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Use of Natural Compounds from Plant Sources as AchE Inhibitors for the Treatment of Early Stage Alzheimer's disease-An Insilico Approach

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Abstract— Traditionally, drugs were discovered by testing compounds manufactured in time consuming multi-step processes against a battery of in vivo biological screens. Promising compounds were then further studied in development, where their pharmacokinetic properties, metabolism and potential toxicity were investigated. Here we present a study on herbal lead compounds and their potential binding affinity to the effectors molecules of major disease like Alzheimer's disease. Clinical studies demonstrate a positive correlation between the extent of Acetyl cholinesterase enzyme and Alzheimer's disease. Therefore, identification of effective, well-tolerated acetyl cholinesterase represents a rational chemo preventive strategy. This study has investigated the effects of naturally occurring nonprotein compounds polygala and Jatrorrhizine that inhibits acetylcholinesterase enzyme. The results reveal that these compounds use less energy to bind to acetylcholinesterase enzyme and inhibit its activity. Their high ligand binding affinity to acetylcholinesterase enzyme introduce the prospect for their use in chemopreventive applications in addition they are freely available natural compounds that can be safely used to prevent Alzheimer's Disease.

Index Terms— Alzheiemr's Disease, Acetylcholinesterase, Binding Affinity, Jatrorrhizine, Clinical Studies, Docking, Rational, Toxicity

1 INTRODUCTION

Alzheimer's disease, most common form of dementia is incurable, degenerative, and terminal disease mostly diagnosed in people over 65 years of age. The disease advances with symptoms include confusion, irritability and aggression, mood swings, language breakdown, long-term memory loss, and the general withdrawal of the sufferer as their senses decline. Gradually, bodily functions are lost, ultimately leading to death. In advanced stages of the disease, all memory and mental functioning may be lost. The condition predominantly affects the cerebral cortex and hippocampus, which lose mass and shrink (atrophy) as the disease advances. These changes, occurring in the association area of the cerebral cortex, the hippocampus and the middle and temporal lobes, are accompanied by decreased concentrations of the neurotransmitter acetylcholine.Acetylcholinesterase is also known as AChE. An acetyl cholinesterase inhibitor or anti-cholinesterase is a chemical that inhibits the cholinesterase enzyme from breaking down acetylcholine, increasing both the level and duration of action of the neurotransmitter acetylcholine.

2 METHODOLOGY

Some small molecules were taken as targeting agent which are responsible for inhibiting biological process in AD. The investigation drug Galantamine was used as a reference drug in the studies.

2W9I was taken as targeting protein and structure for the same was taken from PDB.

Then Initial screening is done by Lipinski's rule of 5. The accepted compounds that were showing better interaction with the target protein and their energy were minimized using Marvin's Sketch.

Then the selected conformations are saved in three formats:

1. SDF Format
2. PDB Format
3. MOL. Format

Then docking is done between the protein and small molecules with QUANTUM. The best three results

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obtained were analysed under HEX and ARGUS. IC50 value is taken by QUANTUM.Then graphs are plotted by seeing the values.

After this, ADME TOX analysis was performed. In this analysis ADME features were showing interaction
With 2W9I in both Argus and Quantum analysis were predicted under ADME test for toxicity prediction.

TABLE 1
Natural compounds and Their Quantum results

S.no	Name of compound	Gbind	Rms
1.	Dichlorfop	-17.66	69.85
2.	Naringenin	-20.45	72.55
3.	Caffeine	-13.02	80.61
4.	Cla	-20.00	66.34
5.	Toluidine red	-12.08	69.48
6.	Polygala	-22.82	73.28
7.	Jatrorrhizine	-25.95	96.90
8.	Sterigmatocystin	-22.54	73.39
9.	Testosterone	-20.03	74.21
10.	Vitamin b6	-21.39	72.23

TABLE 2
Drugs and Their Quantum results

S.no	Name of drug	Gbind	Rms
1.	Cinacalcet	-15.58	95.83
2.	Penicillamine	-16.18	90.59
3.	Selegeline	-21.29	90.18
4.	Amantadine	-23.79	94.72

AMES test is considering for initial screening of the molecule based on their ability to induce mutation.
AMES test is used for determining if a chemical is mutagen. The molecules which showing ability to induce mutation were rejected in toxicity based screening.

