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Study of the Effect of Gedunin against Protein-GTP-Binding Protein RAD to Treat Diabetes: An *In-Silico* Approach

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ABSTRACT

Diabetes mellitus is an autoimmune disorder which causes impairment in the insulin secretion or resistance. The prevalence of Diabetes is increasing day by day worldwide, also this metabolic disorder diseases is associated with high risk of cardiac problem, obesity and many other diseases. Although, there are different medications and treatments are available but still they have proven to be ineffective and associated with severe adverse effects for long term. Therapeutic compounds based on herbal phytochemicals are considered as the most prominent for the treatments. The computational study helps in finding the structural basis analysis of the compounds by rapid screening tools with rational drug designing. This study aims to identify the natural inhibitor of Rad-GTP binding protein to treat Diabetes dysfunctionality.

Therefore, by searching the natural compounds against Rad-GTPase binding protein with 2DPX structure, all the important data was retrieved by using online webservers followed by computing docking score in AutoDock Vina. Out of three selected compounds, Gedunin was used to dock with the protein 2DAX protein structure, as it follows the both criteria for proceeding further, one is to follow lowest binding criteria then ADME (Absorption, Distribution, Metabolism and excretion). The docking score of Gedunin compound shows that, this bioactive agent can go against the Diabetes mellitus and inhibits the expression of Rad-GTPase. By result Gedunin has shown its therapeutic role with -7.9 kcal/mol value. It has been concluded that in future Gedunin may act as a novel drug target.

KEYWORDS: *Diabetes, Rad-GTP ase, herbal phytochemical and Drug discovery*

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I. INTRODUCTION

Diabetes mellitus generally known as diabetes is a metabolic disease which causes high blood sugar level. About 463 million people out of total world population suffers from diabetes. The region of North America and Africa has emerged as the hotspot of diabetes. The spreading rate of diabetes among the young age nearly (10.9%) and Western Pacific region has recorded highest number of young age people suffering from diabetes.

Type of diabetes

Type 1: It is an autoimmune disease condition where immune system destroys own cells in the pancreas where insulin is produced. As a result, less or no insulin is produced.

Type 2: In this type of diabetes body becomes resistant to insulin. Either the body does not make insulin or use it well. This can be developed at any age but is most common among middle aged and older people.

Type I diabetes (insulin dependent) is caused due to insulin insufficiency because of lack of functional beta cells. Patients suffering from this are therefore totally dependent on an exogenous source of insulin while patients suffering from Type II diabetes (insulin

independent) are unable to respond to insulin and can be treated with dietary changes, exercise and medication [2]. Type II diabetes is the more common form of diabetes, constituting 90% of the diabetic population.

During the past few years diabetes has gained a lot of attention among the researchers because of the highest prevalence of diabetes. Some of the diabetes patients do not show any symptoms mainly with type 2 diabetes. The symptoms of diabetes include: -

- Blurry vision.
- Extreme fatigue.
- Increased hunger and thirst.
- Poor muscle strength.
- Frequent urination.

GTP -binding protein Rad

RAD is a member RAS family. It is a GTPase and have low molecular weight. RAD is over expressed in the type 2 diabetes and its over expression in the muscle cells results in reduced uptake of glucose.

Molecular Docking is a crucial tool that plays a major role in docking procedure. The main objective of performing docking is to find out major modes of binding ligand with the target protein. Molecular docking helps us to understand biomolecular interactions between compounds for rational drug discovery. The method helps us in predicting the best binding of ligand molecule with target protein.

The objective of this study is to examine active sites and domains of GTP binding protein RAD to perform autodocking of target protein with selected ligands and analyse the therapeutic effect of ligands against target protein RAD.

II. METHODOLOGY

A. Identification of Protein

The protein was downloaded from the Protein Data Bank (PDB) database, which is a freely available software used for finding out the detailed information of the protein data and structures. In this research work Rad-GTP binding protein was used to target the Type 2 Diabetes Mellitus (T2DM). The protein was chosen on the basis of extensive data collection from the literature. The structure of Rad -GTP binding protein was retrieved in. pdb format from protein data bank RCSB.

B. Preparation of Ligand compounds

After studying the literature three phytochemical compounds were selected for the Molecular Docking study with the Rad-GTP binding protein structure which was downloaded earlier in 2DPX. The selected compounds were as follows: -

1. Gedunin
2. Aglycone
3. Harman

These all are the herbal natural inhibitors of the protein which causes non- union fracture in diabetes patients as well. The ligands were generated by downloading all the compounds in. sdf format with 3D conformation from the PubChem data base.

