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Full Length Original Research Paper

Molecular docking studies of Ellagic Acid and Gallic Acid In diabetic Nephropathy

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Abstract:
The chronic diabetes mellitus leads to diabetic nephropathy, which is one of the major microvascular complication of end stage renal disease worldwide and causes premature death in diabetic patients. The objective of the present investigation was to evaluate the protective effect of phytoconstituents, Ellagic acid and Gallic acid of seeds of *Eugenia jambolana* in diabetic nephropathy by dry lab studies. The nephroprotective effect was studied by molecular docking method using 108 A diabetic nephropathy protein. The results of docking studies revealed that Ellagic acid and Gallic acid showed the bonding score of -8.05708 Kcl/mol and -6.67303 Kcl/mol, for 108 A nephropathy protein for being a good nephro protective in diabetic nephropathy complications.The findings of this investigation concluded that Ellagic acid and Gallic acid has potential protective effect in diabetic nephropathy.

Keywords: Diabetic nephropathy, Molecular docking, Nephropathy protein, Ellagic acid, Gallic acid.

Introduction

The world is facing an explosive increase in the incidence of diabetes mellitus(DM), which is a chronic metabolic disease with highest rates of prevalence and mortality affects more than 100 million people worldwide[(1)].The prevalence rate of diabetes is estimated to be 1-5% in India. The number of people with diabetes in India is currently around 50.8 million and expected to rise to 87 million by 2030 unless urgent preventive steps are taken[(2,3)]. Hence India leads the world with largest number of diabetic patients earning the dubious distinction of being the "diabetic capital of the world"[(4)].

Diabetes is now considered to be a vascular disease. Diabetic nephropathy is one of the majormicrovascular complication of type-2 diabetes mellitus and is the major cause of end-stage renal disease(ESRD). Diabetic nephropathy has been a growing threat in the world and Eastern countries are not an exception. In Australia type-2 diabetes mellitus patients starting dialysis increased 5-fold between 1993 and 2007 and in India diabetic nephropathy, is expected to rise to 6.6 million of the more than 100 million patients suffering diabetes. So it is a major cause of morbidity in diabetic patients [(5,6)]. All of the pharmacological modalities show limited efficacy and certain adverse effects and are expensive particularly for developing countries like Indiaand China. Comparatively very less side effects andlow cost of phytopharmaceuticals from natural resources open new avenues for the treatment of various diseases. Therefore there is a need for phytochemicals that have protective

effect in diabetic nephropathy, which are potent, cost effective and also safe without long-term side effects [1].

The seeds of *Eugenia jambolana*, belonging to the family of Myrtaceaehas been indicated in Ayurveda for its use in diabetes mellitus [7]. It is reported to have hypoglycemic[2, 8,9], hypolipidemic[8], antiulcer [9], antibacterial [10], anti-inflammatory [11],neuropsycho pharmacological [12], anti HIV [13] and antidiarrhoeal activity [14].

Although the seeds of*Eugenia jambolana* has been used in traditional medicine [13] yet scientific validation of its effect on diabetic nephropathy needs to be studied. Hence this investigation was undertaken to evaluate the nephroprotective effect of phytoconstituents, Ellagic acid and Gallic acid present in the seeds of *Eugenia jambolana* by molecular docking studies using 108 A diabetic nephropathy protein.

Materials and methods[15]

Databases used

Protein data bank of 108 A nephropathy protein

The Protein Data Bank (PDB) was a repository for the 3D structural data of large biological molecules, such as proteins and nucleic acids. The data typically obtained by X-ray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world and can be accessed at no charge on the internet. The PDB was overseen by an organization called the World Protein Data Bank.