Using ADME TOX analysis it was found that Jatrorrhizine was showing lower AMES test values than reference molecules. Further health effects of these molecules in blood, cardiovascular system, gastrointestinal system, kidney, liver and lungs were predicted. LD 50 values also were predicted for selecting reliable molecule for ADME analysis.
After ADME analysis,a graph was plotted on ADME-TOX values.

After all analysis, one compound Jatrorrhizine was the best molecule.
This molecule is considered as better ligands for 2W9I based on interaction, Pharmacokinetics, and pharmacodynamic features and can be used for Chemotherapeutic use.

3 BIOCHEMISTRY OF ALZHEIMER'S

The biochemistry of Alzheimer's disease (AD), one of the most common causes of adult dementia, is as yet not well understood. It has been identified as a protein misfolding disease due to the accumulation of abnormally folded amyloid betaprotein in the brains of AD patients. Amyloid beta, also written Aβ, is a short peptide that is an abnormal proteolyticbyproduct of the transmembrane protein amyloid precursor protein (APP), whose function is unclear but thought to be involved in neuronal development. The presenilins are components of proteolytic complex involved in APP processing and degradation. Amyloid beta monomers are soluble and contain short regions of beta sheet and polyproline II helix secondary structures in solution, though they are largely alpha helical in membranes; however, at sufficiently high concentration, they undergo a dramatic conformational change to form a beta sheet-rich tertiary structure that aggregates to form amyloid fibrils. These fibrils deposit outside neurons in dense formations known as senile plaques or neuritic plaques, in less dense aggregates as diffuse plaques, and sometimes in the walls of small blood vessels in the brain in a process called amyloid angiopathy orcongophilic angiopathy.
AD is also considered a tauopathy due to abnormal aggregation of the tau protein, a microtubule-associated proteinexpressed in neurons that normally acts to stabilize microtubules in the cell cytoskeleton. Like most microtubule-associated proteins, tau is normally regulated by phosphorylation; however, in AD patients, hyperphosphorylated tau accumulates as paired helical filaments that in turn aggregate into masses inside nerve cell bodies known as neurofibrillary tangles and as dystrophic neurites associated with amyloid plaques. Although little is known about the process of filament assembly, it has recently been shown that a depletion of a prolyl isomerase protein in the parvulin family accelerates the accumulation of abnormal tau.

4 Disease Mechanism

Although the gross histological features of AD in the brain are well characterized, three major hypotheses have been advanced regarding the primary cause. The oldest hypothesis suggests that deficiency in cholinergic signaling initiates the progression of the disease. Two alternative misfolding hypotheses instead suggest that either tau protein or amyloid beta initiates the cascade. While researchers have not identified a clear causative pathway originating from any of the three molecular hypotheses to explain the gross anatomical changes observed in advanced AD, variants of the amyloid beta hypothesis of molecular initiation have become dominant among the three possibilities.

4.1 The mechanism of action of acetylcholinestarase

Cholinergic nerve transmission is terminated by the enzyme acetylcholinesterase (AchE). AchE is found both on the post-synaptic membrane of cholinergic synapses and in other tissues eg red blood cells. Acetylcholine (Ach) binds to AchE and is hydrolysed to acetate and choline. This inactivates the Ach and the nerve impulse is halted. AchE inhibitors (eg rivastigmine) prevent the hydrolysis of Ach, which increases the concentration of Ach in the synaptic cleft; AchE inhibitors are widely used in the treatment of Alzheimer's disease.