All the important information available of the PubChem was gathered and utilized later for identifying the detailed information of the herbal compounds. Afterwards, the. sdf format files of the ligands were converted into the. pdb format from the online available tool SMILES TRANSLATOR.

C. Virtual Screening through PyRx software

PyRx is the software that is used to screen ligand molecule with the target protein and helps in finding out binding affinity, number of atoms and their E values. This software runs only on '.pdbqt' format. At first, in PyRx software graphical window downloaded protein was uploaded into. pdb format. Then, it was converted into the. pdbqt format my translating into macromolecule. Furthermore, after this all the ligands compounds were imported into the. sdf format and their energy was minimized followed by converting all the ligand molecules into the. pdbqt format as well.

Vina wizard section was opened to select all the. pdbqt files of the ligands and protein macromolecule. Programme was run to analyse the values of the binding affinity of individual value with RMSD lower bound and upper bound values.

D. Drug ADME property analysis

The compatibility of the ligand molecule to act as a drug depend on several factors like its Adsorption activity, Distribution, Metabolism and Excretion, they altogether makes ADME abbreviation. For analysing the value and checking the lipinski rule of Five, SwissADME server was used, which computed the values of Hydrogen donor, acceptor, molecular formula and other properties as well. It also shows the violations factor of Lipinski rule of Five.

Therefore, SwissADME is an online web server. It uses the SMILE notations that are copied from PubChem. It was used to analyse Drug likeliness property. Selected ligands were analysed for Lipinski's rule of five. Lipinski's rule of five states the following points: -

1. Hydrogen bond donors mustbe less than 5.

2. No. of Hydrogen bond acceptors must be less than 10.
3. The molecular weight should be less than 500 Dalton.
4. Partition co-efficient Log P must be less than 5.
5. Not more than 1 rule can be violated.

The ligands that followed the above Lipinski's rule of five were selected for autodocking on AutoDock vina tool.

E. Molecular Docking through AutoDock vina software

For molecular docking of protein and the ligand molecule, initially the target protein in '.pdb' format was loaded on the homepage of AutoDock. The target protein was prepared for docking by removing water molecules, then adding hydrogen polar atoms thereafter by adding kollman charges in the protein molecule. After this, the protein was selected and was saved in '.pdbqt' format. Then, the selected ligand was loaded on homepage of AutoDock, the ligand molecule was also converted into. pdbqt format, which is followed by again adding the protein file i., 2DAX into the same window. But, this time protein. pdbqt file was selected. The Grid box was selected for the region where docking was performed and grid box provides the dimensions values of the docking region and simultaneously config, grif file were also saved into. txt format.

Command prompt (cmd) was opened for giving the commands into the python cell and docking score was generated in nine different modes.

F. Visualization by PyMol 2.5 software

PyMOL is a tool which is used to visualize the molecular surface of the docked protein. In other words, structural visualization was done with the help of this tool. The output in '.pdbqt' format was also uploaded and the result was runned in PyMOL. Molecular surface of the output wasvisualized.

III. RESULT AND DISCUSSION

The protein RAD was downloaded in. pdb format from Protein Data Bank as shown in Figure 1 and additional details of RAD in Figure 2. RAD belongs to signalling protein class, the resolution of protein is 1.80Å, method used is X-ray diffraction. Selected ligands were retrieved from PubChem and were downloaded in ".sdf". By using online SMILEthe downloaded structure was converted into ".pdb" format. 2-D and 3-D structure of ligands are shown in Figure 2 (a),(b),(c), and figure3(a),(b),(c).

