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Screening of Potential Glycogen synthase kinase -3β Inhibitors from Herbal Lead by In silico Docking Technique

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Abstract: Glycogen synthase kinase 3 (GSK3) is an serine, threonine phosphorylating enzyme which plays a significant role in the pathogenesis of many diseases includes type II diabetes mellitus, alzheimer's disease (AD), cancer, and bipolar disorder. In AD this enzyme involved in hyper phosphorylation of tau protein and further promotes the formation of neurofibrillary tangles (NFT). Accumulation of NFT attracts and actives neuronal microglial cells may leads to induction of neuroinflammation and degeneration in the major region of brain. In mammals this enzymes exist in two form GSK-3 alpha (GSK-3A) and GSK-3 beta (GSK-3B).It is evident that GSK-3B plays an active role in brain aging and neurodegeneration in AD patients. Currently donepezil hydro chloride is an approved drug which is a potent inhibitor of enzyme acetylcholinesterase (AChE) being prescribed widely for the treatment of AD. But donepezil being a drug of choice for AD may exert side effects like insomnia, muscle cramps, tiredness, drowsiness and dizziness in patients. Hence it is a right time to explore a new therapeutic lead which can be clinically effective against AD with minimal side effect. Phytoconstituents like Apigenin, Curcumin ,Chlorogenic acid ,Kaempferol ,Quercetin along with standard drug GSK-3B Inhibitor X was selected for docking against the target GSK-3B enzyme. Results obtained from the study projects that all the selected lead shown good binding potential towards GSK-3B in which Apigenin, kaempferol and quercetin shown much significant binding similar to that of standard. Hence it was concluded that lead from traditional medicine with biologically significant properties and structural diversity, have often served as valuable drug candidate for the treatment of AD by replacing the synthetic drug with known side effects.
Key words: Alzheimer's disease, Glycogen synthase kinase 3 beta, Docking, Apigenin, Curcumin , Chlorogenic acid , Kaempferol ,Quercetin.

Introduction

Computer aided drug discovery attains greater importance mainly because of the reliability in the results and also paves a new way for the research focus towards the alternative animal models. Molecular Docking continues to hold greater promise in the field of computer based drug design that screens small molecules by orienting and scoring them in the binding site of a protein. As a result novel ligands for receptors of known structure were designed and their interaction energies were calculated using the scoring functions. Dock score was used to estimate the ligand-binding energies. Apart from these, other input parameters for docking are also considered for evaluating the compounds inhibition efficacy. It is estimated that docking programs currently dock 70 – 80% of ligands correctly[1].

Enzyme hyperactivity in central nervous system promotes certain degenerative disorders like Alzheimer's and Parkinson's disease. Increased level of the enzyme acetylcholinesterase (AchE) leads to depletion in the level of acetylcholine a vital neurotransmitters required for the process of memory and learning may leads to Alzheimer's disease (AD).Similarly increased level of monoamine oxidase (MAO) enzyme involved in degradation of norepinephrine, serotonin and dopamine an neurotransmitters which is required for proper muscle coordination, mood, behavior, learning and adaptation.

Alzheimer's is a primary neurodegenerative disease characterized by selective neuronal cell death, the presence of extra cellular amyloid deposits in the core of neuritic plaques and the formation of neurofibrillary tangles in the brain of affected individuals. Neurochemically, these deficits are associated with dramatic loss of cortically projecting cholinergic neurons and by a reduction in the presynaptic markers of the cholinergic system, particularly in the areas of the brain related to memory and learning[2].

A wide range of evidence shows that acetylcholinesterase (AChE) inhibitors may interfere and delays the progression of AD. The successful development of these compounds was based on a well-accepted theory that the decline in cognitive and mental functions associated with AD is related to the loss of cortical cholinergic neurotransmission. The earliest known AChE inhibitors, namely, donepezil, physostigmine and tacrine, showed modest improvement in the cognitive function of Alzheimer's patients[3]. Donepezil hydrochloride inaugurates a new class of AChE inhibitors with longer and more selective action but recent studies suggested that daily usage of donepezil exhibited symptoms of mania and subjects became hyperverbal with elevated mood and agitation [4]. Currently, there are about 19 new Alzheimer's drugs in various phases of clinical development.

