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# "*In Silico* Study of the effect ofMyrcene against the Glycodelin Protein in the treatment of Retinopathy-a diabetes mellitus complication"

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**Abstract**

Diabetes mellitus is a metabolic disease which has 422 million cases around the world in which 1.6 million people were died annually by the same. This disease makes the body enable to make insulin or can't use it effectively. Diabetes mellitus has two types type 1 & type 2 which is a autoimmune disease and body becomes resistant to insulin respectively. Diabetes mellitus(DM), also known as diabetes, is a metabolic disease caused high blood sugar in the blood. Retinopathy, nephropathy, and neuropathy are some complications which associated with the diabetes mellitus complications. In this study we deal with retinopathy, it is a disease of retina. Retinopathy is a eye disease in which blood vessels which damaged by the diabetes. So, the current research was carried to study the effect of Glycodelin on the target protein molecule with the help of molecular docking to treat the retinopathy which is complication associated with diabetes. Therefore, different compounds or ligands-Alpha-Amyrin acetate, Myrcene, Vascine, Rutin and Quercetin were selected for the treatment of retinopathy. To screen the Glycodelin target protein with ligand compounds using computer aided molecular modelling. Ligand with the target protein was docked using molecular docking software. The protein structure was retrieved by online databases and molecular docking of Myrcene (CID: 31253) compounds with Glycodelin protein was performed by AutodockVina. This it is remarkable to consider the ligand for further validation and future process for the development of drug for the treatment of retinopathy. Therefore, ligand can be used for further study through in vivo and in vitro studies.

**Keywords:** Autoimmune Disease, Diabetes Mellitus, Glycodelin, Molecular Docking.

## Introduction

Diabetes mellitus is a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both(1). There is not just a single factor but several pathogenic processes which are involved in the development of diabetes. Type 1 have autoimmunity disease and type 2 is a resistance to insulin action.Insulin deficiency in turn leads to chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism(6).Diabetes mellitus is not treated fully but can be controlled by combination of diet and exercise [non-pharmacological], or diet with herbal or oral hypoglycaemic agents or insulin [pharmacological]. There are some complications which are specific to diabetes include are retinopathy, nephropathy, and neuropathy.Patients with all forms of diabetes of sufficient duration, including insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM), are vulnerable to these complications, which cause serious morbidity(7).

People with Diabetes mellitus are about 422 million worldwide in which 1.6 million people died by the same according to the World Health Organization. Both the number of cases and of diabetes have been s increasing over the past few decades. Diabetes is a chronic, metabolic disease in which body has high levels of blood glucose. The diabetes mellitus cause serious damage to the heart, blood vessels, eyes, kidneys and nerves over the time. Thecommon symptoms which found in diabetic patients are increased hunger, increased thirst, weight loss, frequent urination, blurry vision, extreme fatigue, sores that don't heal. In Men who has diabetes may face a decreased sex drive, erectile dysfunction (ED), and poor muscle strength. Where as in women with diabetes has symptomssuch as urinary tract infections, yeast infections, and dry, itchy skin.

**Type 1 diabetes,**also known as juvenile diabetes or insulin-dependent diabetes. It is aautoimmune diabetes in which body attacks itself to destroy the β-cell which are responsible for the insulin synthesis. It is a chronic disease in which body face a deficiency of insulinbecause of pancreatic β-cell loss and which further leads to hyperglycaemia. Extreme hunger, increased thirst, Unintentional weight loss, Frequent urination, Blurry vision, Tiredness are some common Symptoms found in type 1 diabetes patients. Type 1 DM is not completely understood yet, but the pathogenesis of the disease is thought to involve T cell-mediated destruction of β-cells(11).

**Type 2 Diabetes,** has common symptomswhich areincreased hunger, increased thirst, increased urination, blurry vision, tiredness, sores that are slow to heal. The two factors which contribute to the multiple pathophysiological disturbances are Environmental factors and genetic factors that are responsible for impaired glucose homeostasis in type 2 Diabetes mellitus(10). Type 2 diabetes mellitus is the most common type of diabetes with more no of cases around the world. In this body enable to make enough insulin either can't use it effectively.

