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Novel drug formulation for inhibition of β-amyloid protein by using Pharmacokinetics and In-Silico modelling studies.

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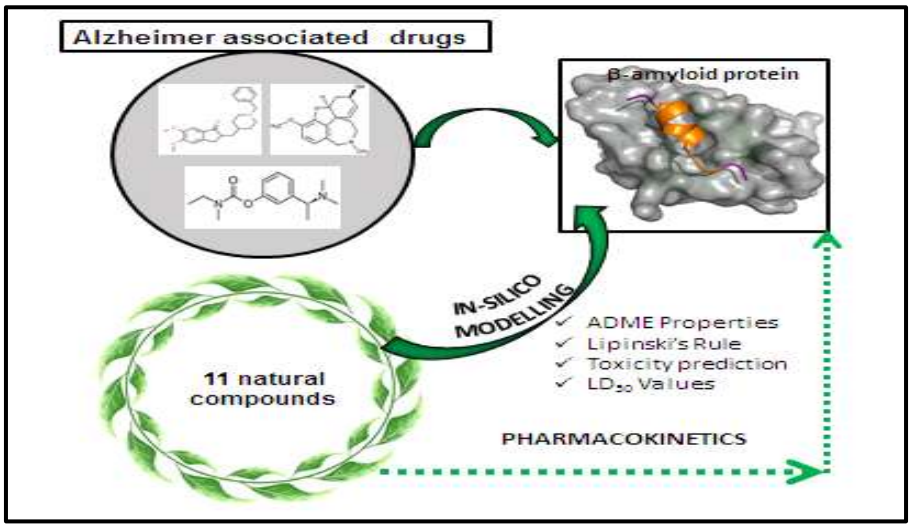
ABSTRACT:
Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory loss, cognitive decline and behavioral changes. The amyloid β peptide (Aβ) is a critical indicator that triggers the progression of AD via accumulation and aggregation.Recently few FDA approved drugs are available in the market for treatment, but their cholinergic adverse effect, potentially distressing toxicity and limited targets in AD pathology limits their use. Therefore, it is crucial step to find an effective compound to combat AD.11 natural compounds that have antioxidant properties to slow down disease progression by quenching free radicals were chosen. Therefore, we performed In-silico studies to investigate the binding interactions between natural compounds and a prime anti-Alzheimer drug target β-amyloid. Three known Cholinesterase inhibitors (Donepezil, Galantamine and Rivastigmine) were taken as reference drugs over natural compounds for comparison and drug-likeness studies. Most of these compounds followed both good anti-oxidant activity and pharmacokinetics properties that make them potentially novel drug for the treatment of Alzheimer's disease.

Keywords: Alzheimer's disease (AD), amyloid β peptide (Aβ), In-silico studies, Cholinesterase inhibitors, anti-oxidant activity, pharmacokinetics.

Graphical abstract



I. INTRODUCTION:

Alzheimer's disease is one of the most common neurodegenerative disorders that normally cause dementia and affect the middle to old-aged persons, around one in four individuals over the age of 85[1]. According to the World Alzheimer report 2021, the country with the largest number of people living with Alzheimer's disease is China with an estimated 12.6 million cases. This is followed by India with 5.7 million cases. Other countries with high number of cases include Indonesia, Brazil and Russia.Thus, this condition

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will bring gigantic financial and personal burdens to current and future generations [2]. In order to deal with this problem, effective therapeutic and preventive interventions should be developed urgently.There are no such drugs/treatments available that can cure AD or any other common type of dementia completely. However, medications have been developed for Alzheimer's disease that can temporarily attenuate the symptoms, or delay it progression. The U.S. Food and Drug Administration (FDA) have approved two medications-cholinesterase inhibitors and Memantine. Over the past decade, much of the research on Alzheimer disease (AD) has focused on oxidative stress mechanisms and its importance in disease pathogenesis. [3-5]

Extensive β-amyloid (Aβ) deposits in brain parenchyma in the form of senile plaques and in blood vessels in the form of amyloid antipathy are pathological hallmarks of Alzheimer's disease(AD) (Figure 1)[6].Reduction in Aβ has been the major target of recent experimental therapies against AD. Unfortunately, human clinical trials targeting Aβ have not shown the hoped-for benefits. Thus, doubts have been growing about the role of Aβ as a therapeutic target.[7-8]Here we need more evidences supporting inhibition of Aβ in AD using different components obtained from nature.