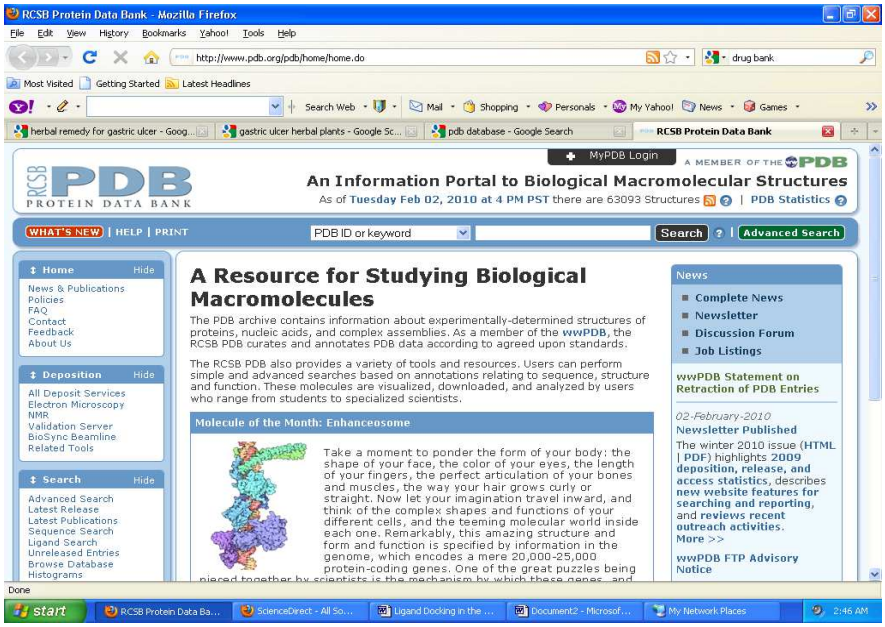


Figure 1: Home page of protein data bank

The PDB was a key resource in areas of structural biology, such as structural genomics. Most major scientific journals, and some finding agencies, such as the NIH in the USA, now require scientists to submit their structure data to the PDB. If the contents of the PDB were thought of as primary data, then there were hundreds of derived (i.e.,secondary) databases that categorize the data differently. For example, both SCOP and CATH categorize structures according to type of structure and assumed evolutionary relations.

The structure files may be viewed using one of several open source computer programs. Some other free, but not open source programs include VMD, MDL Chime, Swiss-PDB viewer, StarBiochem (a Java-based interactive

molecular viewer with integrated search of protein databank) and Sirius. The RCBS PDB website had contained an extensive list of both free and commercial molecule visualization programs and web browser plugins. (http://www.rcsb.org/pdb)

Retrieval of Protein 3D Structure

The following steps were used to retrieve the 3D structure.

Step 1: The Google website was visited and in the search column the keyword RCSB was entered; the search button was clicked.

Step 2: The RCBS homepage was displayed.

Step 3: In the search column enter the protein name as farnesyltransferase and click go, then the list of proteins will be displayed.

Step 4: From the list, protein structure was selected and then 3D structure of receptor was saved

Drug Bank

The DrugBank database is a unique bioinformatics and cheminformatics resource that combines detailed drug (i.e. chemical, pharmacological and pharmaceutical) data with comprehensive drug target (i.e. sequence, structure, and pathway) information. Since its first release in 2006, DrugBank has been widely used to facilitate in silico drug target discovery, drug design, drug docking or screening, drug metabolism prediction, drug interaction prediction and general pharmaceutical education. The latest version of DrugBank (release 2.0) has been expanded significantly over the previous release. With approximately 4900 drug entries. Significantly, more protein target data has also been added to the database, with the latest version of DrugBank containing three times as many non-redundant protein or drug target sequences as before (1565 versus 524). Each DrugCard entry now contains more than 100 data fields with half of the information being devoted to drug/chemical data and the other half devoted to pharmacological, pharmacogenomic and molecular biological data. A number of new data fields, including food-drug interactions, drug-drug interactions and experimental ADME data have been added in response to numerous user requests. DrugBank has also significantly improved the power and simplicity of its structure query and text query searches.

