Molecular Docking Study to Identify Potent Inhibitors of Alpha-synuclein Aggregation of Parkinson's Disease

Ekta Gupta[1], Shradheya RR Gupta[2], Abhijeet Kumar[3], Anmol Kulshreshtha[4], Ravi Ranjan Kumar Niraj[5]

ABSTRACT

Introduction: Parkinson's disease (PD) is the widespread neurodegenerative disorder ranked second in this categories and PD is also the most common movement disorder. PD disorder affects more than 0.1% of the total population older than 40 years of age. Contemporary, therapies of PD are restricted to only symptomatic relief without dealing with the basic disease etiology such as aggregation of αSyn, thus the progression of the disease continues with the current therapies. The major objective of this study was to find out putative inhibitors of human alfa-synuclein to search possible therapeutics of Parkinson's disease.

Material and Methods: Our study included Molecular docking study of 3D-Structure of alfa- synuclein of human retrieved from PDB with their chemical ligands. The protein-ligands docking were performed using AutoDock4.2.5.1. Further, Molecular Dynamic Simulation for protein-ligand complex of best dock complex was carried out using Gromacs16.10.

Result: Total nineteen molecules was selected for docking study out of which Amento flavones molecule shows best binding. The molecular docking simulation results indicate that the protein complexes were stable throughout MD simulations and thus proteins possess the ability to stability.

Conclusion: This study provides an insight of *in-silico* drug designing approach towards alfa- synuclein modulators as a promising therapeutics of Parkinson's's disease.

Keywords: *In-silico,* Molecular Docking, Parkinson's disease.

INTRODUCTION

Parkinson's disease (PD) is the widespread neurodegenerative disorder ranked second in this categories and PD is also the most common movement disorder. PD disorder affects more than 0.1% of the total population older than 40 years of age.[1] Characteristics of Parkinson's disease are successive loss of muscle control, which leads to trembling of the limbs and head while at rest, stiffness, slowness, and impaired balance. Parkinson's disease (PD) is a progressive neurological disorder depends on a number of genetic and environmental factors. The etiology of PD is characterized by the aggregation of α-synuclein (αSyn) known as Lewy bodies. The death and neuronal dysfunction in PD is prominently caused by aggregation of αSyn. It is also associated to neurotransmitter and release of calcium. Contemporary, therapies of PD are restricted to only symptomatic relief without dealing with the basic disease etiology such as aggregation of αSyn, thus the progression of the disease continues with the current therapies. Although, Currently it is not well known how the aggregation of α Synelicit cell death, but probably soluble oligomeric form of αSyn, known as protofibrils, are the majorly responsible. The cell death by protofibrils is caused due to blockage of various cellular targets.[2-6] The secreted αSyn is also may contribute to the disease proliferation. αSyn aggregates can increase oxidative stress in the SNPc region of the PD brain which can lead to vesicular dopaminergic leakage. Abundance of iron in the SNPc region catalyzes oxidative stress which might also promote α Synmisfolding and aggregation in the cytoplasm.[7-8] αSyn can directly interact with dopamine (DA) transporters, which eventually promotes the DA transporters mediated DA uptake and cellular apoptosis.[9] Computational chemistry has always played the central role in current days molecule design including drugs, as computational chemistry provide a cost and time affective solution to in vitro and in vivo assays, and followed by the '3Rs' policy of reducing, refining and replacing animal testing for preliminary studies.[10-11] Our study includes Molecular docking study of 3D-Structure of α synuclein of human retrieved from PDB with their chemical ligands. The protein-ligands docking were performed using AutoDock 4.2.5.1. Further, Molecular Dynamic Simulation for protein-ligand complex of best dock complex was carried out using Gromacs16.10. This study provides an insight of *in-silico* drug designing approach towards α synuclein modulators as a promising therapeutics of Parkinson's disease.

MATERIAL AND METHODS

This study was done in Dr. B Lal Institute of Biotechnology, Jaipur during August, 2018-February, 2019.

