International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 5 Issue 5, July-August 2021 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

The Role of Gedunin Against Aquaporin 2 for the Possible Treatment of Diabetes: An *In Silico* Study

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ABSTRACT

Diabetes is one of the major causes of death in the world, affecting around 422 million people (or 8.5 percent of the world's population). Diabetes mellitus, or diabetes, is a disease that causes excessive blood sugar levels. Diabetes mellitus is a condition where a person does not produce enough insulin or does not link directly to insulin, and resulting in excessively high blood sugar (glucose) levels. Diabetes is classified into several types: Prediabetes, Type 1 diabetes, Type 2 diabetes and gestational diabetes. In diabetes research, numerous bioinformatics technologies are used. The current research was carried to study the effect of naturally occurring compounds against Aquaporin 2 as the target protein molecule with the help of molecular docking for diabetes diagnosis. AQP2 is found in the apical cell of the main ducts of the kidney and in the vesicles (intracellular) in the cells. Docking experiment were performed out by using aquaporin 2 (4nef). The technique of molecular docking was utilized to investigate the potential of naturally occurring compounds such as aglycone, gedunin, and harman with the target protein Aquaporin 2 (4nef). In diabetes, gedunin may function as a therapeutic drug against the disease-causing protein Aquaporin 2 (4nef). Further research and then certain clinical and pre-clinical studies may be allowed to discover drugs that can be used to treat diabetes.

KEYWORDS: *Diabetes, Molecular Docking, Aquaporin 2 (4nef), Gedunin, Autodock*

How to cite this paper: Ayushi Chaudhary | Noopur Khare | Tripti Bhatnagar | Abhimanyu Kumar Jha "The Role of Gedunin Against Aquaporin 2 for the Possible Treatment of Diabetes: An *In Silico* Study" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-5 | Issue-5, August 2021, pp.156-162, URL: www.ijtsrd.com/papers/ijtsrd43788.pdf

IJTSRD43788

INTRODUCTION

Since 1965, the World Health Organization has updated and published recommendations on how to describe diabetes mellitus (also known as "diabetes") on a regular basis [1].

Diabetes is one of the major causes of death in the world, affecting around 422 million people (or 8.5 percent of the world's population). Despite many significant attempts to improve treatment options, the prevalence is expected to continue to rise. This article discusses diabetes mellitus, its symptoms, types, epidemiology, therapy and the *in silico* study for the treatment of diabetes disease.

Diabetes mellitus, or diabetes, is a disease that causes excessive blood sugar levels. Diabetes mellitus is a condition where a person does not produce enough insulin or does not link directly to insulin, and resulting in excessively high blood sugar (glucose) levels [2]. Insulin transports sugar from the bloodstream into cells, where it is stored or utilized for energy. If diabetes is not well controlled can lead to serious impacts, including damage to a variety of organs and tissues in your body, including your eyes, nerves, heart, and kidney [3]-[4].

Symptoms of Diabetes

Diabetes symptoms are caused by rising blood sugar.

The normal symptoms of diabetes include:

- Increased thirst
- Frequent urination
- Weak feeling
- Blurry vision
- Weight Loss
- Increased hunger
- Extreme fatigue
- Sores that don't heal

Others Symptoms

In Men: low sex drive, poor or decreased muscles strength and Erectile Dysfunction (ED).

In Women: frequent yeast infection, dry skin, itchiness, urinary tract infection (UTI).

Classification Of Diabetes

Diabetes is classified into several types:

- Prediabetes arises when the blood sugar is greater than normal, still not more enough for type 2 diabetes diagnosis.
- Type 1 diabetes disease is caused by an autoimmune reaction. The immune system targets and damages insulin-producing cells in the pancreas. This kind of diabetes affects around 10% of diabetic patient.
- Type 2 diabetes develops when the body becomes resistant to insulin and produces high sugar levels in your blood.
- Gestational diabetes is defined as elevated blood sugar levels throughout pregnancy. This type of diabetes causes insulin blocking hormones released by placenta.

Diabetes is a endocrinological disease condition that affects millions of individuals and is able to create numerous health concerns. In diabetes research, numerous bioinformatics technologies are used. To acquire an overall view of bioinformatics and diabetes, a study investigated at the types of tools often used by researchers, both by tool category (e.g., sequence alignment, microarray analysis) and individual tools cited (e.g., GCG Pileup, ClustalW, etc.) [5].

The current research was carried to study the effect of naturally occurring compounds against Aquaporin 2 as the target protein molecule with the help of molecular docking for diabetes diagnosis.

