Original Article

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Implication of Fyn C-Kinase as a Common Therapeutic Target for Alzheimer Disease and Its Inhibition Using Natural Compounds

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Abstract

Background: Alzheimer Disease (AD) is one of the major threatof ageing. In current time it creates a social stigma among people so for this disease is important for research orientations. Herbal medicine offers several options to modify the progress and symptoms of AD hence new trend in the preparation and marketing of drugs based on medicinal plants, and their scientific and commercial significance appears to be gathering momentum in health-relevant areas. Current insilico study signifies the potentiality of Ginkgolide against Fyn C kinase in case of Alzhemier disease. *Material and Methods:* In the current study 3D model of Fyn C kinase receptor was predicted by comparative homology modeling program MODELLER. The computed model's energy was minimized and validated using PROCHECK, ProSa and Errat tool to obtain a stable model structure. Stable model was used for molecular docking against screened phytochemical and available synthetic molecules using AutoDock 4.2, which resulted in energy-based descriptors such as Binding Energy, Intermol energy, vdW + Hbond + desolv Energy and Electrostatic Energy. *Results:* These interactions between the active residues may lead to significant conformational change in that particular portion of the protein. This efficacy and suitability of ligand was determined on the basis of binding energy calculations. Ginkgolide showed minimum binding energy calculations among selected 4 other natural ligands. *Conclusion:* Such information may open new prospects in the use of natural compounds and their derivatives as a potential drug candidateagainst Fyn C kinase for treatment of Alzheimer Disease (AD).

Keywords: Molecular Modeling; Alzheimer; Ginkgolide; Molecular Interaction; Binding Energy; Natural Ligands.

Background

Alzheimer Disease (AD) is the one of the major threat for ageing in current time hence there is a great demand to be focused on this disorder[1]. Further Alzheimer is also creating a social stigma among people so for much important for research orientations. It is characterized with abundance of plaque and tangles in the brain which play a critical role in blocking communication among nerve cells and disrupting major processes that the cells need to survive[2]. Fyn C kinase (member of tyrosine kinase) protein plays a key role in Alzheimer disease hence can be a potential target for novel approaches associated with drug designing for this personal to social troubles due to this disorder. This protein is strategically located at the synapses, where it regulates the activity on several proteins[3,4]. It is reported that increase in Fyn C kinase activity remarkably enhance the susceptibility of granule cells to the Aβ - induced depletion of memory proteins and, in fact, triggered prominent deficits in memory retention [5,6].

Herbal medicine offers several options to modify the progress and symptoms of AD. There has been a new trend in the preparation and marketing of drugs based on medicinal plants, and their scientific and commercial significance appears to be gathering momentum in health-relevant areas. These plant-derived products are carefully standardized, and their efficacy and safety for a specific application have been demonstrated [7]. Ayurvedic medicine is a system of traditional medicine native to India, and Ayurvedic

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practitioners have developed a number of medicinal preparations for the treatment of various ailments. An entire body of literature in the Ayurvedic texts deals with the nervous system and disorders associated with it [8]. Phytochemical studies have shown the presence of many valuable compounds, such as lignans, flavonoids, tannins, polyphenols, triterpenes, sterols, and alkaloids, that show a wide spectrum of pharmacological activities, including anti-inflammatory, anti-amyloidogenic, anti-cholinesterase, hypolipidemic, and antioxidant effects [9,10]. In this current course of study we had predicted a three dimensional model for Fyn C kinase protein of Homo sapiensthrough Modeller 9V11 [11]. Thecomputed model's energy was minimized and reliability ofmodel was checked by Procheck [12], PROSA [13]and ERRAT [14]value. Stable model was docked with the screened phytochemicals through AutoDock 4.2 [15] which gives the idea of most effective and physiologically suitable herbal molecule. As Virtual screening [16,17] is the most important step for drug developmental studies hence standard protocol regarding the same were used in this direction.

Methods

Retrieval of Target Protein Sequence

Amino acid sequence of Fyn C Kinase (ID:P06241) [18] of *Homo sapiens*was obtained from UniProt database (*http://www.uniprot.org/uniprot*) [19]. The sequence was retrieved in FASTA format and is further used tertiary structure analysis.

Template Identification and Alignment

To find out an appropriate template for the modeling of the target Fyn c kinase BLAST program [20] was used against the PDB database [21]. By the BLAST search, we screened structures 2DQ7,2SRC & 1Y57 as the template protein for query sequence. Sequence alignment of target sequence with template wasperformed by using dynamics programming based align2Dmodule in Modeller 9.11 software. Default parameterswere applied and the aligned sequences were inspected andadjusted manually to minimize the number of gaps andinsertions.

Molecular Modeling

The protein sequence was subject for comparative homology modeling via Modeller 9.11 advance modeling predicted putative 3D model. Alignment of query with template protein was used as input for model single script in Modeller program and five comparative models were generated. These models were validated with the help of Modeller objective function and DOPE score [22], which were the statistical parameter for the assessment of model using the standard Modeller energy function. The constructed models were energy minimized by CHIMERA [23]. The quality of 3D structure wasfurther verified by Procheck, Errat and ProSA tools. The predicted model wasvisualized by Chimera.