Protein structure assessment

3D-Structure of alpha- synuclein (PDB ID - 4RIK) of human

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was retrieved from PDB**.** PROCHECK (Ramachandran plot) was used to analyze the stereo-chemical and overall quality of the structure.[12]

Ligand preparation

The chemical ligands of α synuclein (Amentoflavone1, Curcumin2, Epigallocatechin, Etorphine, L-Dopa, Naringenin, Resorcinol, Resveratrol, Rosamarinic acid, Trolox, Jatamanin-A, F, G, H, I, J, K & M) were downloaded from Pubchem chemical database (Table 1). The structures were optimized using Chemsketch and their energy was minimized for lowest energy conformations using UCSF-Chimera.[13]

Molecular docking

The prepared ligands were docked against retrieved structure of alpha synuclein using Autodock 4.2.5.1.[14] Lamarckian model of genetics was used and only polar hydrogen was added to the protein and Kollman and Gastegier charges were assigned. The grid box was set to 126Å, 100 Å, 116Å (x, y and z axis) to include all the amino acid residues that were present in protein and the spacing between grid points was set to default value of 0.558 Å. The study was primarily aimed to decipher the binding domain search of respective ligand hence blind docking was performed for the purpose. A total of 50 independent runs were performed with a step sizes of 0.2 Å for translations and 50 for orientations and torsions. The maximum number of generations was set to 1000 and maximum number of top individuals that automatically survived was set to 1 with mutation rate of 0.02, crossover rate of 0.8, cluster tolerance 0.5 Å, external grid energy 1000.0.

Molecular dynamics simulation

The Molecular Dynamic Simulation was performed using GROMACS 2018.1 package, the force field selected for this study was Gromos43a1. The protein salvation was performed using SPC water model in a cubic box size 10.8 × 10.8 × 10.8 nm^3. The energy minimization was executed using the steepest algorithm up to a maximum 25,000 steps or until the maximum force (Fmax) less than 1000 kJ/ mol nm which is the default threshold. The quantity of steps necessitated to reach minimization criteria were scrutinized to be around 400 for such complex. This step was performed after the equilibration of the complete system by means of both NVT/ and NPT ensembles for 50,000 steps (100 ps) at 300 K and 1 atm. The system was primarily equilibrated using NVT ensemble followed by NPT ensemble. After this equilibration process, the molecular dynamics simulation was executed for 1ns long. Finally, the simulations were evaluated; RMSD and RMSF plots were calculated for complete episode of simulations. The protein-ligand simulation of docked protein was also performed to study interaction pattern of ligands and change in protein after molecular docking with respective ligand. The all steps are kept similar except the final the molecular dynamics simulation was carried out for 10ns long.

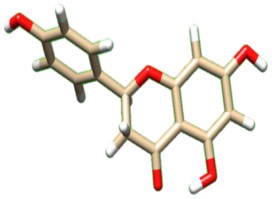
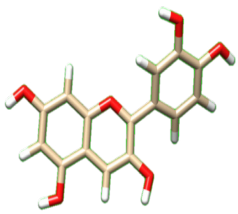
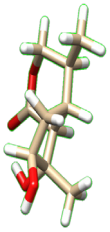
RESULTS

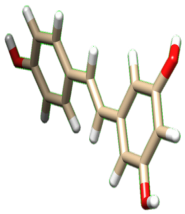
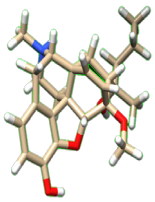
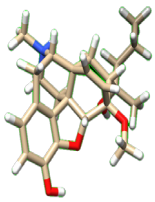
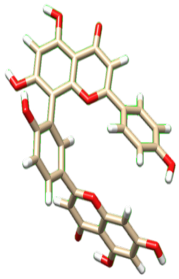
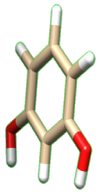
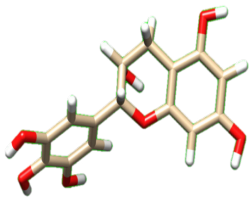
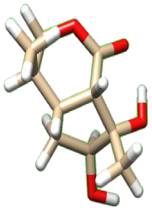
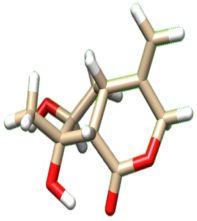
Protein structure assessment

3D-Structure of alpha- synuclein (PDB ID - 4RIK) of human was retrieved from PDB**.** The analyses of model using PROCHECK server revealed the model is of good quality (Figure 1).