Glycogen synthase kinase 3 (GSK3) centrally mediates several physiological functions such as cell proliferation, migration, inflammation, immune responses, glucose regulation, and apoptosis. In contrast the role of GSK3 in the pathogenesis of AD become highly significant as it involves in the activation of β-secretase enzyme which involved in the formation of insoluble amyloid plagues through amyloidogenic pathway[5].

GSK-3B tangled in hyperphospohorylation of tau protein leads to fabrication of neurofibrillary tangles (NFT). Microglia cells' being a primary immune cell in the brain recognizes NFT and promotes the release of inflammatory mediators and free radicals. Neuronal inflammation coupled with oxidative stress further leads to neurodegeneration[6]. Results of preclinical and clinical studies strongly recommends that GSK-3β inhibitors could be useful in the treatment of AD. Development of GSK-3 inhibitor considered to be a new treatment strategy and that will become a milestone in the history of AD research[7].

The leads of Central Nervous System (CNS) active medicinal plants, that have emerged besides *Rawolfia serpentina, Mucuna pruriens* for Parkinson's disease, *Ocimum santum* as an antistress agent, *Withania somnifera* as anxiolytic, *Centella asiatica* and *Bacopa monneria* for learning and memory disorders. *Bacopa monneria* and *Ginkgo biloba* for Alzheimer's disease. The study related to Alzheimer's disease (A.D) is focused towards the traditionally used rejuvenating and neurotonic agents [8]. The recent trends in the pharmacological studies are based on the biochemical and molecular mechanism that leads to the development of CNS active principles from the herbal drugs.

Experimental

Software's required

Various computational tools and software's are used to analyze the protein GSK-3B structure and to study the binding energy properties with Apigenin , Curcumin ,Chlorogenic acid ,Kaempferol ,Quercetin and GSK-3B Inhibitor X a known standard . Glycogen synthase kinase 3 Beta (GSK-3B) enzyme with pdb code 1Q3W sequence was obtained from protein data bank (www.pdb.org/pdb/). To get insight the intermolecular interactions, the molecular docking studies were done for the above mentioned phytoconstituents along with GSK3 X as a standard at the active site 3D space of enzyme of interest GSK-3B using online DOCKING SERVER web tool module.

Ligand preparation

The ligands such as Apigenin , Curcumin ,Chlorogenic acid ,Kaempferol ,Quercetin and GSK-3B Inhibitor X were built using Chemsketch and optimized using Docking server online web tool as shown in

Figure 1 and 2 for docking studies by using Geometry optimization method MMFF94 and charge calculation was carried out based on Gasteiger method at PH 7 as shown in Table 1.