**Gestational Diabetes mellitus,** Isa glucose intolerance with onset or first recognition during pregnancy(8).The diagnosis of GDM is conducted through screening of pregnant women for testing of abnormal glucose tolerance which is mild nad asymptomatic(9). In this women with gestational diabetes don't show any symptoms like pre-diabetic symptoms. Thedetection of GMD is done while during a routine blood sugar test or oral glucose tolerance test which is usually performed in-between the $24^{th}$ and $28^{th}$ weeks of gestation. It is very rare that women with GMD will experience increased thirst and urination.

**Glycodelin: A Glycoprotein**

Glycodelin is a glycoprotein found in human body as human placental protein-14 (PP-14), the gene responsible for this protein is PAEP stands for progestogen-associated endometrial protein. And it is a glycoprotein that inhibits cell immune function and plays an essential role in the pregnancy process[5]. The PAEP gene is present on the long arm of chromosome 9 and encodes for PP-14 protein. It is mainly expressed in 60 organs, but reaches its highest expression level in decidua[3,4]. According to a study (12) the role of glycodelin in the development and progression of retinopathy in type 1 diabetes during pregnancy. A possible causal relationship between low glycodelin levels and progression of retinopathy may be mediated by the immunomodulatory properties of glycodelin. Diabetic retinopathy is a complication of diabetes mellitus complication and it is disease of eyes(retina). In this small blood vessels of retona were damaged by the DM.Mismanagement of blood sugar is a risk factor for the retinopathy.

**Methodology**

**Identification of Protein**

Glycodelin(Gd) is belong to the family of lipocalin along with β-lactoglobulin (βLg) but they both have different functions.The gene of glycodelin is known as PAEP, which stands for progestagen-associated endometrial protein. Gd has no (13) or at least a significantly lower (14,15) affinity for hydrophobic ligands. Gd can extract from its natural sources human amniotic fluid.From the review studies, retinopathy is the specific complication of diabetes mellitus. In this study we take Glycodelin as a target protein.

Obtained the Glycodelin Protein from Protein Data Bank (PDB) https://www.rcsb.org/ and downloaded protein in pdb format.

**Identification of Ligands**

There are five natural compounds- Alpha-Amyrinacetate, Myrcene, Vascine, rutin and quercetin which are selected and used as a ligand in the study.

1. **Alpha-Amyrin acetate (CID: 293754)**
   It is a natural compound found and isolated from epicuticular wax. The amyrin belongs to the triterpene class. They are designated α-amyrin (ursane skeleton)(17). α-amyrin and the β-amyrin are precursor of ursolic acid and oleanolic acid respectively which helps in plant biosynthesis(18). A-amyrinacetate which was isolated via bioassay-guided isolation, shows a good and significant result in inhibited Th17 polarization as revealed when interleukin (IL)-17(16). This shows that alpha Amyrin acetate has a function of inhibitory. For this reason we take this compound in the molecular docking study.
2. **Myrcene (CID: 31253)**
   Myrcene is a alkene natural hydrocarbon in nature. It is classified as monoterpenes. Isoprenoid precursors has monoterpenes as dimers. Myrcenes are the important component of essential oil present in several plants like bay, cannabis and hops(19,20). Myrcene is biosynthesize through geranyl pyrophosphate(GPP) in plants. This compound found in plants and a example of monoterpenes. Present in several plants as a component of essential oil and has a properties of analgesic, sedative, and potentiated barbiturate sleep time(21).

3. **Vasicine (CID: 442929).**
   Vasicine (peganine) is a quinazoline alkaloid. It is found in Justiciaadhatoda. It is additionally found in Peganumharmala(22). The Results of study(23) suggest the use of the extract of A. vasicais an antidiabetic agent and also show the possibility that the compounds, vasicine and vascinol could be a useful treatment for metabolic disorders. This shows that Vasicine plays a inhibitory role in diabetes which makes it a good choice for the further study in molecular docking.