Molecular Docking

The outcome of molecular docking study shown in Table 1,2 and Dock complex was shown in figure2. The Amento flavone was found comparatively more suitable ligand in this study. The docked complex with ligand Amento flavone was

S.No.	Ligand Name	Properties	Chimeric View
1.	Naringenin	Mol. Formula-C15H1205 Mol. Weight-272.25g/mol H-bond donar-3 H-bond acceptor- 5	
2.	Cyanidin	Mol. Formula-C15H1106 Mol. Weight-287.24g/mol H-bond donar-5 H-bond acceptor- 6	
3.	Jatamanin g	Mol. Formula-C10H1604 Mol. Weight-200.23g/mol H-bond donar-2 H-bond acceptor- 4	

4.	Resveratrol	Mol. Formula-C14H1203 Mol. Weight-220.24g/mol H-bond donar-2 H-bond acceptor- 3	
5.	Etorphine	Mol. Formula-C25H33NO4 Mol. Weight-411.53g/mol H-bond donar-2 H-bond acceptor- 5	
6.	Trolox	Mol. Formula-C14H1804 Mol. Weight-250.29g/mol H-bond donar-2 H-bond acceptor- 4	
7.	Amento flavone	Mol. Formula-C30H18010 Mol. Weight-538.46g/mol H-bond donar-6 H-bond acceptor- 10 Lipinski's Rule-No	
8.	Resorcinol	Mol. Formula-C6H6O2 Mol. Weight-110.11g/mol H-bond donar-2 H-bond acceptor- 2	
9.	Epigallocatechin	Mol. Formula-C15H1407 Mol. Weight-306.27 H-bond donar-6 H-bond acceptor- 7	
10.	Jatamanin f	Mol. Formula-C10H1604 Mol. Weight-200.23g/mol H-bond donar-2 H-bond acceptor- 4	
11.	Jatamanin a	Mol. Formula-C10H1404 Mol. Weight-198.22g/mol H-bond donar-2 H-bond acceptor- 4	

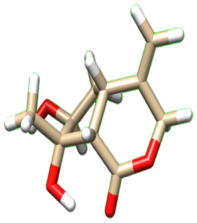
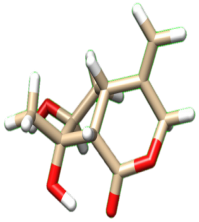
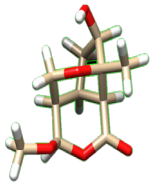
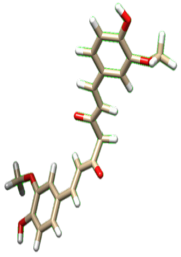
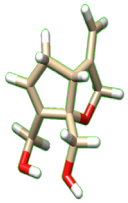
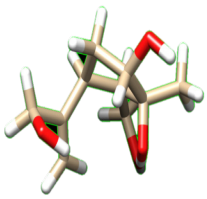
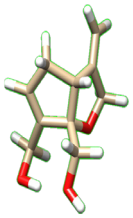
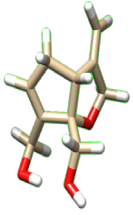
12.	Jatamanin h	Mol. Formula-C11H1605 Mol. Weight-228.24g/mol H-bond donar-1 H-bond acceptor- 5	
13.	L dopa	Mol. Formula-C9H11NO4 Mol. Weight-197.19 H-bond donar-4 H-bond acceptor- 5	
14.	Jatamanin i	Mol. Formula-C11H1605 Mol. Weight-228.24g/mol H-bond donar-1 H-bond acceptor- 5	
15.	Curcumin	Mol. Formula-C21H2006 Mol. Weight-368.38g/mol H-bond donar-2 H-bond acceptor- 6	
16.	Jatamanin k	Mol. Formula-C12H1406 Mol. Weight-254.24 H-bond donar-2 H-bond acceptor- 6	
17.	Jatamanin j	Mol. Formula-C11H1605 Mol. Weight-228.24 H-bond donar-1 H-bond acceptor- 5	
18.	Jatamanin m	Mol. Formula-C10H1403 Mol. Weight-180.22 H-bond donar-2 H-bond acceptor- 3	
19.	Rosmerinic acid	Mol. Formula-C18H1608 Mol. Weight-360.31g/mol H-bond donar-5 H-bond acceptor- 8	