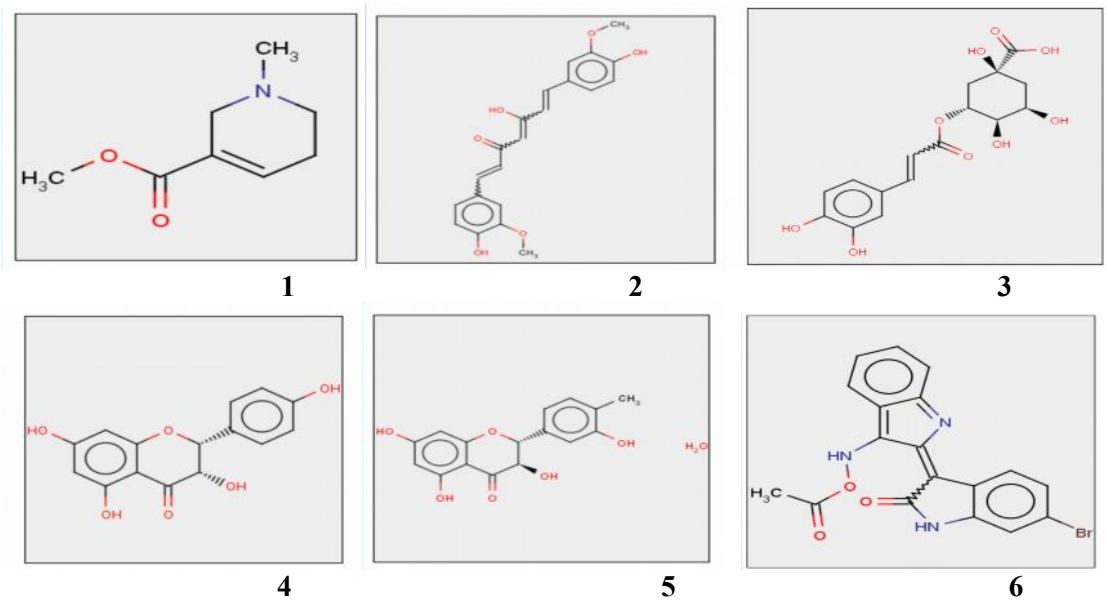

Figure 1: 2D Structure of lead 1.Apigenin 2. Curcumin 3.Chlorogenic acid 4.Kaempferol 5.Quercetin and 6.GSK-3B Inhibitor X

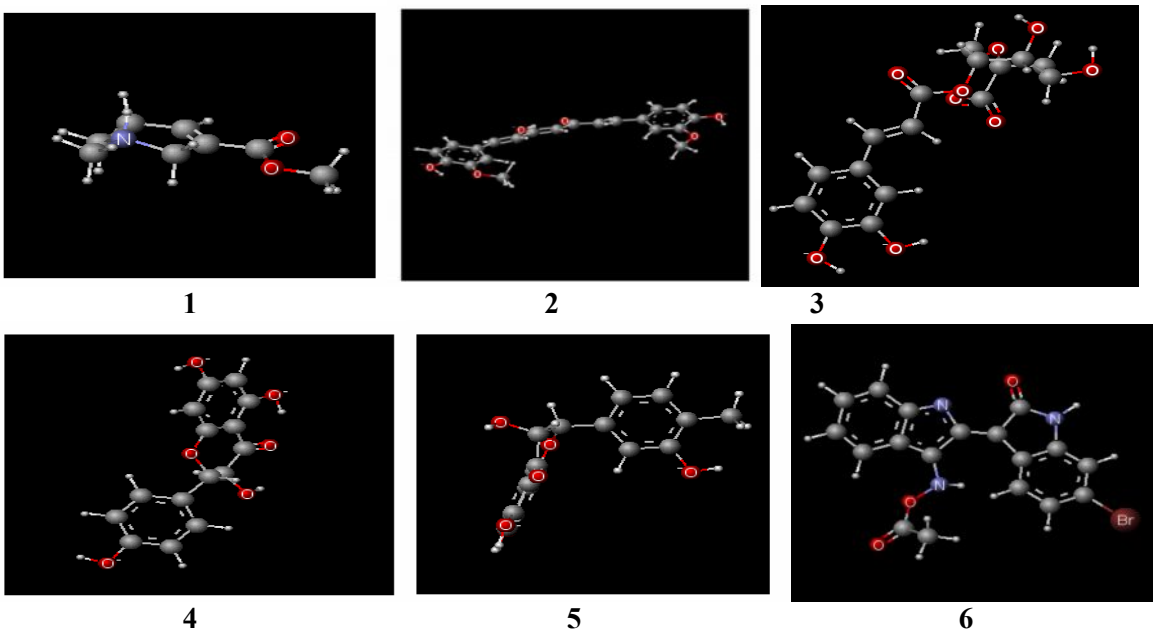

Figure 2: 3D Structure of lead 1.Apigenin 2. Curcumin 3.Chlorogenic acid 4.Kaempferol 5.Quercetin and 6.GSK-3B Inhibitor X

Table 1: Ligand Properties

Compounds	molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds	Log P	pKa
Apigenin	270.24	$C_{15}H_{10}O_5$	3	1	1	1.22	8.23
Curcumin	368.38	$C_{21}H_{20}O_6$	2	6	8	2.85	7.8
Chlorogenic acid	354.30	$C_{16}H_{18}O_9$	6	9	5	0.37	3.9
Kaempferol	286.23	$C_{21}H_{10}O_6$	4	6	1	1.9	6.44
Quercetin	302.23	$C_{15}H_{10}O_7$	5	7	1	1.5	7.15
GSK-3B Inhibitor X	399.21	$C_{18}H_{12}BrN_3O_3$	2	4	3	1.48	9.95