Figure 2: Home page of Drug Bank (www.drugbank.ca)

PubChem compound

PubChem was a database of chemical molecules. The system was maintained by the National Centre for Biotechnology Information (NCBI), a component of National Library of Medicine, which wasa part of the United States National Institutes of Health (NIH). PubChem can be accessed for free through a web user interface. Millions of compound structures and descriptive datasets can be freely downloaded via FTP. PubChem contain substance descriptions and small molecules with fewer than 1000 atoms and 1000 bonds. The American Chemical Society tried to get the U.S. Congress to restrict the operation of PubChem, because the claim it had competed with their Chemical Abstracts Service. More than 80 database vendors contribute to growing PubChem database. PubChem was designed to provide information on biological activities of small molecules, generally those with molecular weight less than 500 daltans. PubChem'sintegration with NCBI's Entrez information retrieval system provides sub/structure, similarity structure, bioactivity data as well as links to biological property information in PubMed and NCBI's Protein 3D Structure Resource.

Figure 3: Home page of PubChem

Pubchem compound was a searchable database of chemical structures with validated chemical depiction information provided to describe substances in PubChem Substance. Structures stored within PubChem compounds are pre-clustered and cross-referenced by identify and similarity groups. (pubchem.ncbi.nlm.nih.gov)

Retrieval of ligand structures

The following were the steps used in the selection of the Ligand.

Step 1: The Google website was visited and in the search column the keyword PubChem Compound was entered; the search button was clicked.

Step 2: PubChem home page was displayed.

Step 3: In search box enter the compound name as Ellagic acid/Gallic acid.

Step 4: Select the structure form the list of compounds displayed in pubchem.

Softwares Used

OpenBabel software

OpenBabel is free software, a chemical expert system mainly used for converting chemical file formats. Due to the strong relationship to informatics this program belongs more to the category cheminformatics than to molecular modelling. It is available for Windows, Unix, and Mac OS. It is distributed under the GNU GPL. The project's stated goal is: OpenBabel is a community-driven scientific project assisting both users and developers as a cross-platform program and library designed to support molecular modeling, chemistry, and many related areas, including interconversion of file formats and data. (sourceforge.net/projects/openbabel/)