5 Results and Analysis

Alzheimer's disease, the most common form of dementia, is a progressive disorder characterized by widespread loss of brain cells called neurons, beta-amyloid deposits in the cerebral blood vessels, development of plaques and the presence of neurofibrillary tangles. Alzheimer's disease (AD) is an irreversible, progressive disorder in which brain cells(neurons) deteriorate, resulting in the loss of cognitive functions, primarily memory, judgment and reasoning, movement coordination, and pattern recognition. There is a very high genetic cause for Alzheimer's disease. In addition to genetics as a cause of Alzheimer's disease, many environmental factors including diet are to be considered. Older adults who smoke have an elevated risk of developing Alzheimer's disease. Nerve signals travel across synapses with the help of chemicals known as "neurotransmitters," including one called acetylcholine. Nerve cell destruction causes a reduction in acetylcholine, leading to impaired transmission of nerve signals and poor communication between nerve cells called neurons. In addition to acetylcholine, the brain of Alzheimer's disease patients have areas of abnormal protein called "plaques" and "tangles," the names reflecting what these abnormalities in the brain look like under the microscope. The underlying cause of Alzheimer's – what actually triggers the changes in the brain – is still not fully known but could partly be due oxidation and damage to nerve cells over time. It is likely that no single factor is responsible, but rather that it is due to a variety of factors, which may differ from person to person.

People whose parents or brothers and sisters develop the disease appear to be at greater risk of developing it themselves, so there may be a genetic component. However, no straightforward pattern of inheritance has been found. It is known that head injury is a risk factor, and also that Alzheimer's disease often affects people with Down's syndrome. Some researchers have suggested that people who exercise their brains (for example, doing crosswords and other mental agility exercises) are less likely to develop the disease. And Omega 3 fatty acids, contained in oily fish such as mackerel and salmon may, also help to prevent dementia. Australian scientists say they have identified a toxin that may play a key role as a potential cause of Alzheimer's disease. The toxin, called quinolinic acid, kills nerve cells in the brain, leading to dysfunction and death. Quinolinic acid may not be the main cause of Alzheimer's disease, but it plays a key role in its progression. Researchers have shown that a common anesthetic gas can cause fragments of a normal brain protein called amyloid-beta to clump together, which is thought to be the main problem underlying Alzheimer's disease. Intravenous anesthetics have less of an effect, the team reports in the journal Biochemistry.

Anesthetics used in long surgery, such as inhaled anesthetics isoflurane and halothane, may be another cause of Alzheimer's disease. Scientists conducted a series of lab experiments using nuclear magnetic resonance to investigate the reaction of amyloid-beta peptides to the inhaled anesthetic isoflurane and the intravenous anesthetics propofol and thiopental. They found that the peptides aggregated together after 10 to 30 hours' exposure to isoflurane, depending on the concentration of the gas and the size of the protein fragments. The effect was seen with propofol after exposure for 48 hours, but no clumping was seen with thiopental. Biochemistry, January 23, 2007.

Having frequent colds or flu or other infections increases the risk for this neurological deterioration due to increased overall inflammation in the body and brain.

There are certain natural and synthetic AchE inhibitors which will prevent the cause of AD by blocking the Biochemical pathway .Some number of natural compounds which are Inhibiting Ache were taken.

Docking of these molecules was performed under QUANTUM 3.3.0.

It was found that the natural compound Polygala, Sterigmatocystin and Jatrorrhizine were showing reliable pharmacokinetics and pharmacodynamics features than the commercial drugs. Hence they were taken out for work.

After analysing the graphs, it was found that Bulbocapnie was the best Acetylcholinesterase Inhibitor.

This molecule is considered as better ligands for Acetylcholinesterase based on ligand-receptor interaction,

Jatrorrhizine is a protoberberine alkaloid isolated from *Enantia chlorantha* (Annonaceae) and other species. Synonyms that may be encountered include jateorrhizine, neprotin, jatrochizine, jatrorhizine, or yatrorizine. It has been reported to have anti inflammatory effect, and to improve blood flow and mitotic activity in thioacetamide-traumatized rat livers. It was found to have antimicrobial and antifungal activity. It binds and noncompetitively inhibits monoamine oxidase (IC_{50} 4 micromolar for MAO-A and 62 for MAO-B).It interferes with multidrug resistance by cancer cells *in vitro* when exposed to a chemotherapeutic agent. Large doses (50-100 mg/kg) reduced blood sugar levels in mice by increasing aerobic glycolysis.