Protein preparation

The target protein Glycogen synthase kinase 3 Beta (PDB Code: 1Q3W) complexed with Alsterpaullone was retrieved from protein Data Bank (www.rcsb.org) and the drug molecule alsterpaullone, crystallographic water molecules were removed from the protein. The chemistry of the protein was corrected for missing hydrogen followed by correcting the disorders of crystallographic structure by filling the valence atoms using alternate conformations and valence monitor options. As shown in Figure 3.

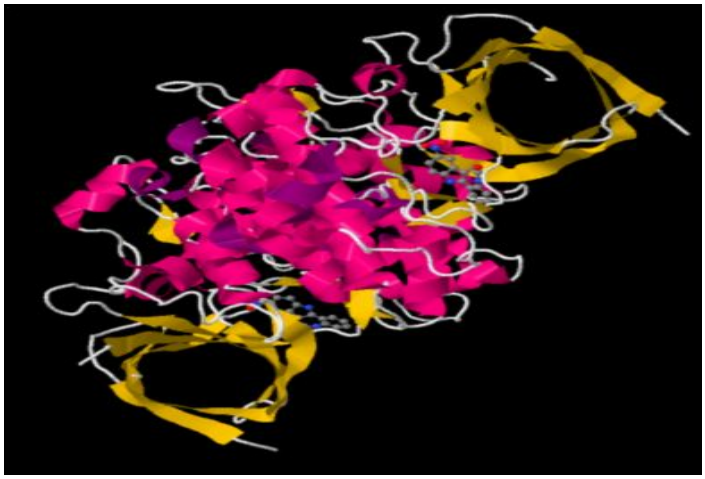

Figure 3:Target protein Glycogen synthase kinase 3 Beta 1Q3W

Active Site Prediction

Active site of enzyme was obtained by LIGSITE web server by using the automatic identification of pockets on protein surface given 3D coordinates of protein. The potential ligand binding sites in GSK-3B target protein is identified using grid space of 1 and probe of radius 5.0 angstrom [9]. Ligand site prediction was performed by using online tool GHECOM and the respective pockets calculations [10, 11]. As shown in Figure 4.

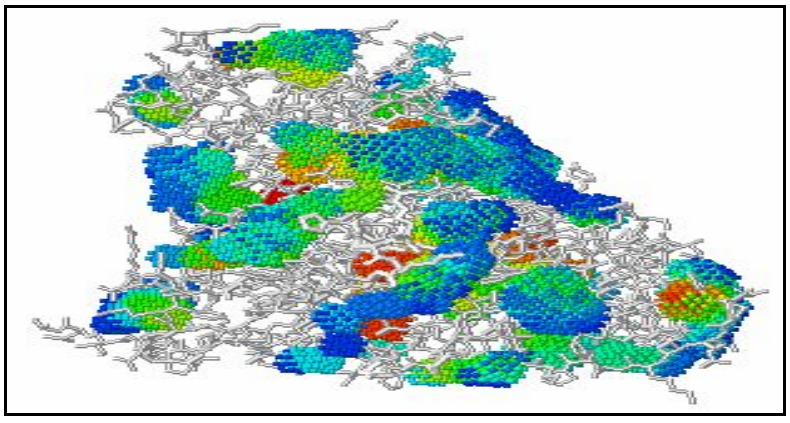

Figure 4 : Possible ligand binding pockets on the surface of target enzyme GSK-3B. Pockets calculated by GHECOM.

Docking Methodology

Docking calculations were carried out using Docking Server[12, 13]. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out based on the binding free energy on the following compounds like Apigenin , Curcumin ,Chlorogenic acid ,Kaempferol ,Quercetin ,GSK-3B Inhibitor X and their binding affinity towards the target protein GSK-3B (PDB Code: 1Q3W)

Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of Auto Dock tools. Affinity (grid) maps of Å grid points and 0.375 Å spacing were generated using the Autogrid program. Auto Dock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were

performed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method [14]. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied[15].