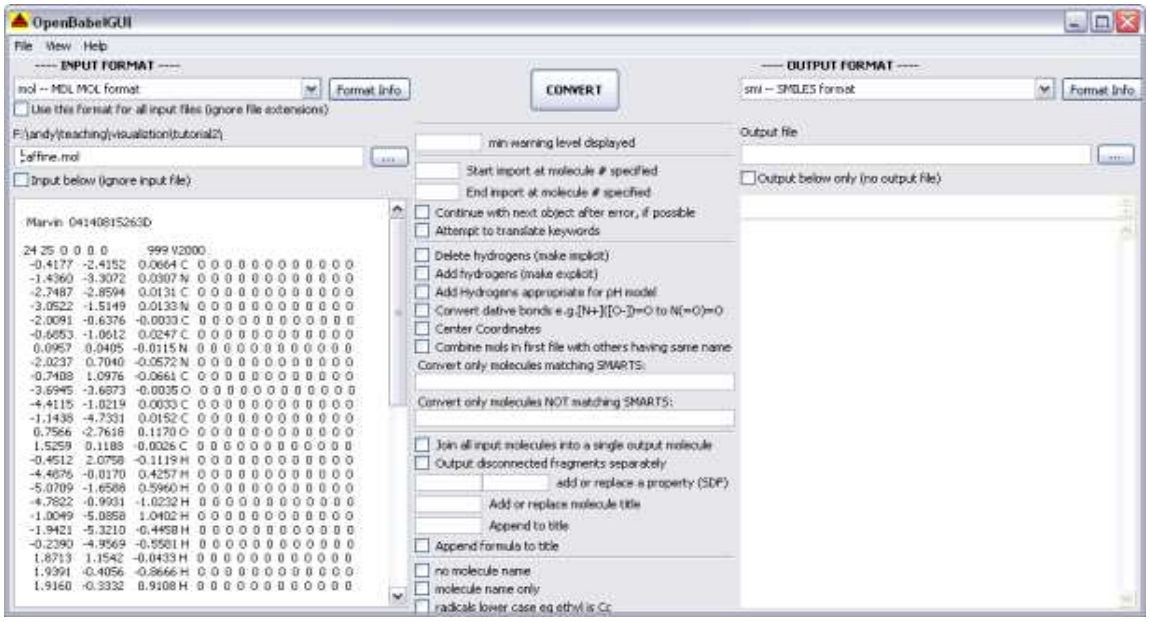


Figure No: 4 Home page of OpenBabel

Features currently include

- A huge variety of common chemical file formats, including SDF/MOL, Mol2, PDB, SMILES, XYZ, CML.
- Recognition of file type based on file name extension
- Chemical MIME support
- SMARTS matcher
- Flexible atom typer
- Flexible bond typer for perception of multiple bonds from atomic coordinates
- Gasteiger partial charge calculation
- Hydrogen addition and deletion
- Isotope support, calculation of average and exact masses
- Feature perception (rings, bonds, hybridization, aromaticity)
- Multiple conformer storage within molecules
- Command line conversion for multiple molecules in one file
- Command line interface
- Bitvector class
- Vector and matrix transformations
- Molecular test suite
- Open-source/Free Software under the GNU General Public License

- Cross platform (Windows, Linux, Mac OS X, SGI, Solaris, Dreamcast...)

Usage: babel (-i<input-type>) <name> (-o<output-type>)

