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In Silico Molecular Docking Analysis of Cinnamon (Cinnamomum verum) as an Insulin Receptor (INSR) Activator for Diabetes Mellitus Therapy

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Abstract: The prevalence of obesity has increased in recent years. Obesity can increase the risk of metabolic and cardiovascular disorders that are deadly. One of the metabolic diseases caused by obesity is type 2 diabetes mellitus. Type 2 diabetes mellitus or also known as non-insulin dependent type is a condition of insulin receptor resistance. Type 2 diabetes mellitus is caused by impaired insulin sensitivity, failure of pancreatic beta cells to maintain the amount of insulin to compensate for the resistance that occurs. The mechanism of insulin receptor resistance that commonly occurs is abnormalities of the insulin receptor that reduce INSR expression on the cell surface and post receptor abnormalities. The presence of a single-nucleotide in INSR. 4IBM affects the function of insulin receptors and increases the possibility of INSR-mediated diseases, one way to overcome this is by increasing the expression of 4IBM. Cinnamon (Cinnamomum verum) is a traditional plant that is often used as a medicinal ingredient for various disorders and based on previous research has been proven as a potential agent for managing diabetes mellitus. This research is an in silico study that aims to examine the potential of active chemicals contained in cinnamon and their role as 4IBM protein inhibitors for therapy in type 2 diabetes mellitus using Autodock tools 1.5.6 and based on the principle of Lamarckian genetic algorithm. Docking results showed binding energy ranged from -6.97 kcal/mol to -5.83 kcal/mol, with Epicathein compound having the smallest binding energy with an inhibitory constant of 7.74. This study can be used as a basis for conducting further research (in vivo and in vitro) related to the active chemicals of cinnamon and their effects as Diabetes Mellitus therapy.

Keywords : Diabetes Mellitus, Cinnamomum verum, 4IBM, Autodock tools 1.5.6

I. INTRODUCTION

The prevalence of obesity in 2017-2018 was 42.4% and the severity increased to 9.2%, an increase compared to 1999-2000 [1]. Obesity is a condition influenced by a complex relationship between genetic, socio-economic, and cultural influences [2]. Obesity can increase the risk of metabolic and cardiovascular disorders associated with mortality. Metabolic disorders caused by obesity include type 2 diabetes mellitus (type 2 DM), hypertension, dyslipidemia, non-alcoholic fatty liver disease (NAFLD), and ischemic heart disease [3] Type 2 DM or also known as non-insulin dependent type is a condition of insulin receptor resistance, usually accompanied by relative insulin deficiency [4]. Type 2 DM is caused by impaired insulin sensitivity and the failure of pancreatic beta cells to maintain the amount of insulin to compensate for the resistance [5]. Higher insulin levels are needed to achieve a reduction in sugar levels [6]

Current therapy for type 2 DM includes lifestyle modification, normalization of blood glucose levels, and management of comorbid diseases (Kao and Sabin, 2016). Metformin is a first-line drug that reduces insulin resistance in the liver and peripheral tissues. insulin receptor sensitization. Another drug to reduce insulin resistance that acts as a PPAR-ɣ agonist is glitazone. However, the use of glitazone as a therapeutic regimen is accompanied by fat accumulation in the subcutaneous tissue [7].

The mechanism by which resistance to the insulin receptor often occurs is that there are abnormalities in the insulin receptor that reduce INSR expression on the cell surface and post-receptor abnormalities that cause

inadequate signal transduction [6]. These receptor abnormalities result in insulin receptor resistance. The presence of a single-nucleotide in the INSR, 4IBM, affects insulin receptor function and increases the likelihood of INSR-mediated disease [8]. One way to overcome this situation is to increase the expression of 4IBM.

Cinnamon (Cinnamomum verum) is a traditional plant that is often used as a medicinal ingredient for a variety of disorders [9]. Based on previous studies, cinnamon has been shown to be a potential agent to treat diabetes mellitus. Cinnamon is an ingredient that contains active compounds that can regulate blood sugar by increasing insuline uptake through activation of insulin receptor kinase, autophosphorylation on insulin receptors, and glycogen synthesis [10]. One of the active chemicals contained in cinnamon is epicathecin. The use of epicathecin can reduce blood glucose levels in patients with diabetes mellitus [11]. This study aims to analyze the potential of epicathecin contained in cinnamon and its role as a 4IBM protein inhibitor for therapy in type 2 DM in silico.

II. METHOD

System configuration

This study was conducted using a laptop with Windows 10 Education 64 bit. The hardware specification used is Intel Core i3-10110 CPU processor with 8 GB RAM. The main software used was Autodock tools 1.5.6.

Macromolecular structure retrieval

Protein structure of insulin receptor-PDB ID: 4IBM was retrieved from the Protein Data Bank (PDB). PDB is a database containing various experimental structures of proteins and nucleic acids. The structure of the 4IBM protein consists of 2 chains, chain A and chain B. In this experiment, only chain A was used for docking. Autodock tools 1.5.6 is a free software used to perform molecular studies by calculating binding affinity on protein molecules [12].

Ligand structure retrieval

The ligands used were active chemicals from cinnamon searched using Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/phytochem/search). The active chemical epicathecin which is contained in large quantities in cinnamon was taken. The molecular structure of epicathecin was retrieved from Pubchem (Pubchem CID: 72276)

Drug scanning

The ligands to be used were tested for their potential as oral drugs based on Lipinski's rule of five using SwissADME. SwissADME is a website that can be used to assess the pharmacokinetics and drug-likeness of a compound [13]. Lipinski's rule includes 4 parameters related to solubility and permeability, namely molecular weight, logP, number of hydrogen bond acceptors, and number of hydrogen bond donors. Ligands that showed violations of 2 or more Lipinski rules were not used in this study [14].