The Role of Aquaporin and Disease

Aquaporin 2 Gene

AQP2 is found in the apical cell of the main ducts of the kidney and in the vesicles (intracellular) in the cells. Human aquaporin 2 (AQP2) is a water channel found in the collecting duct of the kidney, where it contributes in urine concentration. AQP2 transport between intracellular storage vesicles and the apical membrane regulates water absorption. The pituitary hormone arginine vasopressin regulates this mechanism, and improper transport leads to nephrogenic diabetes insipid us (NDI)[6].

The AQP2 gene codes for aquaporin 2, a protein that is produced in the body. This protein creates a pathway for water molecules to pass through cell membranes. It is located in collecting ducts, which are a network of small tubes that easily absorb water from the kidneys and return it to the bloodstream.

The aquaporin 2 water channel is necessary for keeping the body's water balance in control. A hormone called vasopressin, also known as antidiuretic hormone, regulates the location of these channels. The body creates more ADH when a person's fluid intake is low or when a lot of fluid is lost (for example, through sweating). Aquaporin 2 water channels are inserted into the membrane of collecting duct cells as a result of chemical processes triggered by this hormone. Water can be reabsorbed into the bloodstream through these pathways, resulting in more concentrated urine. ADH is produced less when fluid intake is adequate. Aquaporin 2 water channels are deleted from the membrane of collecting duct cells when ADH signals are not present. Water is reabsorbed into the bloodstream less often during these times, resulting in more dilute urine [7].

In humans and animals, the normal vasopressin-regulated water channel of the renal collecting duct, aquaporin-2 (AQP2), is dysregulated in a variety of water balance disorders, including polyuria (e.g. urinary tract blockage, hypokalemia, inflammation, and lithium toxicity) and dilutional hyponatremia. Normal vasopressin regulation of AQP2 comprises two different regulatory mechanisms: 1) short-term management of AQP2 transport into and out of the outer membrane, and 2) long-term control of cell concentration of the AQP2 protein. The majority of water balance problems are caused by a dysfunction of the mechanisms that control the overall amount of AQP2 in collecting duct cells. In overall, the level of AQP2 in a collecting duct cells is regulated by a balance of production via AQP2 mRNA translation and removal via breakdown and/or release into the urine in exosomes. The rise in AQP2 abundance in response to vasopressin is primarily due to enhanced translation after increases in AQP2 mRNA. Vasopressin-mediated control AQP2 gene transcription is inadequately known, yet in proteomic studies multiple transcription-related

binding elements have been identified in the 5' flanking AQP2 region of the gene. A study review research in the field and describe aspects of vasopressin signaling in the collecting duct that may influence AQP2 regulation in health and in the context of polyuric disease cases[8].

