR-238, ARG-296, GLU-313, HIS-405 and **GLN-413 are participated in binding and inhibitory activity with AChE enzyme.**

Indeed, decreased cholinergic activity in the brains of people with Alzheimer's Disease provides justification for the development of Acetylcholinesterase (AChE) inhibitors to treat the dementia associated with Alzheimer's Disease. The basal forebrain cholinergic system offers numerous regulatory inputs to Hippocampus development, and ACh is implicated in both learning and the cognitive deficits associated with ageing and Alzheimer's Disease (AD).

To improve cognitive function, medicines such as Donepezil, Tacrine, and Rivastigmine are being used in the treatment of mild to moderate Alzheimer's Disease. The disadvantages of using the afore mentioned medications are their side effects, which include Diarrhoea, Nausea, Vomiting, Lethargy, Sleeplessness, Muscle Cramps, loss of Appetite, and Hepatotoxicity. Given these constraints, a variety of plants or plant-derived substances with anticholinesterase characteristics are being studied for potential use as a new therapeutic alternative in the symptomatic treatment of Alzheimer's Disease **(Orhan and Sener, 2003).**

Therapies designed to reverse the cholinergic deficit in AD is mostly based on inhibitors of AChE, which increase the availability of acetylcholine through inhibition of its destruction, hence an enhancement of cholinergic transmission in the brain and improvement in the symptoms ofAD.48,49 AChE inhibitory activity of *E. officinalis* and EoT were shown in both in vitro50 and in vivo scopolamine induced amnesia.

The oral administration of *E. officinalis* fruit juice enriched with Emblicanin-A and Emblicanin-B was effective in reducing the Iron, Cadmium and Arsenic induced toxicities through its chelation properties. *Emblica officinalis* fruit extract has been reported to alleviate aluminum-induced clastogenicity and genotoxicity by the combined action of ingredients in the crude extract, rather than to ascorbic acid alone, which also supports our present findings.

In the present research programme, a good correlation between the wet-lab studies on the Cholinergic systems and the *in-silico* analysis on AChE enzyme was established. As such, the present study provided comprehensive *in-silico* evidence for potential neuro-protection against the Alzheimer's Disease. Hence, EoFME can be suggested as one of the most efficient

compound to improve memory aspects in all memory-deficient diseases in general and Alzheimer's Disease in particular, only after ascertaining with extensive clinical trials.

CONCLUSION:

In the present study, Chronic intraperitoneal D-Gal administration to albino rats resulted in a significant reduction in ACh content and an increase in AChE activity in brain regions, indicating oxidative damage. However, EoFM extract dramatically reversed these AD-Induced effects thus exhibiting, a counteracting impact therapeutic and neuroprotective properties of EoFM extract on the cholinergic system. From above observations, it was concluded that the oral administration of E. officinalis fruit extract plays an important role to reduce AChE and maintain the brain normal levels of ACh AD patients.

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