COMPARATIVE *INSILICO* DOCKING STUDIES OF RUTIN AS DIABETIC INHIBITOR AGAINST PROTEIN TYROSINE PHOSPHATE

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ABSTRACT:

Rutin, a bioflavonol compound was one of vital nutritional component present in many medicinal plants and exhibit a several pharmacological property. Diabetes is one of the most serious metabolic disorders across the world which affects all age groups. According to World Health Organization (WHO), more than 230 million individuals were affected worldwide by diabetes; they also expected that it will reach upto 350 million by end 2025. Currently in India more than 62 million individuals were diagnosed by diabetes, therefore there is an urgent need for the discovery of novel natural anti-diabetic drugs without any side effects. The present study was designed in order to evaluate its binding efficiency with anti-diabetic protein target protein tyrosine phosphates (PTP) through *in silico* studies. The function of Insulin receptor, the protein tyrosine phosphatase was to the remove the phosphate group from the tyrosine amino acid which was present in the functional region thereby decreasing the production of insulin. The crystal structure of protein tyrosine phosphate was selected as target from the protein data bank and further docking studies were performed using Argus lab software. Among the 14 protein structure of protein tyrosine phosphatase, the compound rutin exhibited significant binding energy with 2BO7 and also exhibited the better binding affinity with other protein structures. The future studies could be designed accordingly to highlight the efficiency of rutin towards drug development in the treatment of diabetes.

Keywords Diabetes, Rutin, Protein Tyrosine Phosphates, Molecular Docking.

1. INTRODUCTION

Natural small molecules Rutin was one of important flavonoid (plant pigment) compound found in various parts of several medicinal plants especially in leaves [1, 2]. The major plant sources of compound rutin are as follows: *Elderflower cordial, Eucalyptus macrorhyncha, Fagopyrum esculentum, Ginkgo biloba, Hypericum perforatum* and *Malus pumilai*. In ancient days all the above parts of plants were used as the medicine in treating various diseases and on analysing the reason for medicinal properties of those plants, it was observed the compound rutin was responsible for its therapeutic properties [3, 4]. Through various literature survey on rutin revealed that the flavonoid compound exhibit several pharmacological properties like antioxidative activity, anti-inflammatory effects, anticonvulsant potentials, neuroinflammation, anti-cancer activity and anti-Alzheimer activity [5].

In the 21st century. Diabetes mellitus is considered to be an important challenging health problem across the world [6]. The latest report on Diabetes stated that especially in developing countries more than 70 million people were suffering from any one type of Diabetes [7]. Diabetes is a chronic metabolic disorder with altered carbohydrate, lipid and protein metabolism due to insufficient amount of insulin secretion in the human body [8]. The abnormal glucose level in the blood was mainly due to the insulin hormone produced by the organ pancreas, whether the body does not produce enough hormones or cells present in the human body do not react to the hormone [9].

Insulin is the key hormone which promotes the uptake and storage of glucose from blood to cells of the body especially liver, adipose tissue and muscle except smooth muscle [10]. Hence the deficiency of insulin secretion in the body leads to all manifestations of diabetes mellitus. The occurrence of diabetes mellitus is a mystery, even though environmental and genetic changes play a vital role [11].

In the case of the insulin receptor, the protein tyrosine phosphatase is responsible for the removal of phosphate from the tyrosine residues in the regulatory domain, hence inactivation of the receptor is unknown [12]. Protein tyrosine phosphorylation is mediated by protein tyrosine kinases (PTK) whereas the removal of phosphate is carried out by protein tyrosine phosphatases. PTP like PTK can serve both positive and negative roles in the modulation of cell function. PTP1B was the first protein tyrosine phosphatase to be purified and characterized [13].

Now-a-days pharmaceutical companies uses a variety of computational methods i.e Bioinformatics tools and databases to select the drug candidates based on its efficacy and safety level and also progress that molecule into clinical trial candidates [14, 15]. Docking technique is one of the most important and frequently used methods in structural - based drug designing, which predict the binding affinity of small molecules to their applicable target binding sites there by inhibiting the target functions [16]. Molecular docking is extensively used to predict the conformation of a receptor - ligand complex where receptor is usually protein or nucleic acid and ligand is usually small molecule or another protein [17].

The docking analysis were consider to very essential in selecting the specify drug lead candidate against the target. The purpose of the current study is to investigate the small molecule rutin as diabetic inhibitor against insulin receptor (Protein Tyrosine Phosphate) through *in silico* docking analysis.