ArgusLab

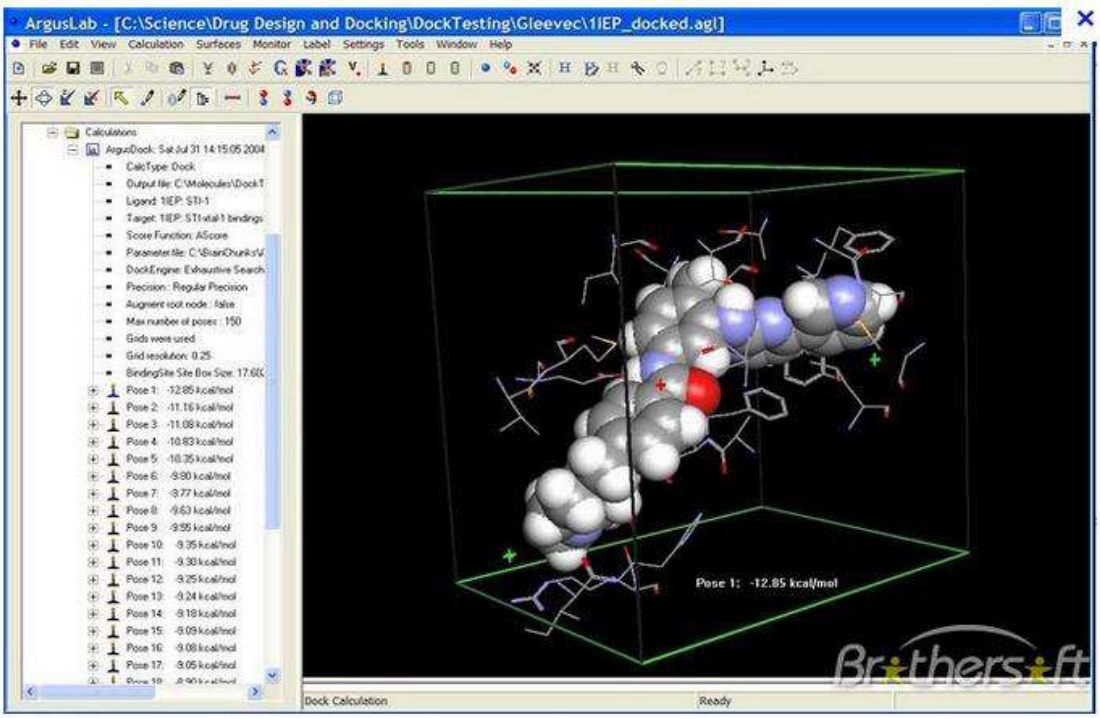


Figure 5: ArgusLab

The ArgusLab Molecule Builder allows to construct new molecules and to modify existing molecules. It is a very useful, highly-featured and easy-to-use molecular modeling, graphics, and drug design program. The program contains two docking engines and a simple scoring function, based on an enhancement of the X-Score method. ArgusLab first generate the scoring grids used during the docking, then the various search phases will occur and finally the candidate poses are processed and the calculation is done. ArgusDock is advantageous in terms of the computational time it provides.

Procedure

Step 1: Open the ArgusLab program.

Step 2: Go to file and open the protein.

Step 3: Select the active site residues in the model.

Step 4: Make a binding site group from the selected residues.

Step 5: Open the inhibitor molecule.

Step 6: Make a ligand group from selected residues.

Step 7: Select the binding site group and ligand group.

Step 8: Size is calculated and grid is generated.

Step 9: Start the Docking process.

Step 10: Save the docked molecule as .Pdb extension.

Receptor-Ligand Interactions

The interactions between a receptor and a ligand were fundamental to drug discovery. ArgusLab provided a set of methods for predicting and analyzing the interactions between protein receptors and ligands. These methods allowed us to carry out structure based

design, or even to examine possible interactions with theoretical structures such as homology models. A common technique central to receptor-ligand interactions was docking.

Dock Ligands (Ligandfit)

The Dock Ligands (Ligandfit) protocol

Docking: During docking, an attempt was made to dock a ligand or series ofligands into a user defined binding site.

In-situ ling and minimization: In this stage, the ligands may be energy minimized in the presence of a fixed or partially flexible receptor.

Scoring

During scoring, various scoring functions may be applied to ligands. The Dock Ligands (Lingand fit) protocol had allowed us to combine docking, minimization, and scoring in one protocol run. Groups of parameters had allowed us to control the three phases of the protocol: docking, minimization, and scoring.

Protein preparation

The ligands and crystallographic water molecules were removed from 108 A protein and the chemistry of the protein was corrected for missing hydrogen. Crystallographic disorders and unfilled valence atoms were corrected using alternate conformations and valence monitor options.

Ligand preparation

The three dimensional structures of the compounds were retrieved from Databases and converted it to .mol format using OpenBabel software suit. The ligand preparation was then carried out by adding hydrogen bonds and lowering energy using CHARMm force field.

Docking Process

Before beginning the docking, it was necessary to specify a binding site of the receptor. Ligandfit uses a method based on protein shape searching for cavities. Often the largest cavity was part of the ligand – binding site.