Results and Discussion

Dock scores

Interaction of lead with the protein target plays a significant role in structural based drug designing. In the present study, protein glycogen synthase kinase 3 Beta (GSK-3B) was docked with phytochemical leads derived from the indigenous plant of Indian system of traditional medicine. The different score such as binding free energy, inhibition constant, intermolecular energy and electrostatic energy values represented in Table 2.

Table 2: Summary of the molecular docking studies of compounds against GSK-3B Enzyme

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki μM	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol
Apigenin	-5.72	64.26	-0.31	-6.23
Curcumin	-4.81	299.43	-0.01	-6.33
Chlorogenic acid	-4.35	652.85	-0.02	-5.27
Kaempferol	-5.65	72.03	-0.32	-6.59
Quercetin	-5.15	168.41	-0.09	-5.85
GSK-3B Inhibitor X	-7.69	2.31	-0.02	-8.46

The results showed that all the selected compounds showed binding energy ranging between -5.72 kcal/mol to -4.35 kcal/mol when compared with that of the standard (-7.69 kcal/mol). Electrostatic energy (-0.32 kcal/mol to -0.01 kcal/mol) of the ligands also coincide with the binding energy. All the phytochemical lead compounds contributed GSK-3B enzyme inhibitory activity because of its structural parameters.

The docking calculations of all five compounds at the active sites of GSK-3B revealed that the compounds bound to the active site of enzyme with lower docking (D energy) when compared with standard GSK-3B Inhibitor X. Compound Apigenin exhibited quite tight binding against GSK-3B enzyme with binding energy -5.75 Kcal/mol and ranks first in the compound series.

The second best score was ranked by compound Kaempferol with binding energy -5.65 Kcal/mol followed by this compound Quercetin with binding energy -5.15 Kcal/mol when compared with standard compound GSK-3B Inhibitor X with binding energy -7.69 Kcal/mol.

Inhibition constant is directly proportional to binding energy. Inhibition constant ranges from (652.85 μM to 64.26 μM). Thus from the report it was clear that all the phytoconstituents having promising GSK-3B inhibition activity when compared to standard with inhibition constant 2.31μM. Intermolecular energy of all five compounds ranging between -6.59 to -5.27 kcal/mol which was lesser when compared to the standard -8.46 Kcal/mol. Intermolecular energy is also directly proportional to binding energy. We found a decrease in intermolecular energy of all the selected compounds coincide with the binding energy.

Hydrogen bond interaction

By enlarging this interaction analysis the hydrogen bond interaction is contributed as major parameter. The Hydrogen bonding interaction of the compounds (Fig 5, 6,7,8,9 and 10 and) was analyzed for possible involvement of hydrogen bond formation with amino acid residues on receptor protein surface.

The result obtained from the hydrogen bond interaction study shows that the phytoconstituents such as Apigenin , Curcumin ,Chlorogenic acid ,Kaempferol and Quercetin possess great GSK-3B inhibition activity by binding with the active site pocket on target protein. Further these compounds may have a direct action on target enzyme by binding to the potentially active amino acid residue in the same way as that of the standard GSK-3B Inhibitor X as listed in the Table 3.