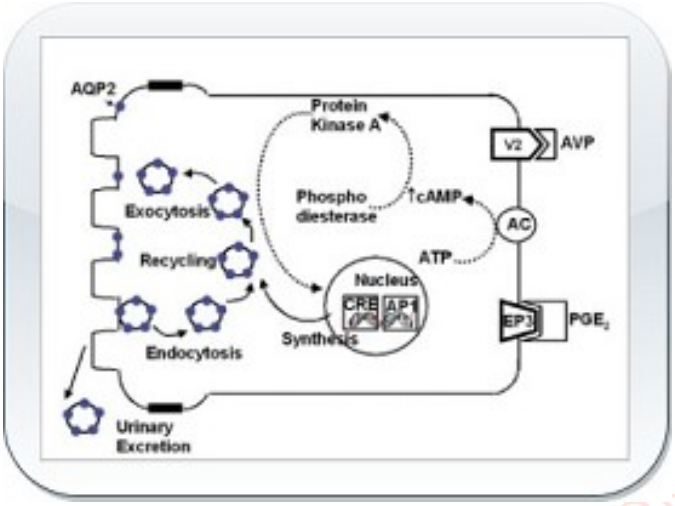


Fig.1 Aquaporin 2 in principle cell

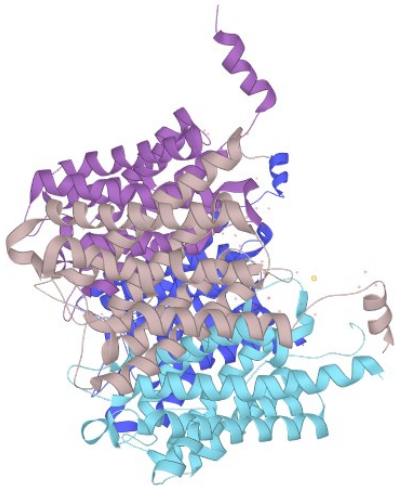


Fig.2 Protein structure of Aquaporin 2

Gene name: AQP2, ***Chains:*** A, B, C, D. ***Length***: 242 amino acids, ***Theoretical weight*****:** 25.24 KDa, ***Source organism:*** Homo sapiens, ***Expression system:*** Komagataella phaffii GS115, **UniProt:** Canonical: Protein Data Bank in Europe - Knowledge Base P41181 (Residues: 3-241; Coverage: 88%), ***Sequence domains***: Major intrinsic protein, ***Structure domains:*** Glycerol uptake facilitator protein.

Some Natural Compounds For Diabetes Diagnosis

Since ancient times, natural compounds have been utilized for all types of treatments in India and around the world. According to a WHO report, more than 60% of the worldwide people uses plants and their by products as medicine. There are many natural compounds which is known for more effective treatment of diabetes disease. In this study we have been taken some natural occurring compounds or products which is helpful in diabetes diagnosis.

Gedunin: Gedunin is a pentacyclic triterpenoid natural substance found mostly in *Azadirachta indica&Cedrela odorata*. It is isolated from the Indian neem tree. It functions as an antineoplastic, antimalarial, Hsp90 inhibitor, and a plant metabolite. It is a limonoid, acetate ester, epoxy, enone, furans, triterpenoid, heteropentacyclic chemical molecule and lactone[9].

In various Meliaceae family genera, Gedunin is a significant limonoid, especially in seeds. Gedunin has been linked to a variety of biological activities, including antibacterial, insecticidal, antimalarial, antiallergic, anti-inflammatory, anticancer, and neuroprotective properties. The identification of gedunin as a heat shock protein (Hsp) inhibitor was a turning point in the drug's development as a biological therapeutic agent. This study is a critical evaluation of literature based on the numerous biological activities for gedunin that have been described so far, their therapeutic effects on certain human conditions and recommendations for future research for this organic compound [10].

Dammarane: Dammarane is a tetracyclic triterpene present in *sapogenins* (triterpenoid saponins) such as those found in *ginseng* (ginsenosides: panaxatriol and protopanaxadiol). Dammar resin, a natural resin from tropical plants in the Dipterocarp family, was used to isolate andname compounds in the series [11]-[12].

Dammarane triterpenoids are very important natural metabolites with exceptional biologic activity, the primary secondary metabolites of *Panax* ginseng. The plants of the *Panax* or other genus and modifying some natural compounds can be isolated. This review consists of a collection of a number (2005-2014) of patents which describe dammarane triterpenoids for therapeutic and preventive usage in several prevalent ailments [13].

Aglycone: It's an organic component (such as phenol or alcohol) mixed with the sugar portion of a glycoside. An aglycone (aglycone or Genin)[14] is a residual compound that has been replaced by a hydrogen atom with a glycosyl [15]. An example is a steroid molecule, which may be a cardiac aglycone. A cardiac glycoside aglycon is an example of a steroid molecule. H-NMR and Heteronuclear multiple bond correlation (HMBC) investigations will be used to extract aglycone from desert plant species. The HMBC experiment is frequently paired with other techniques, including as mass spectroscopy, to better understand the structure and function of aglycone [16]. A study of molecular markers in human arteria epithelial tissue cells found that aglycone inhibited cell migration but not white blood corpuscle

adhesion, which is the first stage in the formation of arterial sclerosis plaques [17].

Harman: Harmane (harman) is a heterocyclic amine present in a range of foods such as coffee [18], sauces [19], and cooked meat [20] It can also be found in tobacco smoke[21]. Harmane has the chemical formula C12H10N2 and is a methylated derivative of -carboline.

Harman, commonly known as *aribin or locuturin*, is an organic chemical that belongs to the harmala alkaloids class. Harmala alkaloids are substances that have a structure that is based on harmaline, harmine, harmalol, harman, or a product of those parents. These parents are beta-carbolines, which are formed by fusing a pyrimidine to the pyrrole molecule of an indole to generate pyrido[3,4-b]indole. Harman is a powerful basic compound (based on its pKa).

Methodology

Material & Methods

Softwares and Tools:

Uniprot, PubChem, SMILES Translator, PyRx,, SwissADME. AutoDock Vina 1.1.2, MGL tools, NCBI, Protein Data Bank (PDB) and PyMOL.