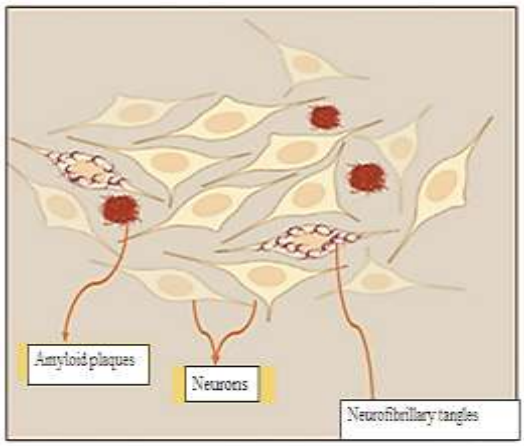

Figure 1: Graphical representation of accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles

Nature has gifted us lots of natural remedies in the form of fruits, leaves, bark, vegetables and nuts, etc. The various ranges of bioactive nutrients present in these natural products play a vital role in prevention and cure of various neurodegenerative diseases, such as AD, Parkinson's disease and other neuronal dysfunctions.[8-11] Previous studies suggested that the naturally occurring phytochemicals, such as polyphenolic antioxidants found in fruits, vegetables, herbs and nuts, and fermentation of natural products may potentially hinder neurodegeneration, and improve memory and cognitive functions.[12]

In our research work, we choose 11 natural compounds and some fermentative natural products from different databases for Insilicomodelling and pharmacokinetics studies. Recent studies have demonstrated that the natural derivatives and their fermentative products possess wide range of biological activities like anti-tubercular, anti-fungal, anti-bacterial anti-malarial, anti-inflammatory and antioxidant activities which proves to be an important formula for formulation of a novel drug for Alzheimer's disease. [13]

In our study, all the 11 compounds have antioxidant properties and it has been shown that treatments with these compounds certainly contribute to their neuroprotective effects and it is a potential approach for slowing disease progression. Therefore, we further screened our compounds against Alzheimer, which is caused by oxidative stress and it is one of the main factors in progression of Alzheimer. Natural compounds that have antioxidant properties exhibit their antioxidant effect by quenching free radicalsof amyloid β peptide (Aβ) protein target.[8] [14-16]

In our study we also took three cholinesterase inhibitors (Donepezil, Galantamine and Rivastigmine), which are commonly prescribed drugs in Alzheimer, as reference compounds over our natural compounds for drug-likeness studies. Mechanism of action of these drugs by preventing an enzyme called acetylcholinesterase, which breaks down acetylcholine in the brain and enhances antioxidants effects and attenuates oxidative stresses.[17-19] As a result of our investigation, we found that our natural compounds showed promising inhibitory activity also. Some of them were found to have even better activity than prescribed drugs against AD targets. Any compound cannot be directly considered as a drug molecule unless it is validated by several parameters like pharmacokinetic properties, ADME properties, and potential toxicity. Therefore, with the help of various bioinformatics tool, we validated all our compounds. [20-22]

In-silico modelling studies are used to find out the interaction between a ligand/drug and a protein at the atomic level which allows us to characterize the behaviour of our compounds in the

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binding site of targets as well as to explain fundamental biochemical processes [23-24]. Each of the natural derivatives and their fermentative productswas docked with β-amyloid target protein to determine the best binding affinity using Autodock4.2 [25]. Further, these compounds could be useful for the identification and development of novel therapeutic drug against Alzheimer disease.

II. MATERIALS AND METHODS:

2.1. Natural Compounds Selection

11 natural derivative compounds were selected as common reported antioxidant natural compounds from various database and literature**(Table 1).**

Table 1: List of studied natural compounds and their sources

Compounds	Sources
Resveratrol	Red Wine, Green tea
Flavonoids	Ginkgo biloba
Terpene-Trilactones	Ginkgo biloba
Gallocatechin	Green tea, Bananas, Pomegranates
Coenzyme Q10	Grapes, Peanuts
Omega-3	Fish- Sardine, Mackrel
Curcumin	Turmeric
Huperzine A	Chinese club moss
Lauric acid	Coconut oil
Ketone bodies	Coconut oil
Caproic acid	Coconut oil

2.2. Basic Pharmacokinetics Parameters Calculation

A compound has to be passed through multiple filters to be considered as a novel drug. Most of the compounds that fail in pre-clinical trials do so because they do not show the required pharmacological properties to be a drug molecule [26]. A pharmacokinetics property such as absorption, distribution, metabolism, excretion, and toxicity (ADMET) hasplay a very crucial role in development of drug design to the final clinical success of a drug candidate [27]. Therefore, prediction of ADMET properties was done earlier with the aim of decreasing the failure rate of the compound for further process in future. Pharmacokinetics properties of natural compounds such as MW (molecular weight), LogP, HBD (number of hydrogen bond donors), HBA (number of hydrogen bond acceptors), nrtB (number of rotatable bonds), nViolation (violations of Lipinski's rule of five)[28] werecalculated by DruLito (Drug LiknessTool)and Molinspiration Online tool.