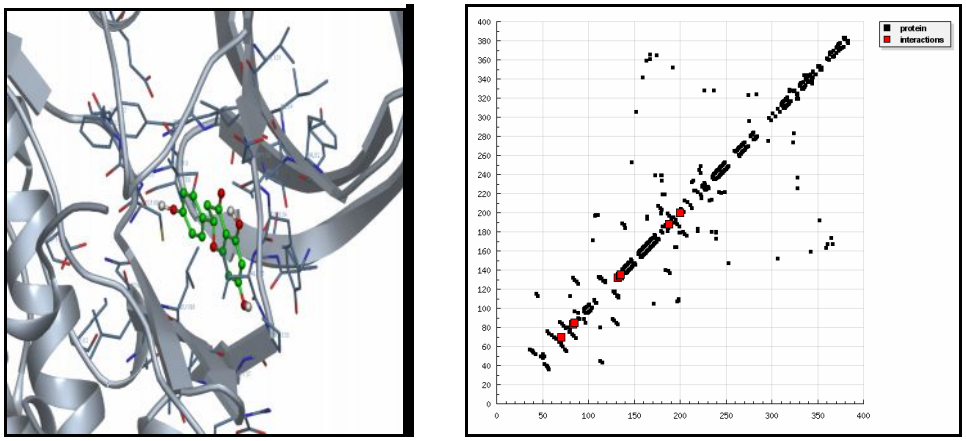

Figure 5: Hydrogen bond interaction between GSK-3B-1Q3W with Apigenin

Figure 5: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme GSK-3B (1Q3W) and the ligand Apigenin involved in hydrogen bond formation with amino acid residues on the protein like 85 LYS, 134 TYR, 135 VAL and 200 ASP. Total interaction surface of about 657.2.

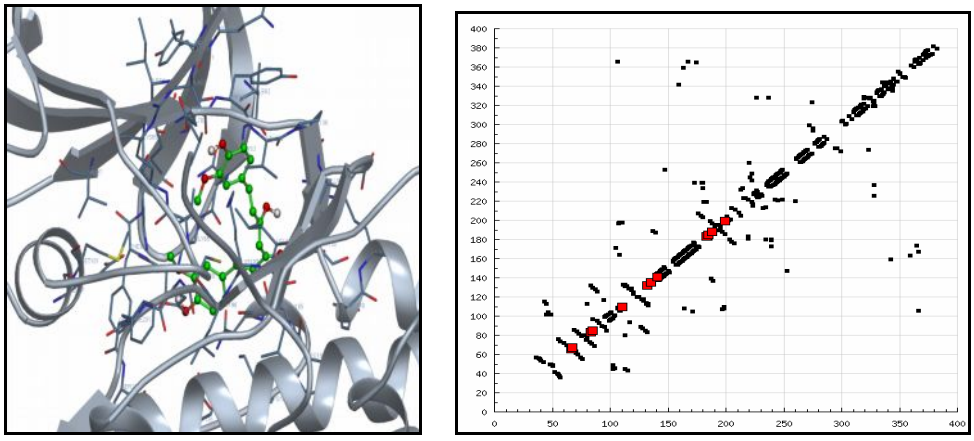

Figure 6: Hydrogen bond interaction between GSK-3B-1Q3W with Curcumin

Figure 6: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme GSK-3B (1Q3W) and the ligand Curcumin involved in hydrogen bond formation with amino acid residues on the protein like 85 LYS, 135 VAL, 185 GLY and 200 ASP. Total interaction surface of about 1008.94.

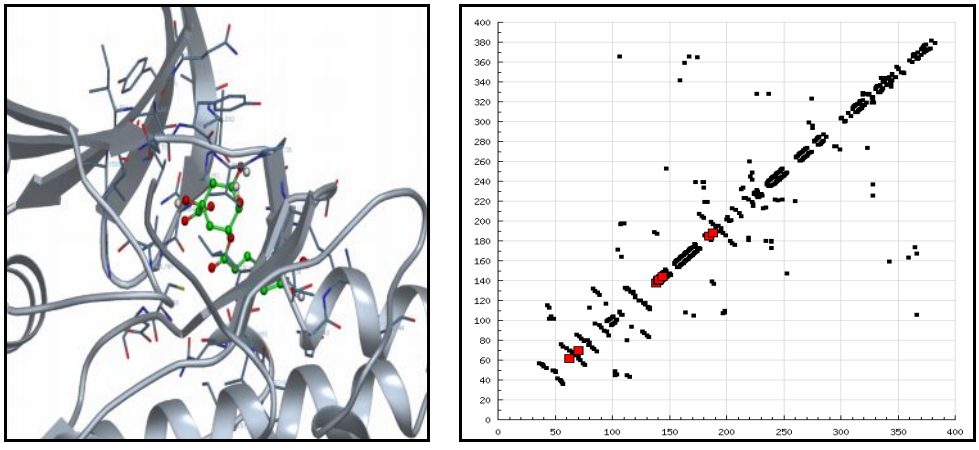

Figure 7: Hydrogen bond interaction between GSK-3B-1Q3W with Chlorogenic acid

Figure 7: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme GSK-3B (1Q3W) and the ligand Chlorogenic acid involved in hydrogen bond formation with amino acid residues on the protein like 85 LYS, 135 VAL, 185 GLY and 200 ASP. Total interaction surface of about 701.5.