Experiment process following steps as: -

A. Protein Identification

After Studing various literatures and texts, the disease-causing protein {Aquaporin 2 (4nef)} **was** retrieved and protein structure was downloaded from RCSB Protein Data Bank (PDB) and downloaded protein was in .pdb format [22].

B. Ligand Identification

According to the literature, all-natural compounds were chosen (named Aglycone, Gedunin, Dammarane, Harman). These natural compounds were obtained in .sdf format from PubChem [23]. After that, all ligands were converted from .sdf to .pdb format and were downloaded using the online SMILES translator[24].

C. Virtual Screening of protein and ligand molecule via PyRx tool

PyRx software was used to perform virtual screening of the ligands. Using virtual screening, the PyRx software determined the affinity and binding energy of each ligand. On PyRx window protein molecules was loaded in pdb format. The protein molecule was transformed from pdb to pdbqt format. Then, ligand molecules in sdf format were imported. All ligand energies were minimized, and all ligand molecules were converted to pdbqt format. The results were examined based on their binding affinity.

D. Analysis of Drug likeness property

Accordingly, natural substances were chosen for molecular docking study based on their drug-like properties. Swiss ADME [25], an internet web server, was used to analyze the Lipinski's rule of five.

The ligands' SMILE notation from PubChem were copied and were pasted to Swiss ADME, The ligand molecules were screened using Lipinski's rule of five.

- H-Bond (Hydrogen Bond) acceptor must be <10.
- H-Bond (Hydrogen Bond) donor must be <5.
- Molecular weight must not >500 Da.
- LogP (MLOGP) partition of coefficient should not be more than 5.
- Only one rule can be violated.

E. Docking via Auto Dock Vina

The target protein in .pdb format was loaded on the Auto Dock Vina graphical window. The protein was prepared by removing water molecules and adding them with hydrogen polar atoms, then adding kollman charges to the protein molecules and were saved in the "**.**pdbqt" format. The ligand molecule was imported in pdb format. The ligands were converted from pdb to the pdbqt format. Protein and ligand molecules were imported onto a graphical interface of auto dock tool, the grid box boundaries were adjusted, and was saved in the grid configurations in grid.txt format.

F. Visualization of structure by PyMOL

PyMol software was used to visualize the protein's structure. The protein in .pdbqt format was manually saved with the name of the output pdbqt file in a selected folder after Auto dock vina were loaded on the PyMol tool's graphical screen. Following that, the protein-ligand interaction was observed and evaluated.

Result

The Aquaporin 2 (PDB ID: 4nef) was downloaded in pdb format from Protein Data Bank. The resolution of the protein was 2.75 Å. With X-ray diffraction as the method, Aquaporin 2 (4nef) has R-Value Free: 0.225, R-Value Work: 0.202, R-Value Observed: 0.203. Aglycone, Gedunin, Dammarane, Harman were downloaded in 3D conformer in **.**sdf format. Virtual screening of the protein and ligand molecules was done via PyRx tool. The binding affinity values of all 4 ligands were: Aglycone was -8.5, Gedunin was -9.3, Darmmane was-6.9 and Harman was -7.1 (Table 1). Accordindly after the PyRx result, less minimum binding affinity ligand were screened and taken for next process. Gedunin ligand shows the minimum binding affinity (-9.3) and can be chosen for the further analysis whereas the Dammarane compound have maximum binding affinity (-6.9) in all selected compounds. The ligands which were selected after the

PyRx result were Aglycone, Gedunin and Harman. These ligands were further analyzed for drug likeliness property analysis through Swiss ADME (Table 2).

Table 1: PyRx result of various ligands with protein molecule (binding affinity, Mode, RMSD lower, RMSD upper)

S. no.	Ligand Molecules	CID Value	Binding Affinity (Kcal/mol)	Mode	RMSD lower bound	RMSD upper bond
1.	Aglycone	139597845	-8.5	0	0.0	0.0
2.	Gedunin	12004512	-9.3	0	0.0	0.0
3.	Dammarane	9548714	-6.9	0	0.0	0.0
4.	Harman	5281404	-7.1	0	0.0	0.0

Table 2: Swiss ADME result

Ligand	Mol. Weight <500 Kda	Number of H-bond Acceptors<10	Number of H-bond donor<5	Log Po/w (MLOGP)	Lipinski
Aglycone	414.75 g/mol	8	3	2.54	Yes; 1 violation(MlogP>500)
Gedunin	482.57 g/mol	7	0	2.56	Yes; 0 violation
Harman	182.22 g/mol	1	1	1.9	Yes; 0 violation

According to the Lipinski's rule of five, the ligands were screened and the Swiss ADME result showed that compound Gedunin qualified for all Drug likeness/Drug properties.