RESULTS

108 A nephropathy protein retrieved from protein data bank

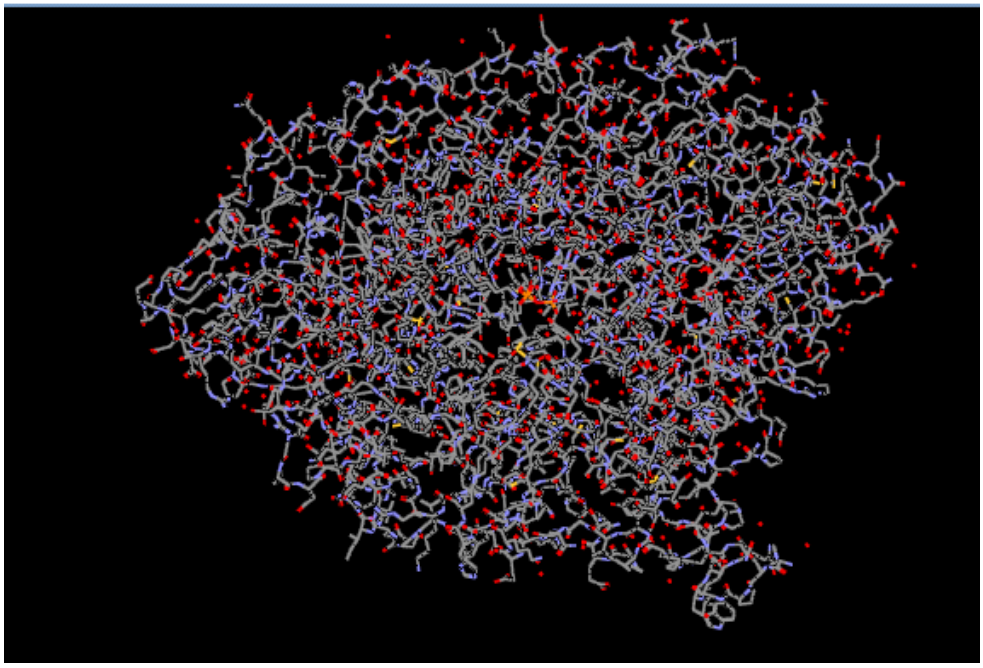


Figure 6: 108 A nephropathy protein retrieved from protein data bank

Structure and molecular formula of compounds for docking

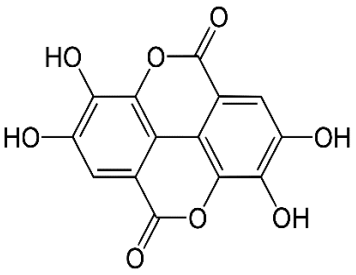
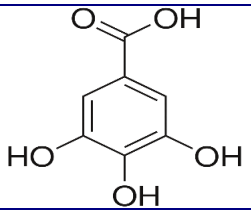
Sl. No.	Compound name	Molecular formula	Structure
1	Ellagic acid	$C_{14}H_6O_8$	
2	Gallic acid	$C_7H_6O_5$	