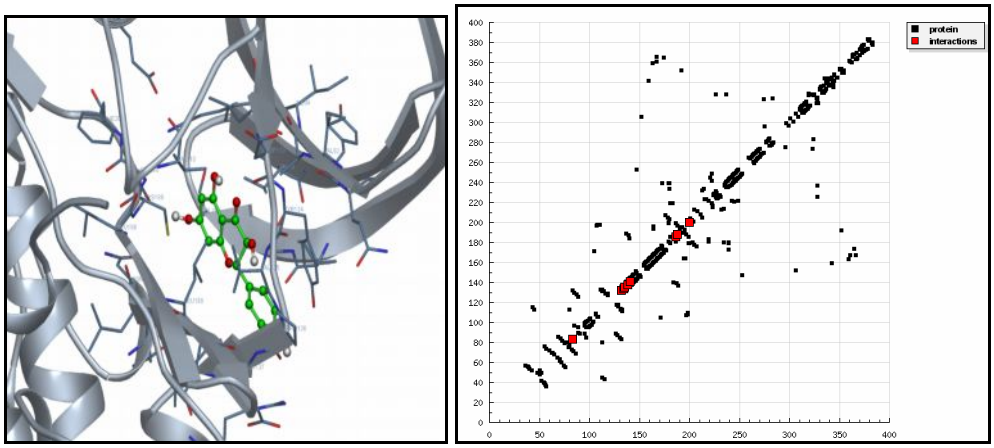

Figure 8: Hydrogen bond interaction between GSK-3B-1Q3W with Kaempferol

Figure 8: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme GSK-3B (1Q3W) and the ligand Kaempferol involved in hydrogen bond formation with amino acid residues on the protein like 135 VAL,134 THY, 185 GLY and 200 ASP. Total interaction surface of about 657.63.

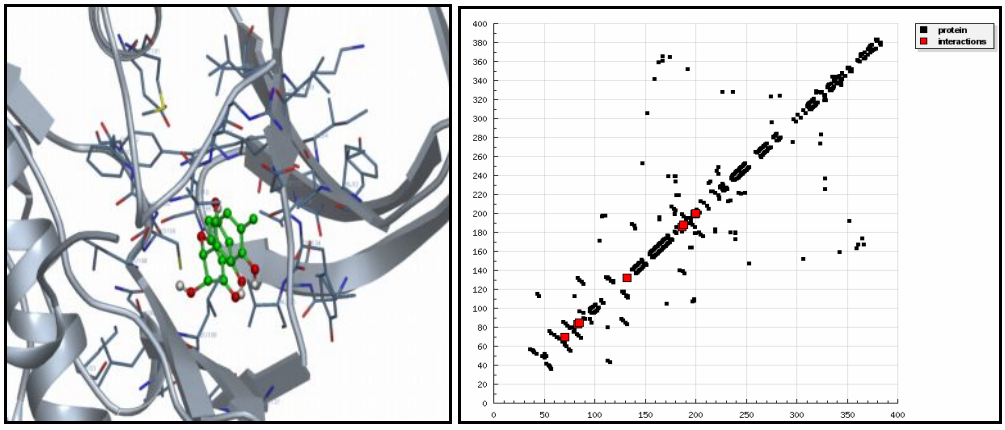

Figure 9: Hydrogen bond interaction between GSK-3B-1Q3W with Quercetin

Figure 9: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme GSK-3B (1Q3W) and the ligand Quercetin involved in hydrogen bond formation with amino acid residues on the protein like 85 LYS,134 THY, 185 GLY and 200 ASP. Total interaction surface of about 681.81.