2. MATERIALS AND METHODS:

2.1 Preparation of Ligand:

The pubchem database (https://pubchem.ncbi.nlm.nih.gov/) contains collect informations about the chemical structures and its properties, biological activities, safety and toxicity of compound, etc. The two dimensional structure of the Rutin was downloaded in .sdf file format from Pubchem databases and converted to .mol file format using Pymol software and now the compound Rutin was ready for docking process [18].

2.2 Accession of target protein:

The protein data bank (PDB) database (https://www.rcsb.org/) contains a collection of 3D structure and sequence of macromolecules like proteins and nucleic acids. The 14 different 3D receptor structures of Protein Tyrosine Phosphate with its PDB IDs (1C87, 1EEN, 1KAK, 1L8G, 1LN9, 1ONZ, 1PXH, 2AZR, 2BO7, 2CMB, 2F6W, 2H4G, 2H4K, 2VEU) were retrieved [19]. Except chain A, other chains present in the receptors, the metal ions bound to the receptor molecules, water molecules present in the structure and finally the heteroatoms were removed from the 14 3D structures of the receptors using PyMol Software [20].

2.3 Analysis of target active binding sites

The active sites of 14 receptors were predicted through metapocket database (http://projects.biotec.tu-dresden.de/metapocket/). The database predicts the possible ligand binding regions on receptor surface for protein-ligand interactions [21]. The predicted regions were considered as the binding sites for the compound Rutin.

2.4 Docking of receptors with ligand:

A computational receptor-ligand docking studies were preformed to analyse the structural complexes of the Protein Tyrosine Phosphates (receptor) with Rutin (ligand) in order to diagnose the specify structural relationship with the ligand. Protein Tyrosine Phosphates receptors were docked with rutin using Argus Lab 4.0.1 docking software program [22]. The following parameters were set for docking process 1) Population size - 50, 2) Grid resolution - 0.35 Å, 3) Binding site box size - 17.137 × 18.5 × 16.5 Å, 4) Maximum generation - 1,000, 5) Crossover rate - 0.8, 6) Mutation rate - 0.2, 7) Elitism - 5, 8) Dock engine used Lamarckian Genetic Algorithm (GA Dock). For predicting the docking calculations, the parameter should be was set as “Dock” and “Flexible” ligand docking mode for each docking run [23].

2.5 Analysis of docking result:

Finally the docking interaction between receptor and rutin were explored through PyMOl software. PyMoL is one of the important molecular visualization software which was specifically used to visualize binding interaction between small molecules and protein. The software also predict the distance of hydrogen bond formations between ligands and receptors, which clearly revealed the stability of inhibition of ligands towards target [24].

3. RESULT AND DISCUSSION

3.1 Structure of the ligand and target proteins:

The two dimensional structure of the Rutin with its pubchem ID: 5280805, Chemical formula: $C_{27}H_{30}O_{16,}$ Molecular Weight: 610.521 g/mol were retrieved (Figure 1). The three dimensional structure of 14 different targets were retrieved from the Protein Data Bank and all the structures were determined by X-Ray crystallography. The best ligand binding site was predicted for 14 proteins using Metapocket were tabulated in Table 1.

Figure1: Two dimensional structure of the Rutin

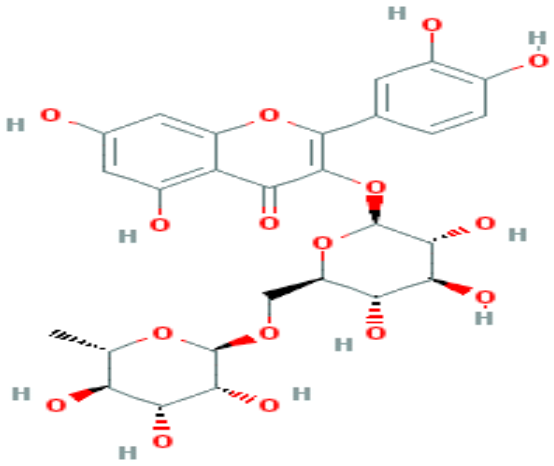


Table 1: Predicted active sites of protein tyrosine phosphate using Metapocket.