Table-1: Detail of Ligands selected for docking study.

S. No.	Ligand name	Binding energy (Kcal/Mol)	Ligand efficency	Inhibition constant (mM)
1	Naringenin	-6.69	0.33	12.56
2	Cyanidin3	-6.44	0.31	19.09
3	Jatamanin g	-6.39	0.46	20.68
4	Resveratrol	-6.32	0.37	23.15
5	Etorphine	-5.99	0.2	40.92
6	Trolox	-5.44	0.3	103.08
7	Amento flavone	-5.44	0.14	**103.28**
8	Resorcinol	-4.75	0.59	327.42
9	Epigallocatechin	-4.68	0.21	373.16
10	Jatamanin f	-4.4	0.31	595.4
11	Jatamanin a	-4.37	0.31	629.6
12	Jatamanin h	-4.29	0.27	721.62
13	L dopa	-3.98	0.28	1.2
14	Jatamanin i	-3.81	0.24	1.6
15	Curcumin	-2.73	0.1	10.03
16	Jatamanin k	-2.67	0.21	11.02
17	Jatamanin j	2.19	0.16	24.68
18	Jatamanin m	2.19	0.18	4.59
19	Rosmerinic acid	1.96	0.08	36.73

Table-2: Showing result of Molecular docking study.

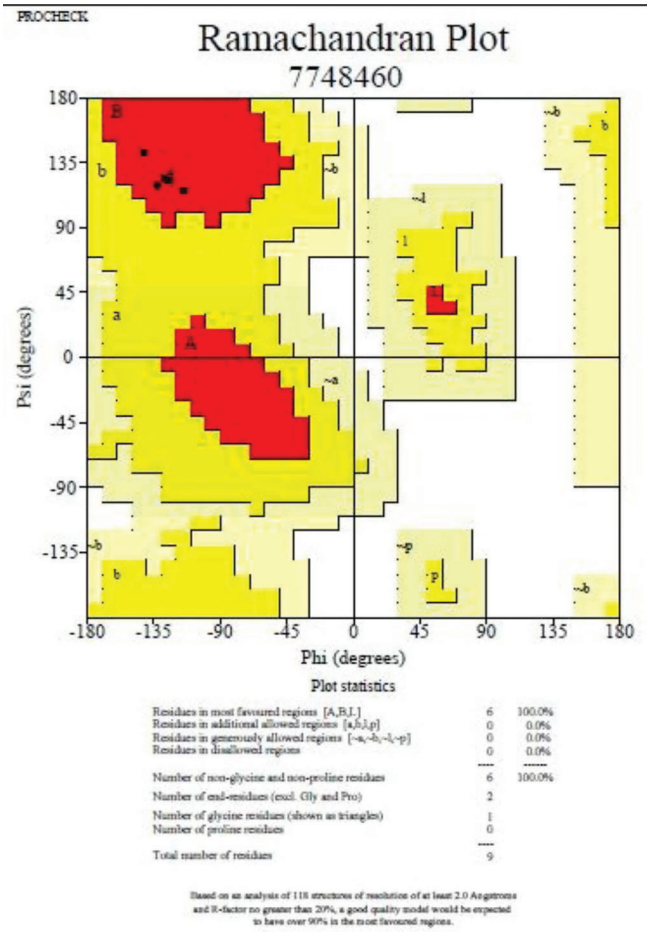


Figure-1: Showing Ramachandran Plot for human alpha synuclein.

further preceded for molecular dynamic simulation study.