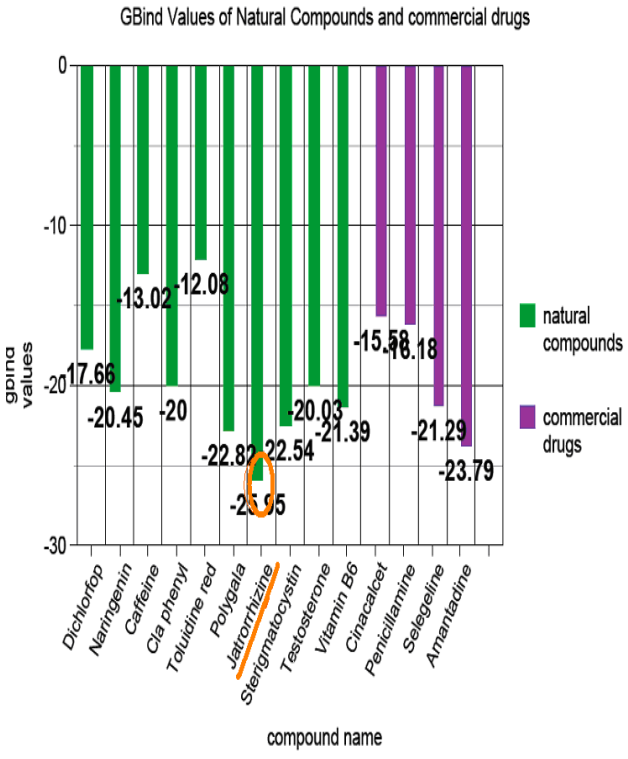

Fig. 1: Graph showing QUANTUM Results of natural compounds and commercial drugs. Greens are for natural compounds and red is for commercial drugs.

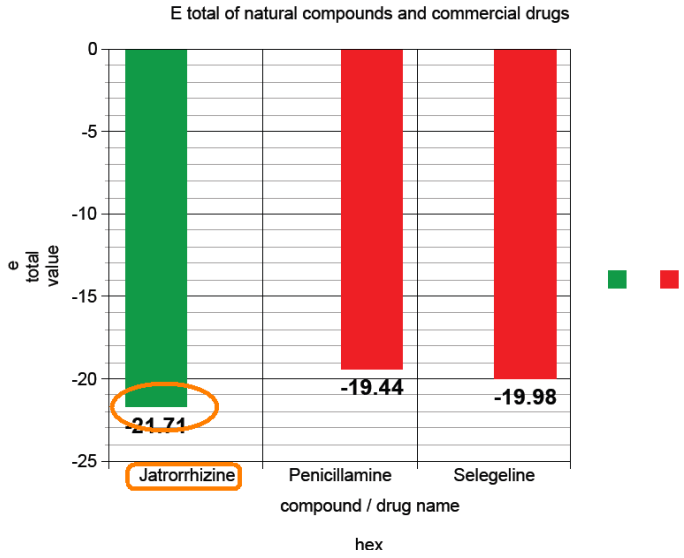

Fig.2: Graph showing Hex results of best two natural compounds and best two commercial drugs based on quantum. Greens are for natural compounds and red is for commercial drugs.

6 Conclusion

Alzheimer's disease, the most common form of dementia, is a progressive disorder characterized by widespread loss of brain cells called neurons, beta-amyloid deposits in the cerebral blood vessels, development of plaques and the presence of neurofibrillary tangles. Alzheimer's disease (AD) is an irreversible, progressive disorder in which brain cells (neurons) deteriorate, resulting in the loss of cognitive functions, primarily memory, judgment and reasoning, movement coordination, and pattern recognition.There are certain natural and synthetic AchE inhibitors which will prevent the cause of AD by blocking the Biochemical pathway .Some number of natural compounds which are Inhibiting Ache were taken.Docking of these molecules was performed under QUANTUM. It was found that the natural compound POLYGALA, STERIGMATOCYSTIN AND JATRORRHIZINE was showing reliable pharmacokinetics and pharmacodynamics features than the commercial drugs. Hence they were taken out for work.

Further, it was found that among three natural compounds JATRORRHIZINE is the best natural compound and better than commercial drugs and hence can be taken as AD chemopreventive agent and it can be effective inhibitors for AchE in AD pathway.

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