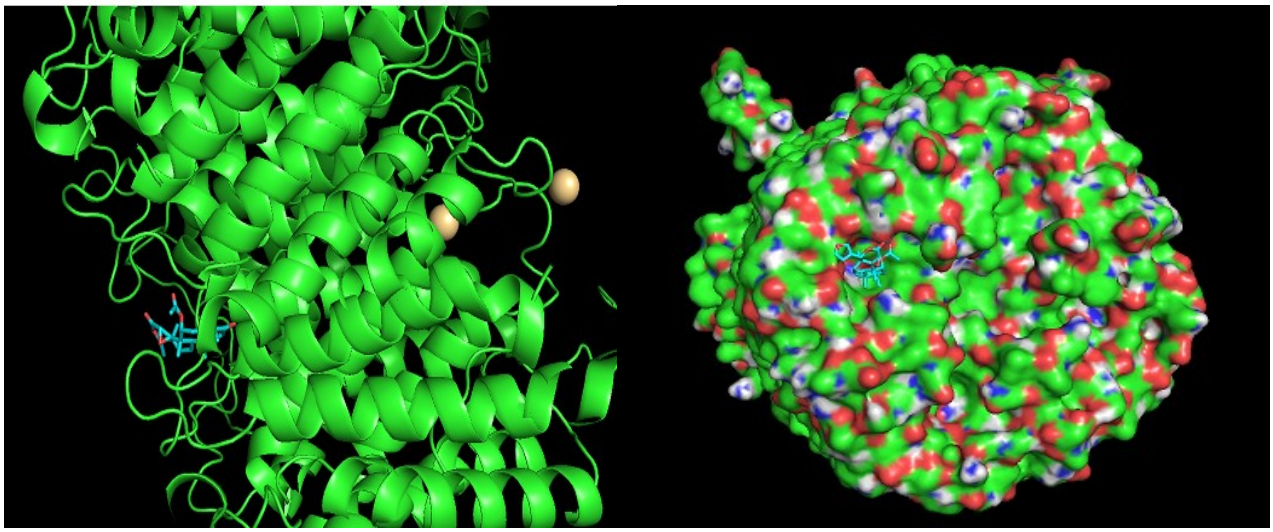
Next, the qualifying molecule named Gedunin was docked with target protein Aquaporin 2 (4nef) via the Autodock Vina tool (MGL tool). The autodock vina results showed 9 different value of binding affinities (Kcal/mol), RMSD lower, RMSD upper in Table 3.

Table 3: Autodock Vina results

Mode	Binding Affinity (kcal/mol)	Distance from best mode	
		RMSD lower bound	RMSD Upper bound
1	-8.4	0.000	0.000
2	-8.1	4.121	7.853
3	-8.0	2.736	5.006
4	-7.9	3.289	6.845
5	-7.6	2.478	5.098
6	-7.6	3.281	6.111
7	-7.5	2.178	7.048
8	-7.4	37.330	42.228
9	-7.3	31.033	33.908

The protein target (Aquaporin 2) and Gedunin interaction was visualized via a PyMOL software (Fig.3)

Fig.3 PyMOL results {interaction between gedunin and aquaporin 2(4nef)}



Conclusion

Diabetes mellitus, or diabetes, is a disease that causes excessive blood sugar levels. Docking experiment were performed out by using aquaporin 2. The technique of molecular docking was used to investigate the potential of naturally occurring compounds such as aglycone, gedunin, and harman with the target protein Aquaporin 2. Docking observations were evaluated to determine the best ligands based on their drug-likeness property. Gedunin was revealed to be the best ligand in this research study, with the lowest binding affinity value, and this compound also fulfilled Lipinski's rule of five. The results of this study were effective in assessing the structural properties needed to enhance the inhibition activity. In diabetes, gedunin may function as a therapeutic drug against the disease-causing protein Aquaporin 2. Further research, certain clinical and pre-clinical studies may be allowed to discover drugs that may be used to treat diabetes.

Conflict Of Interest Statement

The authors declare that there is no conflict of interest.

Acknowledgments

The authors acknowledge the help provided by the Department of Biotechnology, Faculty of Life Sciences, Institute of Applied Medicines and Research, Ghaziabad, Uttar Pradesh, India.

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