4. **Rutin (CID: 5280805)**
   Rutin is a natural compound found in plants like citrus as a flavonoid. It is the phenolic compounds found in the invasive plant species Carpobrotusedulis and have the antibacterial(24) properties of the plant. Rutin (RUT) is a bioflavonoid found in plants and foods and has biological activities of neuroprotective and antidiabetic effects. In the result of a study(25), RUT 50 mg/kg induced significant hypoglycemic effects in both diabetic and non-diabetic rats.This shows that this natural compound has antidiabetic properties.
5. **Quercetin (CID: 5280343)**
   It is a natural compound found in fruits, seeds, leaves and vegetables. It belongs to the flavonoid group of polyphenols. Quercetin(26) is found inred onions and kale in a good amount. This compound shown that it helps in decreasing the numbness, irritation and jolting pain in patients with type 2 diabtes neuropathy(27 The antidiabetic qualities of quercetin involve the stimulation of glucose uptake through an MAPK insulin-dependent mechanism. It has antidiabetic properties which make him a good choice for the further study.

All-natural compounds have been selected according to the literature. By using PubChem https://pubchem.ncbi.nlm.nih.gov/., These natural compounds are retrieved in SDF format. After that Online SMILES translator https://cactus.nci.nih.gov/translate/, is Used for converting all ligands, from SDF to pdb format and downloaded.

**Virtual Screening by PyRx**

Virtual screening of the ligands has been done through PyRx software. The PyRx software has shown the affinity and binding Energy of every ligand using virtual screening. Firstly, open a PyRx window and loaded the protein molecules which were in pdb Format. The protein molecule was converted from pdb format to pdbqt format. Then, ligand molecules that have sdf format were Also imported. Minimized all the energies of ligands and converted all ligands molecules from sdf format to pdbqt format. On the Basis of their binding affinity, the results were analyzed.

**Drug Likeliness property analysis**

Based on drug likeliness properties, natural compounds were selected for studies of molecular docking. Based on the Five rules of Lipinski screened the ligands.

Following Lipinski's rules of five states are [2]:-

1. Hydrogen bond [H-bond] acceptors less than 10.

2. Hydrogen bond [H-bond] donors less than 5.

3. Molecular mass not more than 500Da.

4. Must be less than 5 partitions co-efficient (LogP).

5. More than one rule cannot be violated.

Analyzed the five Lipinski's rules using SwissADMEhttp://www.swissadme.ch/., an online web server. Firstly copy the SMILE Notation of ligands from PubChem. This SMILE notation was submitted on SwissADME and the analysis of the five rules of Lipinski.

**Docking by AutoDockVina through MGL tools**

Load the protein targets on the graphical window of Auto dock Vina in pdb format. Water molecules of protein molecules were Deleted. The polar hydrogen atoms and Kollman charges are also added to protein molecules. Then, the protein molecule was Converted into pdbqt format and saved. The ligand molecule in pdb format was imported. Convert the ligand molecule from pdb Format to pdbqt format. Protein molecules, as well as ligand molecules, were loaded on a graphical screen and set the boundaries Of the grid box. Both protein and ligand molecule to be docked, using command prompt was carried out and analyzed the result And grid box was analyzed and prepared as shown in fig 2.

**Structural Visualisation**

Structural visualization of protein was done by PyMol software which is freely available on the internet. The protein Pdbqtformat. That automatically saved with the name of the output pdbqt file in a selected folder after Auto dock vina were loaded on the Graphical screen of the PyMol tool. After that protein and ligand interaction was visualized and analyzed.

**Result and Discussion**

The Glycodelin Protein was retrieved in pdb format from Protein Data Bank at 2.45 Åresolution in 2D and 3D respectively in [figure 1&2].

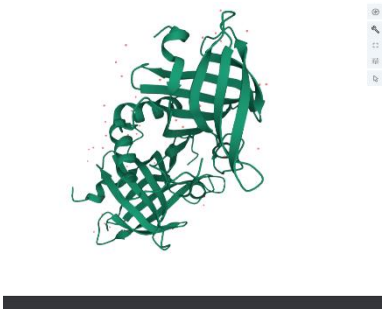



**Figure 1: 2DStructure of Glycodelin Protein Figure 2: 3DStructure of Glycodelin Protein**

Alpha-Amyrin acetate (CID:92842 ), Myrcene (CID:31253 ), Vascine (CID: 442929),Rutin(CID:5280805 ) and Quercetin(CID: 5280343)were downloaded in sdf format and 2D And 3D structure [Table: 1].