2.3. Compound Toxicity Prediction

The compounds toxicities prediction is an important sector of the drug design development process. In silico toxicity assessments are not only faster, but can also reduce the amount of animal experiments. So, calculated LD50 values of all our natural compounds. LD50 value is the amount of doses given to kill 50% of a test population (lab rats or other animals). It is an index determination of medicine and poison's virulence. Thelower the LD50 dose, the greater is the toxicity of the substance. These LD50 values were calculated by an online tool ProTox [29].

2.4. In-silico Modelling

2.4.1. Target preparation

Target protein amyloid β peptide (Aβ) associated with Alzheimer was downloaded from Protein Data Bank (PDB ID= 4DJU).For crystal structure of the target, the crystallographic water molecules were removed, the missing hydrogen atoms were added and the energy level was minimized using Swiss PDB viewer tool [30].

2.4.2. Ligand preparation

The structure of all the 11natural derived compounds were obtained from Pubchemand were saved in PDB format for further docking studies.

2.4.3. Target-Ligand docking

Docking studies yielded crucial information concerning the orientation of the inhibitors in the binding pocket of the target protein. During the molecular docking process, all the natural compounds bound in the groove of their respective targets. Each of the compounds was docked with amyloid β peptide (Aβ) target. Then we used 3D sorting method to filter out the best possible compounds from the pool of 11 natural compounds.

The docking studies were performed using Autodock4.0 [31]. The inhibition constant (Ki) of natural compounds against Alzheimer associated targets was calculated from docking energy using the following equation:

$$Ki = exp(\Delta G * 1000)/RT)$$

Where ΔG = docking energy; R = 1.98719 cal K^{-1} mol^{-1},

T = 298.15°K, Ki = inhibition constant (nM)

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III. RESULTS:

3.1. Pharmacokinetics Properties

Pharmacokinetics properties of natural compounds to be considered as drug candidates were based on Lipinski's rule of five. This rule is formulated for most orally administered drugs, it uses four criteria to determine if a molecule is drug-like; to have a molecular weight of ≤ 500, a LogP (logarithm of partition coefficient) ≤ 5, five or fewer hydrogen bond donor sites, and ten or fewer hydrogen bond acceptor sites. Molecules violating more than one of these rules may have problems with bioavailability.[32] The entire set of compounds well followed the RO5 (Rule of 5) except 3 of the compounds (Flavonoids, Coenzyme Q10 and Omega-3) violating more than one of these rules that created the Lipinski's rule violation by having molecular mass > 500 and log P> 5 that can create a problem in oral bioavailability.

Table 2:Shows the drug likeness of compounds and violation of Lipinski's rule are highlighted in red color.

Sr. No	Ligand	MolWt (g/mol)	Log P	H-bond acceptor	H-bond donar
1	Resveratrol	228.24	3.1	3	3
2	Flavonoids	594.5	2.3	6	4
3	Terpene-Trilactones	287.06	0.5	1	5
4	Gallocatechin	306.67	-0.128	1	6
5	Coenzyme Q10	862.68	18.454	4	0
6	Omega-3	909.39	19.66	5	1
7	Curcumin	368.38	0	6	4
8	Huperzine A	242.32	2.47	1	2
9	Lauric acid	200.31	1.915	2	4
10	Ketone bodies	284.07	2.031	1	2
11	Caproic acid	116.15	-0.479	2	3

3.2. Toxicity Prediction

The computational prediction of toxicities, drug score profiles of natural compounds are promising. An online software PROTOX was used for the prediction of the LD50 of the new compounds. Most of the compounds in our study fell in non-toxic zone (above the 1000 mg/kg) results are shown in the graph in (Figure. 2).