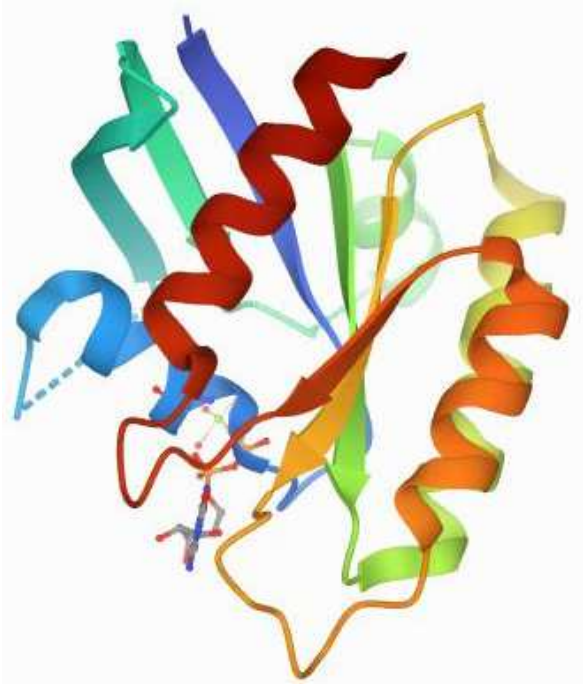
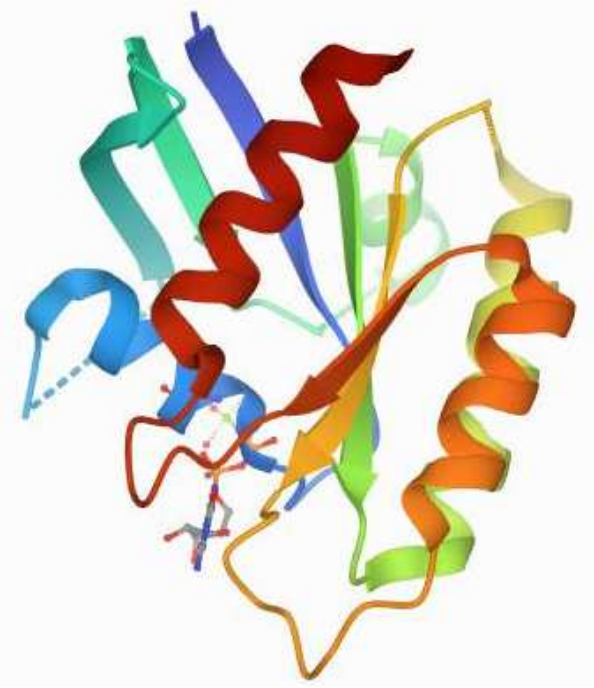
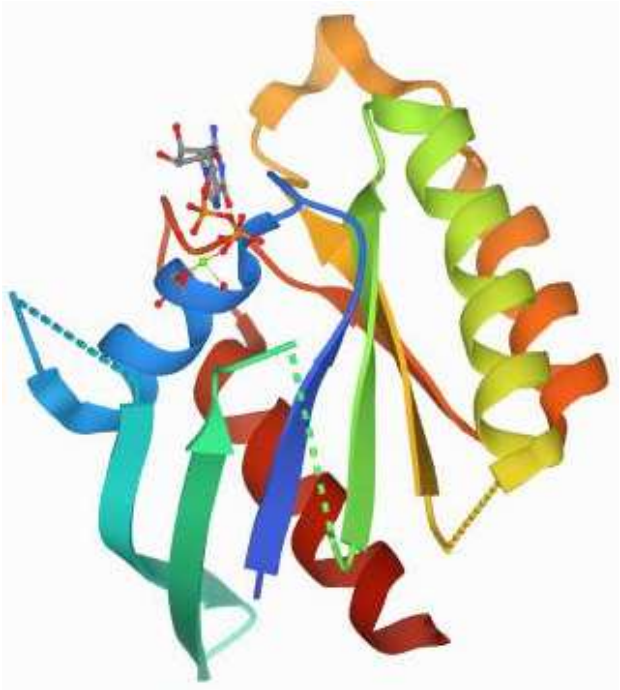


Figure 1: the crystal structure of human RAD GTPase

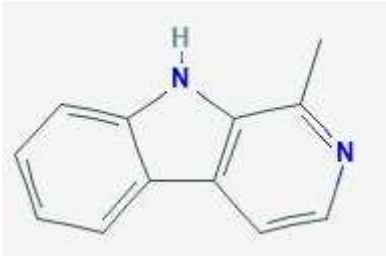
- Name of protein - GTP -bindingProtein RAD
- Gene –RRAD, RAD
- Protein Databank no –2DPX
- Classification- signalling protein
- Organism(s)- *Homosapiens*
- Expression system –*Escherichia coli*
- Mutation- no
- Length of sequence - 174