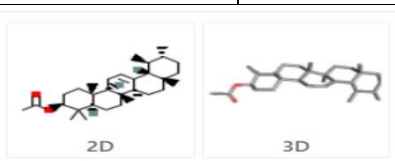
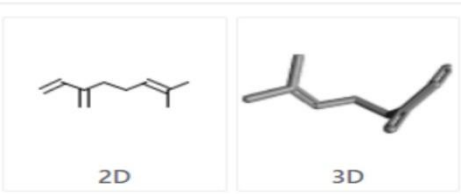
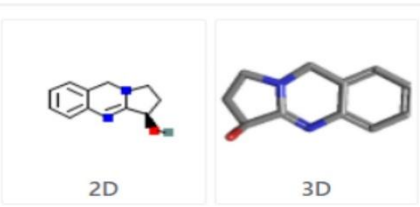
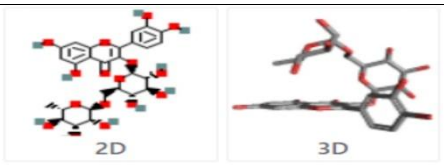
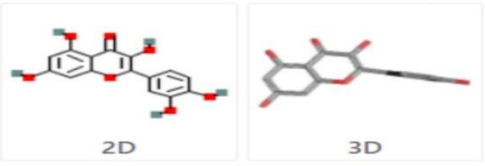
| Name of ligands | CID | Molecular formula | Molecular weight | 2D structure | 3D structure |
|---|---|---|---|---|---|
| Alpha-amyrin acetate | 92842 | $C_{32}H_{52}O_2$ | 468.8 |   | |
| myrcene | 31253 | $C_{10}H_{16}$ | 136.23 |   | |
| vascine | 442929 | $C_{11}H_{12}N_2O$ | 188.23 |   | |
| rutin | 5280805 | $C_{27}H_{30}O_{16}$ | 610.517 |   | |
| quercetin | 5280343 | $C_{15}H_{10}O_7$ | 302.236 |   | |

**Table 1: STRUCTURE OF LIGANDS**

Through PyRx software, the virtual screening of all five ligand molecules such as Alpha-Amyrin acetate,Myrcene, Vascine, rutin and quercetinwas done. The binding affinity values of all five ligands are: -7.0, -6.7,-6.3,-6.2,-6.0 respectively as shown in the [Table: 2]. After the PyRx result, the selected ligands were Myrcene and vasicine. After that drug likeliness Properties were analyzed of these selected ligands. The drug-likeness property analysis of these ligands was done with the help Of SwissADME software [Table: 3]. According to Lipinski's Rule of Five, the ligands were screened. The SwissADME result Showed that myrcene is selected for molecular docking with the glycodelin protein.

| NAME OF COMPONDS | LIGANDS | BINDING AFFINITY | MODE | RMSD LOWER BOND | RMSD UPPER BOND |
|---|---|---|---|---|---|
| Alpha-amyrin acetate | | -7.0 | 0 | 0.0 | 0.0 |
| myrcene | | -6.7 | 1 | 2.067 | 4.795 |
| vasicine | | -6.3 | 2 | 1.56 | 5.533 |
| Rutin | | -6.2 | 3 | 2.157 | 3.47 |
| quercetin | | -6.0 | 4 | 2.168 | 3.1 |

**Table 2: PyRx Result**

| Ligands | Molecular weight | No. of H- bond acceptor less than 5 | No. of H-BOND donars less than 5 | Log po/w (MLOGP) less than 5 | Lipsinki |
|---|---|---|---|---|---|
| myrcene | 136.23 | 0 | 0 | 3.56 | 0 violation |
| vasicine | 188.23 | 2 | 1 | 1.57 | 0 vioation |

**Table 3: SwissADME Table**

The qualifying molecule Myrcene was docked with target protein glycodelin through Auto dock Vina (MGL tool). The Auto Dock Vina results show 9 different values of binding affinity, (RSMD lower bound), (RMSD upper bound) [Table 4]. The protein target Glycodelin and Myrcene interaction was visualized through PyMol software [Figure 3].