S.No	Pubchem ID of PTP	Resolutions	Active Site of the protein PTP
1.	1C87	2.1 Å	ARG 24, HIS 25, ALA 27, SER 28, ASP 29, ASP 48, ILE 219, ARG 254, MET 258, GLN 262.
2.	1EEN	1.9 Å	ALA 17, SER 28, ASP 29, PHE 30, TYR 46, ARG 47, ASP 48, VAL 49, PHE 52, ALA 217.
3.	1KAK	2.5 Å	TYR 20, GLN 21, ARG 24, HIS 25, GLU 26, ALA 27, SER 28, ASP 29, ARG 254, GLN 262.
4.	1L8G	2.5 Å	TYR 46, ASP 48, LYS 120, ASP 181, SER 216, ALA 217, ILE 219, ARG 221, GLN 262, MET 258.
5.	1LN9	-	TYR 46, VAL 49, GLY 183, CYS 215, ALA 217, ILE 219, GLY 220, ARG 221, GLN 262, GLN 266.
6.	1ONZ	2.4 Å	LYS 116, TRP 179, GLY 183, VAL 184, ILE 219, ARG 221, GLN 262, THR 263, ASP 265, GLN 266.
7.	1PXH	2.15 Å	TYR 46, ASP 48, VAL 49, SER 50, LYS120, PHE 182, SER 216, ALA 217, ILE 219, MET 258.
8.	2AZR	2 Å	TYR 46, VAL 49, LYS 120, GLU 115, ASP 181, PHE 182, SER 216, ALA 217, ARG 221, CYS 215.
9.	2BO7	2.95 Å	SER 105, PRO 130, GLU 213, GLY 214, LYS 215, ALA 216, HIS 217, HIS 262, ILE 263, ARG 266.
10.	2CMB	1.7 Å	TYR 46, ASP 48, VAL 49, ASP 181, PHE 182, SER 216, ALA 217, ILE 219, GLY 220, GLN 262.
11.	2F6W	2.2 Å	GLU 26, ALA 27, SER 28, LYS 73, MET 74, GLU 75, ALA 77, THR 230, LEU 251, GLU 252.
12.	2H4G	2.5 Å	ARG 45, TYR 46, VAL 49, LYS 120, ASP 181, PHE 182, SER 216, ALA 217, ARG 221, CYS 215.
13.	2H4K	2.3 Å	ARG 45, TYR 46, VAL 49, GLU 115, LYS 120, ASP 181, PHE 182, SER 216, ALA 217, ARG 221.
14.	2VEU	2.4 Å	TYR 20, GLN 21, ARG 24, ALA 27, SER 28, ASP 29, PHE 52, ARG 254, MET 258, GLN 262.

3.2 Docking analysis and its binding interaction between Protein Tyrosine phosphate with rutin:

The above predicted active residues of the 14 proteins were used as the catalytic sites for small molecules rutin for docking analysis. The results of the docking interaction between the binding site residues of target protein tyrosine phosphate and rutin compound were shown in the Table 2.

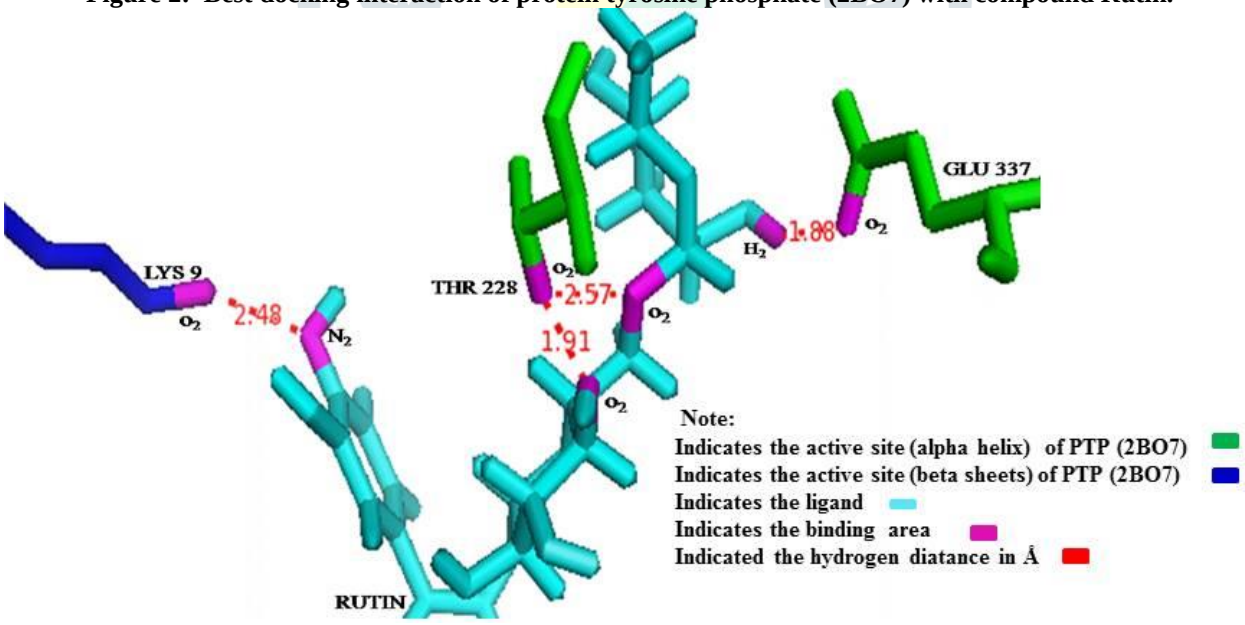
Table 2: Docking energy and binding information of protein tyrosine phosphate with rutin.