Biological Assembly 1

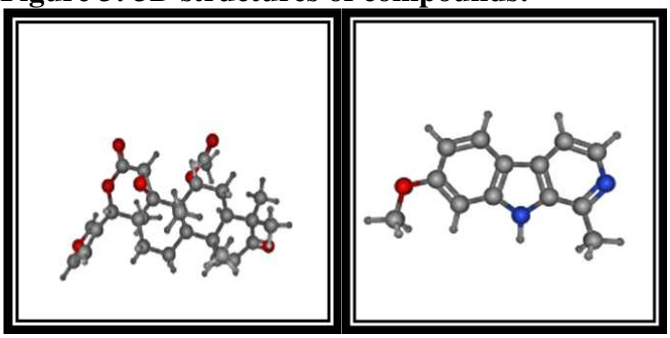


Biological Assembly 2



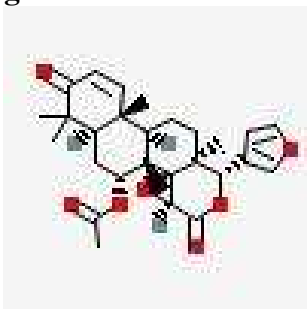
3. Harman

Figure 3: 3D structures of compounds:

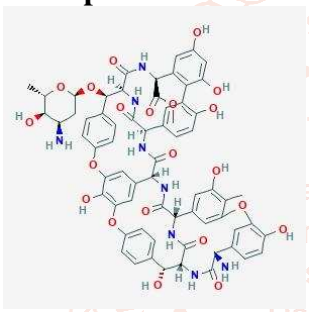


1. Gedunin. 2.Harman

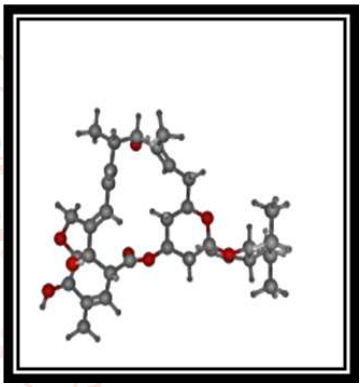
Figure 2: 2D structures of compound



1. Gedunin.



2. Aglycone



3. Aglycone

LIGANDS	PCID	MOL.F	MOL.WT
Gedunin	12004512	$C_{28}H_{34}O_7$	482.6 g/mol
Aglycone	16131419	$C_{34}H_{48}O_8$	584.7 g/mol
Harman	5281404	$C_{13}H_{12}N_2O$	212.25 g/mol

Table.1 Detailed information of the ligand compounds from PubChem

Ligand Name	Another name	Pubchem CID	M.weight (g/mol)	H-Bond Donor	H-Bond acceptor	ILogP Value	Lipinski rule
Gedunin	Gedunin	12004512	482.6	0	7	4.2	Yes
Harman	Harmane	5281404	182.22	1	1	3.6	Yes
Aglycone	Ristocetin psi-aglycone	16131419	1303.2	16	23	1.9	No

Table.2 Drug likeliness Property analysis by SwissADME

Virtual screening of all three ligands Gedunin, Harman and Aglycone were done through Pyrx software.

Ligand molecules	Binding energy
Gedunin	-7.9
Harman	-6.3
Aglycone	-8.1

Table.3 Binding energy of the compounds by PyRx software

After reading the PyRx results, binding energy of selected ligands are concluded and noted in Tabular form. Then after these molecules were runned in SwissADME for inspection of Drug likeliness property

Name of ligand	M. weight	H.bond donor	H.bond acceptor	Partition coefficient MlogP	Violation
Gedunin	482.6g/mol	0	7	2.56	Yes; 0 violation
Harman	182.22g/mol	1	1	1.90	Yes;0 violation
Aglycone	1303.2g/mol	16	23	-3.46	No;3 violation

Table.4 Swiss ADME result analysis

The docking of Gedunin together with Rad was done using Autodock Vina software. According to Autodock Vina result gedunin showed greatest binding against RAD protein as shown in table 5. The interaction between gedunin and Rad was visualized through PyMOL tool.