| | | Distance from the best mode | |
|---|---|---|---|
| Mode | Affinity (kcal/mol) | Root Means Square deviation lower bound [RMSD LB] | Root Means Square deviation Upper bound [RMSD UB] |
| 0 | -4.4 | 0.0 | 0.0 |
| 1 | -4.2 | 0.832 | 1.717 |
| 2 | -4.1 | 1.255 | 2.084 |
| 3 | -4.1 | 1.589 | 3.759 |
| 4 | -4.1 | 1.709 | 3.291 |
| 5 | -4.1 | 1.734 | 3.793 |
| 6 | -4.0 | 1.85 | 4.133 |
| 7 | -3.9 | 1.267 | 2.224 |
| 8 | -3.7 | 1.336 | 2.651 |

**Table: 4 Result of Auto dock Vina**

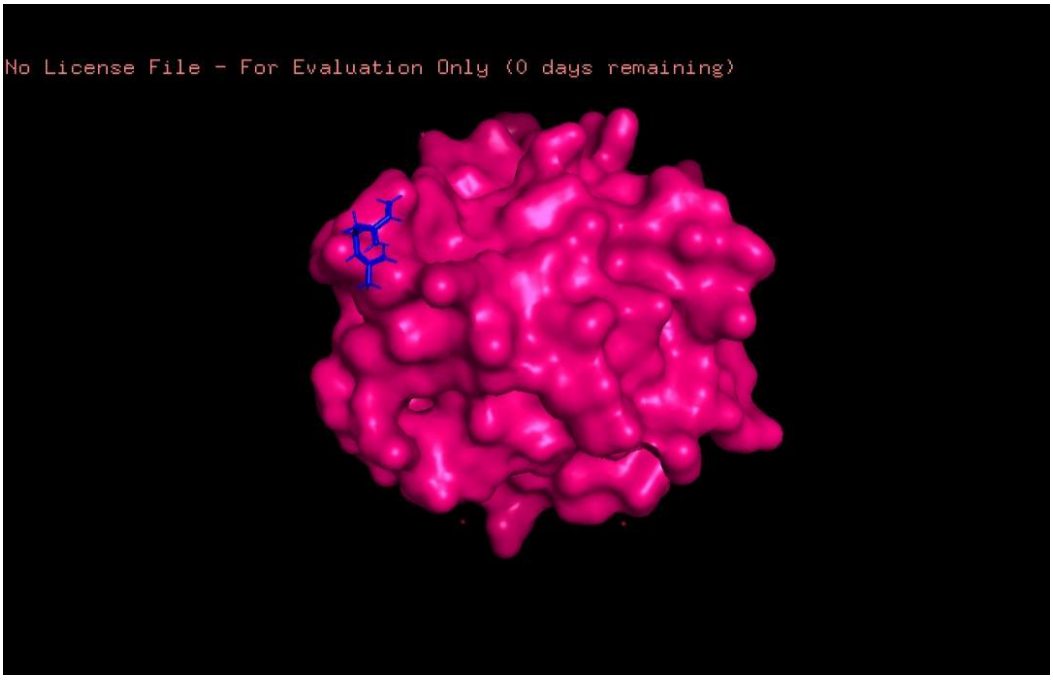



**Figure 3: Interaction of Glycodelin and Myrcene with the help of PyMol software.**

## Conclusion

Glycodelin Protein was used for docking studies. Molecular docking is a useful tool for the drug discovery. This approach was used to study the potential of naturally occurring compounds such as Alpha-Amyrin acetate, Myrcene,Vascine,rutin and quercetin with target Glycodelin-protein. Docking results were analysed for the best ligands on the basis of drug likeliness property analysis. In this study, Myrcenewas found as the best ligand with minimum binding affinity value and this compound is also qualified Lipsinki's rule of five. The result of this study was helpful in understanding the structure characteristics required to improve inhibiting activities. Myrcene may act as a drug against the Retinopathy- a diabetes complication.Retinopathy is a disease of eyes which effects the retina. After both in vivo as well as in vitro, Myrcene may be a promising drug for treating retinopathy complication of diabetes disease in the future.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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