Table 1: Structure and molecular formula of compounds for docking

Docking of Ellagic acid

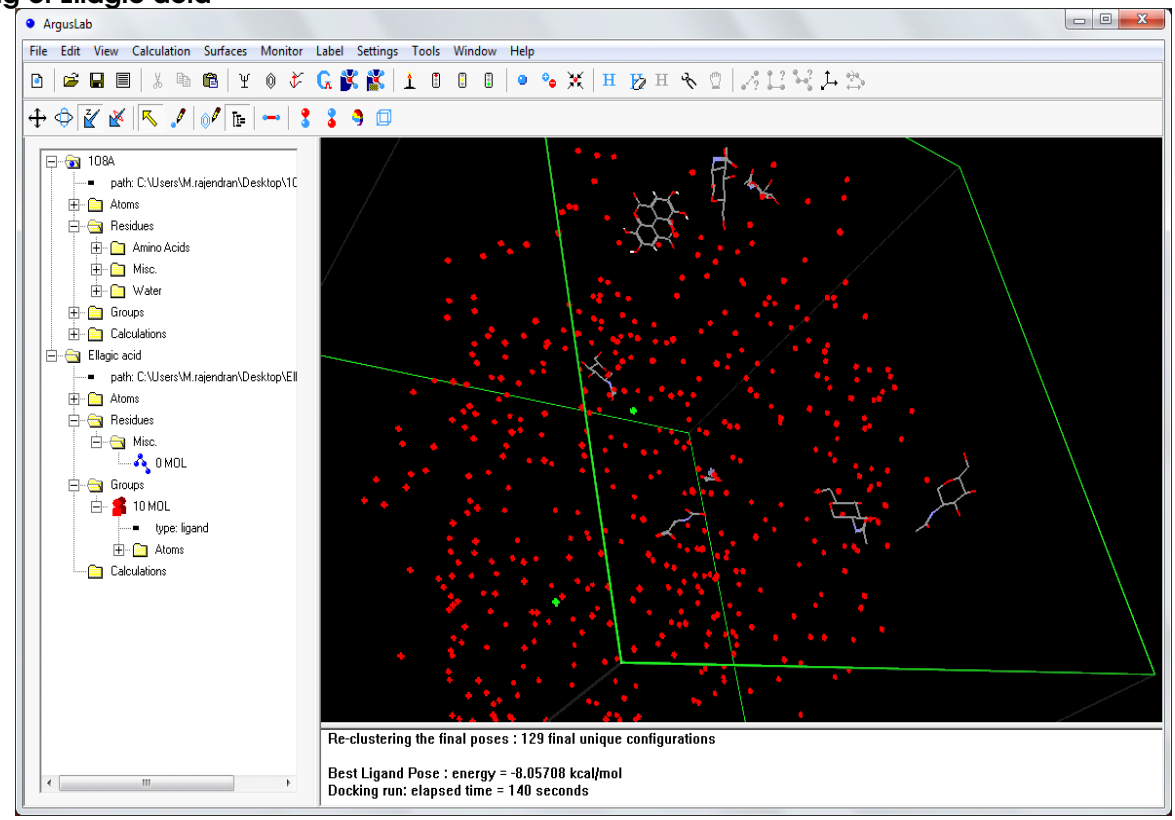


Figure 7: Ellagic acid bind with protein 108 A which represents the docked structure Ellagic acid withprotein108 A and poses involved in this structure was -8.05708 kcl/mol

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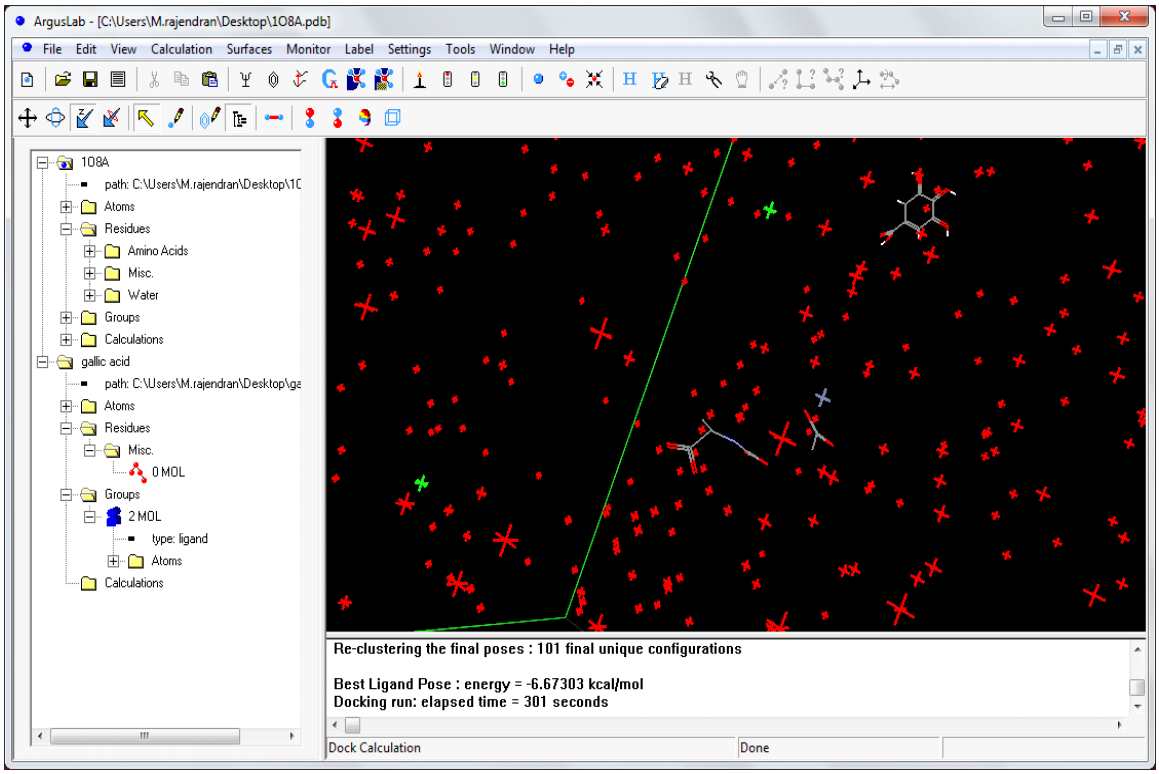