Molecular dynamics simulation

The Molecular Dynamic simulation study revel that, the protein deviated about 0.4 nm in the 30 ns then acquired a stable conformation for the rest of trajectory. RMSF per residue and RMSD throughout simulations were calculated. It was found that, the protein was stable for most part of the simulation trajectory. The root mean squared fluctuation (RMSF) is the time-average of root mean squared deviation (RMSD) for each residue was also calculated (Fig.3a-b). In most cases, residues lying in the core protein regions have low RMSF while exposed loops have high RMSF values. As observed, the peaks in the graph possess a value between 0.2 and 0.6 nm. Both these results indicate that the protein complexes were stable throughout MD simulations and thus proteins possess the ability to stability.

DISCUSSION

Homology modeling, molecular docking and Molecular docking are proven tools for in silico inhibitor identification. Molecular docking approaches are frequently used in computational drug designing to understand ligands-receptor interaction. Earlier studies shows that these computation techniques strongly support in designing novel, potent inhibitors by deciphering the mechanism of drug receptor interaction.[15] The Amento flavone was found comparatively more suitable ligand in this study. These results revealed that amento flavone has the ability to bind towards the active site of α-synuclein and prevents the self-association of protein to form a drug for Parkinson's disease. Earlier report suggest diverse pharmacological activities of Amentoflavone.[16] The therapeutic effect on cardiovascular and central nervous system was also reported in earlier studies.[17-19] Alpha-synuclein is the characteristic property of Parkinson's disease; hence, it has an important application in the clinical diagnosis and therapeutics of Parkinson's disease.[20]

CONCLUSION

The present *in-silico* study has given a good insight to structure and function of alpha synuclein. Present study also