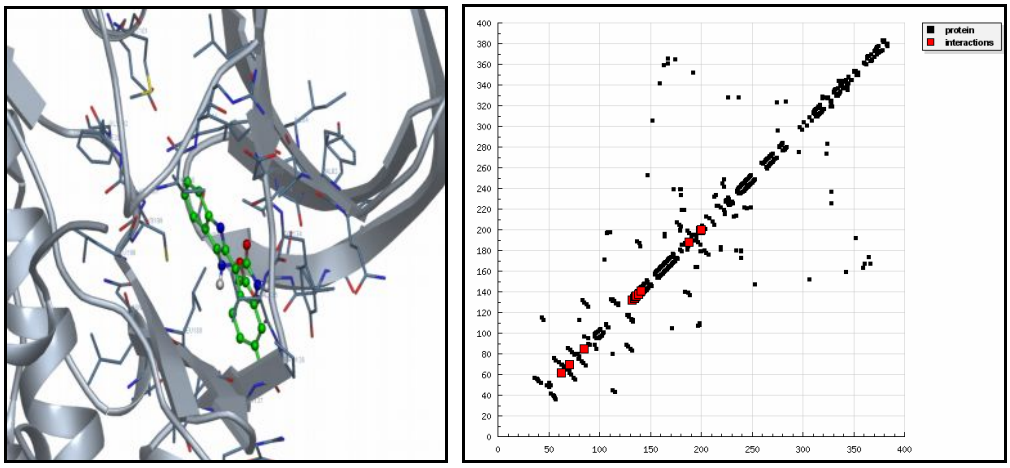

Figure 10: Hydrogen bond interaction between GSK-3B-1Q3W with GSK-3B Inhibitor X

Figure 10: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme GSK-3B (1Q3W) and the ligand GSK-3B Inhibitor X involved in hydrogen bond formation with amino acid residues on the protein like 62 ILE, 85 LYS, 134 TYR, 135 VAL and 200 ASP. Total interaction surface of about 709.98.

Table 3: Interaction of lead compounds with active site amino acid residue of GSK-3B Enzyme

Compounds	Target binding Amino acid residue
Apigenin	85 LYS, 134 TYR, 135 VAL, 200 ASP
Curcumin	85 LYS, 135 VAL , 185 GLY, 200 ASP
Chlorogenic acid	85 LYS, 135 VAL , 185 GLY, 200 ASP
Kaempferol	135 VAL ,134 THY, 185 GLY, 200 ASP
Quercetin	85 LYS ,134 THY, 185 GLY, 200 ASP
GSK-3B Inhibitor X	62 ILE ,85 LYS, 134 TYR ,135 VAL , 200 ASP

Conclusion

Hyper active GSK-3B may increases the deposition of insoluble amyloid plagues which contributes to neurodegeneration on the important region of brain. Further it is also evident that amyloid plagues deposit accelerates the production of reactive oxygen species (ROS). ROS being an unstable moiety requires try to attain stability by gaining an electron from the nearby molecules. Brain contains rich source of protein and lipids which ultimately becomes a source for electron donor. As a result of this event some important structures in the brain like neurons will be getting degenerated due to consequence of membrane damage by ROS. This setup a condition called oxidative stress even may damage the mitochondrial DNA thus liberate more free radicals. DNA repair deficiency is also noted in AD since higher levels of DNA breaks, DNA nicking and fragmentation are observed in AD patients [16].

The World Health Organization (WHO) estimates that 4 billion people, 80 percent of the world population, presently use herbal medicine for some aspect of primary health care. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects against various neurological disorders like AD [17].

In conclusion, the results obtained from the current investigation clearly demonstrated the in silico molecular docking studies of GSK-3B Inhibitor X and selected phytoconstituents exhibits good binding interaction with GSK-3B and warrants further studies needed for the development of potent GSK-3B Inhibitor for the treatment of AD. Further computational screening shows that all these leads have high potential of inhibiting GSK-3B.

These results clearly indicates that the leads especially Apigenin, Kaempferol and Quercetin shows similar binding sites and interactions with GSK-3B enzyme compared to the standard drug GSK-3B Inhibitor X. This in silico studies is actually an added advantage to screen the potential lead against GSK-3B inhibition activity. Now a day's phytoconstituents from the natural derivatives may serve as therapeutic leads in the development of clinically effective GSK-3B inhibitor. Further investigations on the above compounds on preclinical and clinical studies are necessary to develop potential drug entity for the treatment of neurodegenerative disorders like AD.

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