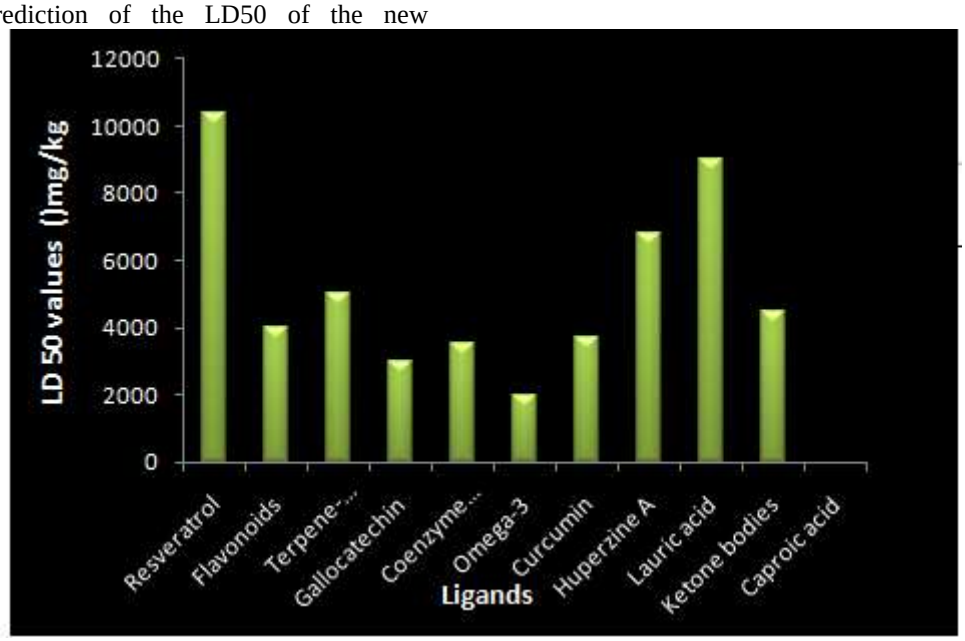

Figure 2:Histogram representation of values of natural compounds compared with Commonly prescribed drugs.

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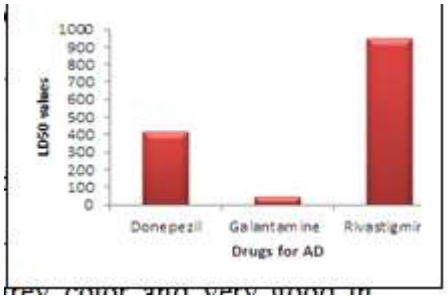
3.3. In-silicomodeling Studies

To ensure the interaction between the natural compounds and Alzheimer disease associated amyloid β peptide (Aβ) target, we performed molecular docking analysis using Autodock4.2. Each of the compounds was docked with amyloid β peptide (Aβ) targetindividually. These compounds showed very good binding affinity with amyloid β peptide (Aβ) target. Natural compounds such as Resveratrol, Terpene-Trilactones,Lauric acid, Caproic acid showed very good binding ability to β-amyloid target while the few compounds such as Coenzyme Q10, Omega-3 showed very poor binding ability.



In this way, all of these results suggest that our natural compounds potentially interfere with Alzheimer associated targets, which should prompt further investigations to expose the mechanism of our compounds against Alzheimer disease in vivo.

Table 3: Calculated K (nm) value from docking energy of 11 natural compounds against amyloid β peptide (Aβ) target. Compounds that have very poor values are highlighted in grey color and very good in green color.

Compounds	β-amyloid (4DJU) target
Resveratrol	**2.82E-08**
Flavonoids	6.25E-06
Terpene-Trilactones	**2.49E-08**
Gallocatechin	3.18E-06
Coenzyme Q10	0.00011
Omega-3	0.000359
Curcumin	3.76E-06
Huperzine A	2.53E-07
Lauric acid	**1.09E-08**
Ketone bodies	4.46E-06
Caproic acid	**2.05E-08**

IV. DISCUSSION:

Natural products have been used since ancient times and are well recognized as sources of drugs in several human ailments. The healing ability of these herbs and medicinal plants draw attention to study natural products as a potentially valuable resource of drug molecules, they are evolutionarily optimized as drug-like molecules and remain the best sources of drugs and drug leads. In our study, we chose 11 natural compounds that have remarkable antioxidant property and acts mainly by scavenging free radical ofamyloid β peptide (Aβ) target. Natural derivatives such as Resveratrol, Terpene-Trilactones,Lauric acid, Curcumin, Huperzine A and Caproic acid showed drug-likliness by Lipinski's rule and good binding affinity. Therefore, the following 6 natural compounds can be used in the formulation of a novel drug for inhibition of β-amyloid protein in Alzheimer's disease(AD) with higher concentration of Resveratrol, Terpene-Trilactones,Lauric acid, Caproic acid as it showed very good binding affinity with β-amyloid target and low toxicity.

V. CONCLUSION:

Several natural products are used alone or in combination with other neuroprotective compounds to improve memory and cognition in AD patients as supported by various experimental studies. Altogether, this pioneering study was used to preliminarily investigate the potential compounds (drug candidates) from natural products and conventional docking study to analyze the best binding affinity mode of compounds. Binding energies of the drug–targets interactions are important to describe how fit the drug binds to the target protein. Further studies are essential to explore the target specific effect of these natural compounds on various signalling pathways, mode of action in various brain regions, the ability to cross the blood brain barrier and the mechanism behind the synergistic action of the antioxidant

agents on the target. Using novel Pharmaceutical technology and medicinal chemistry approach to prepare novel formulations or design new compounds based on natural templates, opens up a new window into using natural therapeutic agents against AD.

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