PDB ID	Docking Energy With Rutin Kcal/Mol	Binding Information		
		Amino Acid	Bond Type (D-H…A)	Distance (Å)
1C87	-6.16042	GLY 220 LYS 120 TYR 46 ASP 181	N…O N…O C…O C…O	2.7 2.6 2.8 2.6
1EEN	-6.13253	GLN 262 GLY 259 ARG 254	N…O N…O N…O	3.0 3.0 2.9
1KAK	-7.26523	GLN 262 GLY 259 ARG 254	N…O N…O N…O	2.3 2.8 2.1
1L8G	-6.67647	SER 118	O…O	2.6
1NL9	-6.73001	LYS 116 GLN 266 ARG 22 ASP 48	N…O N…O N…O O…H	2.3 2.9 2.8 2.3
1ONZ	-6.7035	LYS 116 GLY 183 TRP 179 GLN 266 ILE 219	N…O N…O N…O N…O N…O	2.7 2.8 2.8 2.5 1.9
1PXH	-6.4756	NO INTRACTION		

2AZR	-6.6115	GLY 86 LYS 120 ASP 181 GLY 220	N…O O…H O…H O…N	3.0 2.4 2.3 3.0
2BO7	-10.0019	GLU 337 THR 228 LYS 9 THR 228	H...C O…O O…N O…O	1.8 2.5 2.4 1.9
2CMB	-6.18854	ARG 257 LYS 120 TYR 46 TYR 46	N…O N…O O…O O…O	2.9 2.6 2.6 2.5
2F6W	-6.94722	LYS 255 LYS 73 GLU 75	N…O N…O N…O	2.9 3.0 2.7
2H4G	-6.961	GLY 220 PHE 182 GLN 262	N…O N…O N…O	2.6 2.5 2.9
2H4K	-7.57221	LYS 120 TYR 46 LYS 120 SER 216	N…O O…O N…O N…O	3.0 2.9 2.7 2.8
2VEU	-6.61293	ALA 27 ARG 254 TYR 20 GLN 262	O…H N…O O…O N…H	2.3 2.9 2.3 2.9

In general, the higher negative value of docking score predicted between receptor and ligand expected to hold more binding affinity towards each other especially through hydrogen bonding interaction [25]. By analysing the docking result it was revealed that protein 2BO7 exhibited the higher negative value of -10.0019 Kcal/mol for rutin with strongest hydrogen bond interactions which indicates better binding affinity with the active sites (LYS 9, THR 228, GLU 337) of protein there by strongly inhibiting the function of the protein (Figure 2). The other 13 docking interaction values ranges from -6.1 Kcal/mol to -7.5 Kcal/mol indicates the good binding affinity towards rutin and also exhibits the strong hydrogen bond interactions.

Figure 2: Best docking interaction of protein tyrosine phosphate (2BO7) with compound Rutin.



Thus all the 14 docking results and binding interaction were evaluated and finally reported that protein 2BO7 exhibits the best binding interaction with the compound rutin and other 3D structure proteins also exhibit the better binding interactions. The previous molecular docking studies of small molecules rutin on Matrix Metalloproteinase [26], as Galectin-1 Inhibitors [27] also predicted the similar binding values > -7 Kcal/mol and they also concluded that the rutin exhibited the better inhibitory activity of towards specific proteins. The result of current study revealed that the compound rutin may act as good inhibitors for the diabetic protein tyrosine phosphate. The results of docking study could be useful for the designing of novel antidiabetic drug with small molecules rutin.

4. CONCLUSION:

The molecular docking analysis was performed to explore the structural binding mechanism between receptor and ligand. The current *insilco* research on rutin as the diabetic inhibitor concluded that the compound might act as the novel chemical inhibitor for protein tyrosine phosphate there by increasing insulin secretion in the human body. Further *invivo* animal model studies have to be executed to confirm this current study and in future rutin molecule may possibly expected to develop as the one best diabetic inhibitor.

5. CONFLICT OF INTEREST:

The authors declare they have no competing interests.

6. ACKNOWLEDGEMENT:

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