Retrival and Preprocessing of Natural and Synthetic Compounds

The 3D structures of the selected natural and synthetic compounds with known anti neurodegenerative properties were retrieved from PubChem compounds database. These compounds included bilobalide (PubChem ID: 12308750), curcumin (PubChem ID: 969516), ginkgolide-B (PubChem ID: 65243), withaferin-A (PubChem ID: 265237) and donepezil (PubChem ID: 3152).

Docking

To gain better insight for the interactions between Fyn C Kinase with screened herbal and synthetic ligands molecular docking studies were carried out. AutoDock software along with a graphical user interface, AutoDock tools (ADT) was utilized to generate grids, calculate dock score and evaluate the confirmations. ADT requires the receptor and ligand coordinates in either Mol2 or PDB format. The receptor PDB file was transformed into the PDBQT format file containing the receptor atom coordinates, partial charges and salvation parameters. The ligand file was transformed into a PDBQT file, merged nonpolar hydrogen atoms and torsions were defined. The grid calculations were set up and maps were calculated with the program AutoGrid. All docking runs were performed using the Lamarckian genetic algorithm and obtained dock score were reported in kcal / mol. Docking of herbal and synthetic molecules on the predicted binding pocket, where the residues LEU225, CYS229, ILE246, SER258, THR290, TYR292 and ASP300were being particularly important.

Results and Discussion

The sequence alignment of the query and template was showing good similarity. The query sequence was made up of 537 residues. The result of alignment was employed to build new homology model. After the optimization and energy minimization process,

the best model was selected among five 3D structures generated for FynC kinase protein on the basis of Modeller scores and the Molecular objectives function of the selected model was 13855.59961kcal / mol and the DOPE score result was -49641.066406 Kcal / mol (Fig.1a). Energy minimization of 3D structure is vital for providing the maximum stability to the protein. Ramachandran plot drawn through PROCHECK program validated the model with 91.2% of total residues in most favoured regions, 6.2% residues in additional allowed regions, 2.1% generously allowed regions and 0.5% in the disallowed regions (Figure 1b). Quality assessment of the model via ProSA revealed that the predicted model matched with NMR region of the plot with a Z score of -9.09 (Figure 1c). This score gives authenticity about good quality of models generated in this study. ERRAT (*http://services.mbi.ucla.edu/ERRAT/)* is a protein structure verification algorithm that is specially well suited for evaluating the progress of crystallographic model building and refinement. The program works by analysing the statistics of non-bonded interactions between different atom types. A single output plot was produced by errat program that gave the value of the error function vs. position of a 9-residue sliding window. By comparison with statistics from highly refined structures, the error values have been calibrated to give confidence limits. This was extremely useful in making decisions about reliability. After the errat the overall quality factor was 73.118 which have been shown in the (Figure 1d).

Molecular docking was used to investigatepossible binding modes of selected natural compounds withinthe active site of Fyn C kinase. Several possible binding modeswere detected and were ranked on the basis of their bindingfree energy. It was revealed that Ginkgolide-B was capable of binding within the binding site of Fyn C kinasewith higher affinity as compare to their other compounds. Ginkgolide-B is a leaf extract of *Ginkgo biloba* plant possesses several biological activities like antioxidant, anticancer, antiinflammatory, and neuroprotective effects**.** For instance, Ginkgolide-B was found to bind within the active site of Fyn C kinase protein with binding free energy of -16.77 Kcal / mol that was higher than other compounds. Further the binding efficacy of Ginkgolide-B was compared with known Donepezil inhibitor of Fyn C kinase protein. It was found that this inhibitor of Fyn C kinase protein interacts with binding free energy of -5.81 kcal / mol within the active site of Fyn C kinase. The binding mode of Ginkgolide within the active site of Fyn C kinase is shown in (Figure 2a-e). Table 1 illustrates the binding score of all the compounds used in this study against Fyn C kinase. In the present study, all the compounds were found to interact mainly through seven amino acid residues LEU225, CYS229, ILE246, SER258, THR290, TYR292 and ASP300. Interaction of all the compounds against Fyn C kinase was observed to be dominated by both hydrogen bonds as well as hydrophobic interactions. Hence, the present study reveals that Ginkgolide-B is an efficient phytochemical of Fyn C kinase in terms of amino acid interaction and Autodock binding free energy.