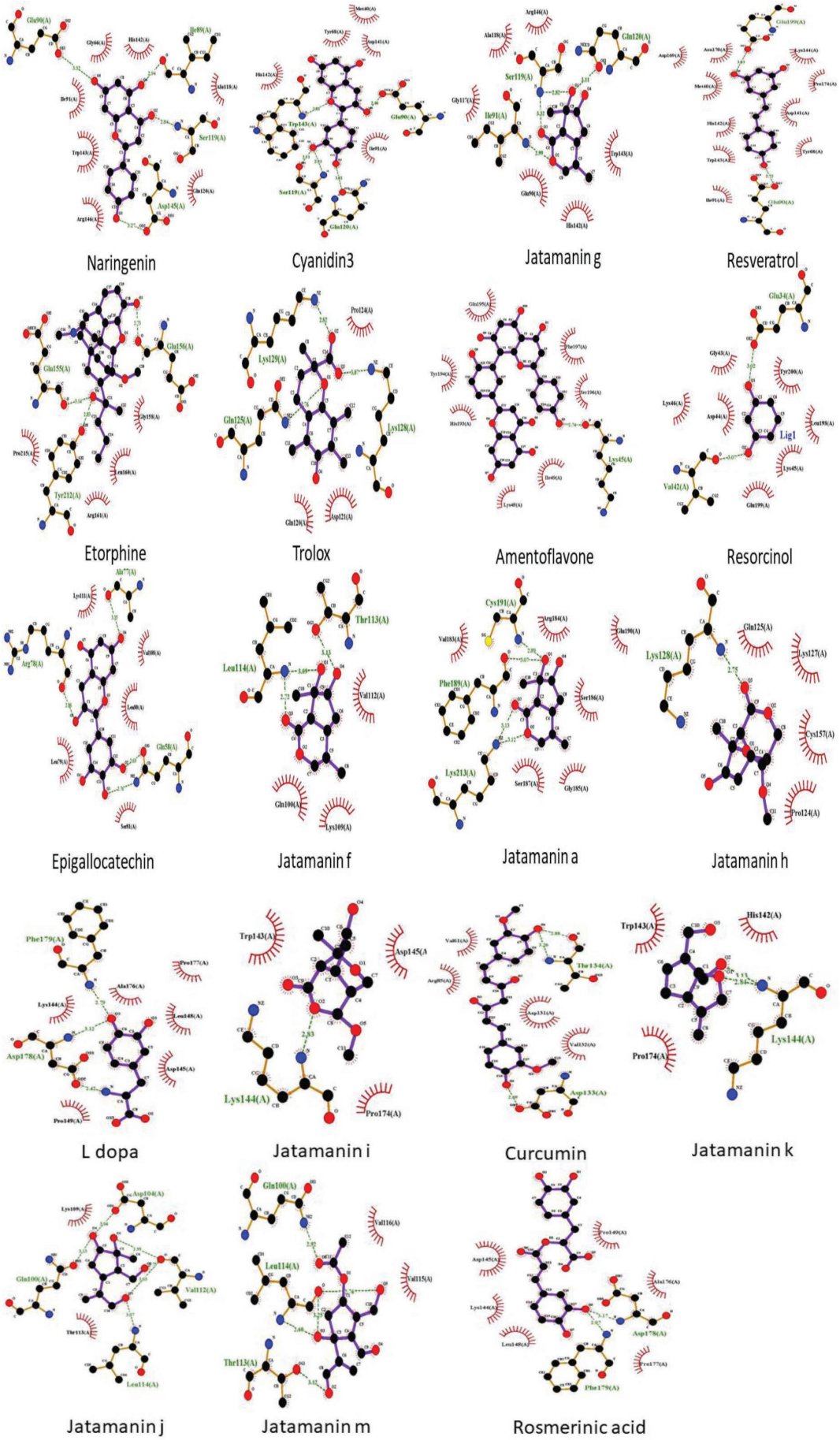


Figure-2: Molecular docking study of human alpha synuclein with ligands respectively. Docking pose analysis via Ligplot, where Oxygen (O), Nitrogen (N) and Carbon (C) atoms are represented in red,blue and black circles.

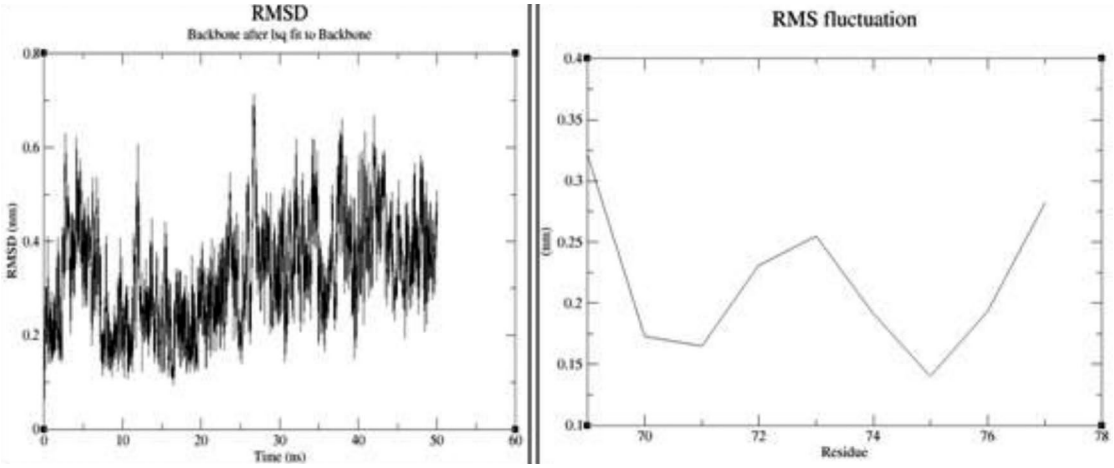


Figure-3: MD simulation RMSD and RMSF graph of human alpha synuclein

states about energy stabilization of alpha synuclein protein with inhibitor (Amento flavone) using Molecular Dynamic Simulation. This study may provide a good plate form for designing structure based inhibitors of alpha synuclein aggregation.

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