Figure 8: Gallic acid bind with protein108 A which represents the docked structure Gallic acid with protein108 A and poses involved in this structure was -6.67303 kcl/mol

Docking scores of Ellagic acid and Gallic acid

The dock score and other scores were observed, out of which Ellagic acid showed -8.05708kcl/molhighest dock score and the compound Gallic acid showed -6.67303kcl/mol is not higher than ellagic acidbut both Ellagic acid and Gallic acid qualified all the important parameters for being a good protective in diabetic nephropathy for 108 A nephropathy protein.

Table 2: Docking scores of Ellagic acid and Gallic acid

Sl. No.	Compound name	Molecular formula	XLogP3	H-Bond donor	H-Bond Donor	Docking Score
1	Ellagic acid	$C_{14}H_6O_8$	1.1	4	8	-8.05708 kcl/mol
2	Gallic acid	$C_7H_6O_5$	0.7	4	5	-6.67303 kcl/mol

Discussion

Molecular docking is used to predict the active site of the intermolecular complex formed between two or more molecules. The most interesting case is the protein ligand interaction, because of its application in medicine. Ligand is small molecules which interact with protein binding site at the areas of the protein, known to be active of forming of compounds. Drug protein interactions involve one or more of the following types of bonding and the stability of these types of bonds hardly permit the formation of an easily reversible drug receptor complex. An important type of bonding between drug and receptor is a weak and easily broken H-bond. Since many drugs contain hydroxyl, amino, carboxyl and

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carbonyl groups, they can form hydrogen bonds with the receptor complex. Hydrogen bonds are a type of dipole-dipole interaction formed between the proton of a group X-X , where X is electronegative atom and other electronegative atoms (Y) containing a pair of non-bonded electrons. Hydrogen bond is unique to hydrogen because it is the only atom that can carry a positive at physiological pH while remaining covalently bonded in molecules[15].

Chronic diabetes mellitus causes multiple complications like diabetic nephropathy and premature mortality, accountingfor atleast 10% of total health care expenditure in many countries[1].Screening methods were routinely and extensively used to reduce the cost and time of ArgsLab. It has been clearly demonstrated that the approach utilized in the docking study was successful in finding novel diabetic nephropathy inhibitors from the phytoconstituentsEllagic acid and Gallic acid of seeds of *Eugenia jambolana*. The plant compounds that targeted the 108 A diabetic nephropathy protein were screened and ranked based on their dock score. The Lipinski prediction helped in the identification of more suitable ligand towards target protein. The dock score and other scores were observed out of which Ellagic acid showed -8.05708 kcl/molhighest dock score. (Table No:2, Figure No:7) Lipinski rule of five was used as a first step filter to perform virtual screening of compound libraries, in an effort to quickly eliminate lead candidates that have poor physicochemical properties for oral bioavailabilty. However the compound Gallic acid showed -6.67303 kcl/mol dock score, was not higher than Ellagic acid(Table No:2, Figure No:8)but both Ellagic acid and Gallic acid qualified all the important parameters for being a good protective for diabetic nephropathy[15].

Conclusions

In this investigation, the molecular docking studies of Ellagic acid and Gallic acid showed a bonding score of -8.05708 Kcl/mol and -6.67303 Kcl/mol, for 108 A nephropathy protein. Therefore, this investigation concluded that phytoconstituents, Ellagic acid and Gallic acid of seeds of *Eugenia jambolana* may be used as nephroprotective for chronic diabetes mellitus patients to prevent the nephropathy complications in diabetic populations, after confirming its efficacy and safety in well-controlled clinical trials. If it is confirmed in humans, Ellagic acid and Gallic acid may be a potent, safe and cost effective phytomedicine to prevent nephropathy-induced premature death in diabetic patients.

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