Conclusions

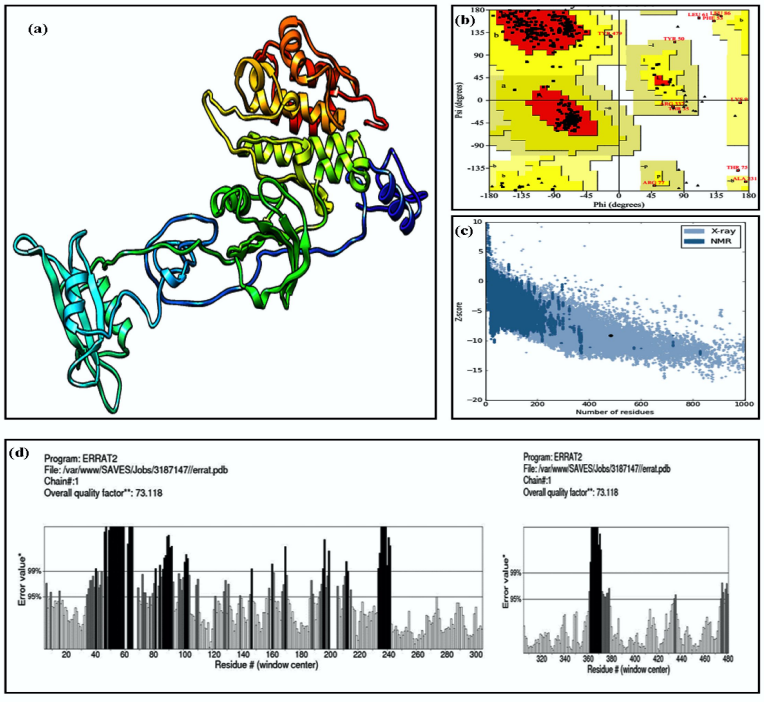

Fig. 1 (a): Modeled 3D structure of Fyn C kinase protein using Modeller 9v11 **(b)** Ramachandran plot of Fyn C kinase protein predicted model **(c)** ProSA analysis of modeled protein structure of Fyn C kinase protein **(d)** Errat plot for Fyn C kinase receptor structure. Error values for residues as predicted by ERRAT for Fyn C kinase receptor. Y axis presents the error value and X axis presents the amino acid sequences of Fyn C kinase receptor. The overall quality factor assigned to Fyn C kinase receptor is 73.118.

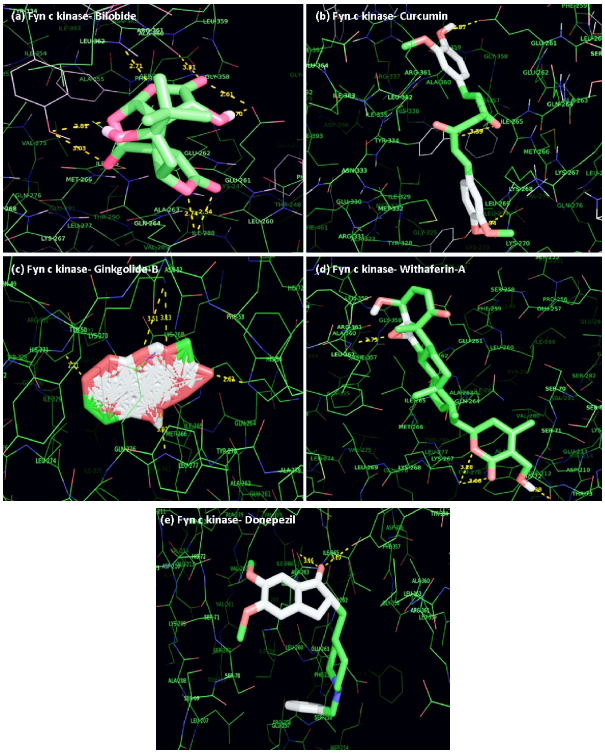

Fig. 2 (a-e): Molecular Interaction between Fyn C kinase receptor with phytochemicals and synthetic molecules. Yellow dotted line displayed the hydrogen bond between the receptor and phytochemical.

This study explores the molecular interactions through docking of some screened potentialnatural compounds in comparison with available standard synthetic chemical with Fyn C kinase protein. Selection of these compounds were done on the basis of their anti-neurodegenerative properties reported earlier in literature along with standard validation of ligands. Out of all the selected compounds, Ginkgolide-B was found to be the most potentialphytochemical against the Alzheimer's important target protein of Fyn C kinase in terms of binding energy. As compared with donepizel (Synthetic Drug) the binding energies values of Ginkgolide-B were also well to the mark in progressive range. Such findings are quite promising and this information may open new prospects in the use of natural compounds and their derivatives as a potential drug candidate against Fyn C kinase for the betterment of Alzheimer Disease (AD). Further experimental studies need to be performed to validate these data. Though, it can be safely stated that Ginkgolide-B is efficient phytochemical againstFyn C kinase can be more productive for having safe with limited or no side effects in the management of this social trauma of Alzheimer's Disease.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Abbreviations

AD: Alzheimer Disease; ProSA: Protein Structure Analysis; UniProt: Universal Protein Resource; BLAST: Basic local alignment search tool; DOPE: Discrete Optimized Protein Energy; PDB: Protein Databank

Competing Interests

The authors declare that they have no competing interests.

Authors' Contribution

AT: designed and performed experiments, analysed the data and drafted the manuscript; SS: designed and performed experiments, analysed results and drafted the manuscript; PS: conceived and designed the project, analysed the data, and edited the manuscript.

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