Mode	Binding affinity(kcal/mol)	RMSD upper bound	RMSD lower bound
1.	-7.9	0	0
2.	-7.7	7.599	2.533
3.	-7.6	3.929	2.547
4.	-7.4	7.3	1.823
5.	-7.3	4.214	2.344
6.	-7.3	4.583	2.283
7.	-7.3	7.615	2.664
8.	-7.2	5.734	3.182
9.	-7.2	17.401	13.843

Table.5 AutoDock Vina Result

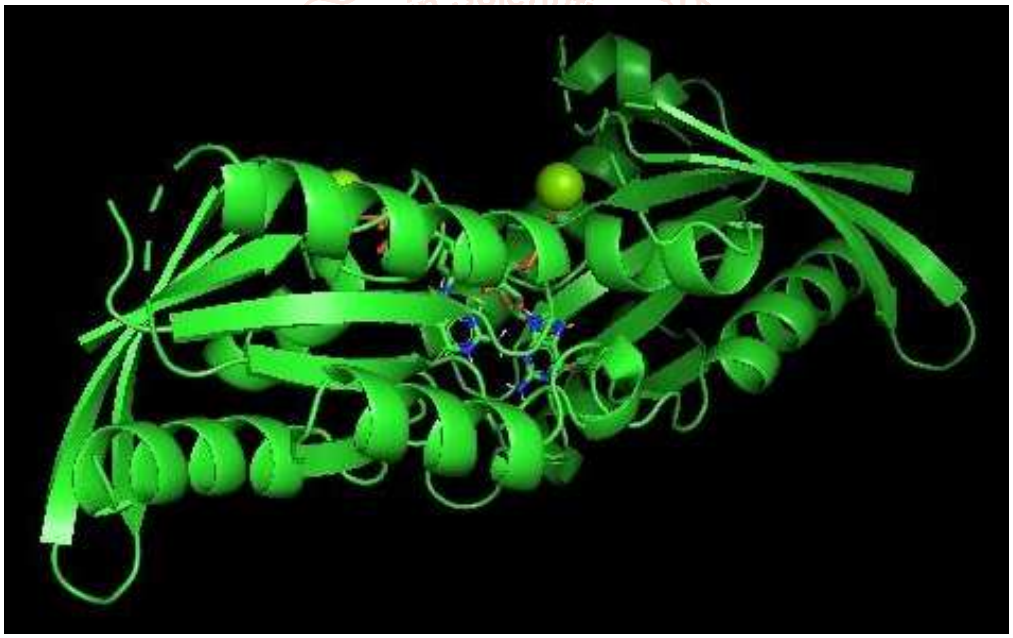


Fig.4 Result visualization in PyMOL

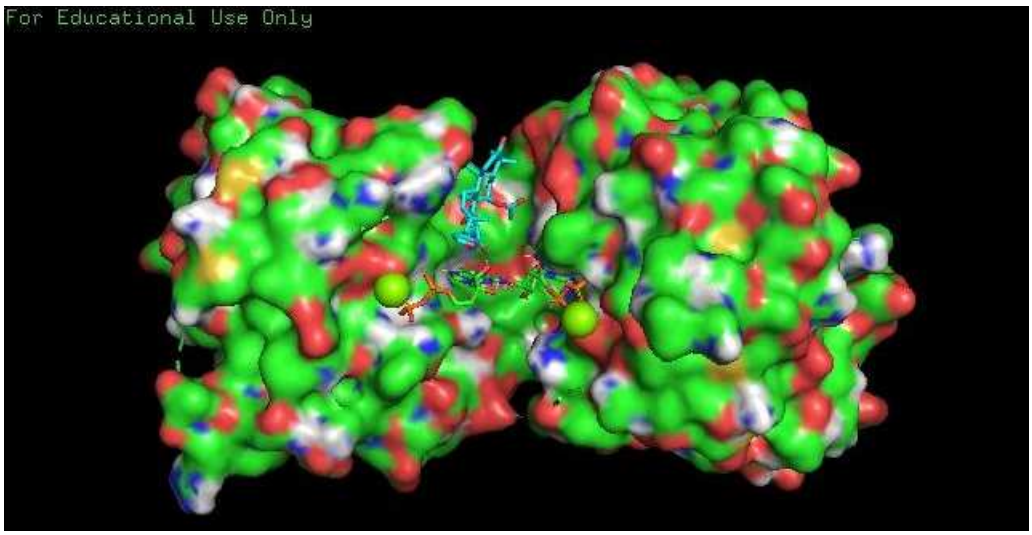


Fig.5 Interaction of RAD with Gedunin through PYMOL visualizer

CONCLUSION

As per*insilico* analysis, gedunin may act as inhibitor and can be used as a drug in treatment of diabetes. Therefore, this drug may play an effective role in the treatment of diabetes. Strong affinity of gedunin had been shown towards target protein of diabetes through molecular docking studies.

CONFLICT OF INTEREST

The author declare that there is no conflict of interest

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