Determination of active site

Amino acids involved in active site formation were determined using Computed Atlas for Surface Topography of Proteins (CASTp). CASTp is a site that can be used to determine the location and position of active sites on protein structures taken from PDB [15]. This active site determination is used to determine the position of the grid box before docking.

Ligand Preparation

The ligand structure taken is a 3D conformation in SDF format and then optimized geometry using the Avogadro application and then saved back in SDF format. Next, the ligand was converted into PDBQT format for further processing with Autodock tools 1.5.6 software. Polar hydrogen addition and non-polar hydrogen incorporation were carried out.

Macromolecular Preparation

Macromolecules retrieved via PDB are prepared by removing other chains and unnecessary water molecules with Autodock tools 1.5.6. Next, the protein molecule is added polar hydrogen and Kollman charge.

Validation of molecular tethering method

Validation of the molecular tethering method was performed by re-molecular tethering of the natural ligand into the active side of its receptor. The grid box size is (24 x 22 x 30) with grid box coordinates (x,y,z) of 3.517 Å; -10.108 Å; and 6.836 Å, respectively. The validity of the molecular tethering method parameters was evaluated based on the RMSD (root mean square deviation) value. The validation of the molecular tethering method is declared valid if the RMSD value is smaller than 2.0 Å10 (Nursamsiar, 2020).

Molecular tethering simulation

The test compound is then tethered to the active side of the receptor with the same grid box and coordinates when validating the parameters of the molecular tethering method. Molecular docking was performed using Autodock tools 1.5.6 program with *.pdbqt file format. The docking process was performed using Lamarckian genetic algorithm [16]. The results of the analysis are in the form of bond free energy, hydrogen bonding, and binding patterns with other amino acid residues on the active side of the receptor. The selected protein-ligand complex conformation is the complex that has the smallest binding free energy value for further analysis [17]. The most negative ΔG results indicate the strongest bond between the ligand and protein [18]. The test ligand data that has been collected is compared with the natural ligand. The docking results in ".pdbqt" format were then converted to ".pdb" using BIOVIA Discovery Studio 2019 software in order to be visualized using PyMol software.

III. RESULT AND DISCUSSION

This study uses proteins in the insulin receptor, which plays a role in the regulation of glucose levels in the blood. The structure of the protein used was taken from the PDB. The protein has 2 monomeric chain arrangements, namely the A and B chains. In this study, only the A chain of the insulin receptor (PDB-ID: 4IBM) was tested along with the active compounds in cinnamon obtained from Dr. Duke's website and the structure was taken from pubchem.

The active compound tested was (-) Epicathecin contained in cinnamon bark. This compound was further evaluated with Lipinski's rule of five to determine its compatibility as a medicinal substance. The parameters evaluated include: molecular weight, logP, number of hydrogen bond acceptors, and number of hydrogen bond donors. The study did not find any violation of Lipinski's rule of five (Table 1).

Table 1

Molecules	Molecular Weigtt	Hdrogen bond acceptor	Hydrogen bond donor	MLOGP
(-) Epicathecin C15H14O6	290.27 g/mol	6	5	0.24

(Source : research data)

The next process is the determination of the active site of the insulin receptor protein which is carried out with the help of CASTp website (Figure 1). From CASTp the position of the active site occupied bt the INSR amino acid is recorded and will be used as a reference for grid box placement in Autodock tools 1.5.6

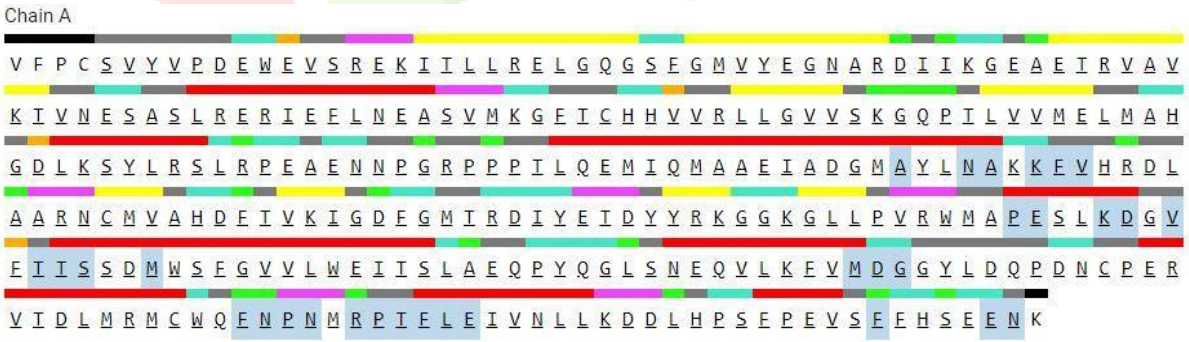

Figure 1. Amino acids involved in the active site of the INSR gene, blue color, indicates active site. (source : research data)

Table 2. INSR gene active site

Amino Acids	Sequences Location
ALA	1121
ASN	1124
ALA	1125
LYS	1127
PHE	1128
VAL	1129
PRO	1178
GLU	1179
LYS	1182
ASP	1183
VAL	1185
THR	1187
SER	1189
MET	1223
ASP	1224
GLY	1225
PHE	1248
ASN	1249
PRO	1250
ASN	1251
ARG	1253
PRO	1254
PHE	1256
GLU	1258
PHE	1276
GLU	1281
ASN	1282

Based on the docking result between ligand and protein using Autodock tools 1.5.6 the binding energy ranged from -6.97 kcal/mol with an inhibitory constant of 7.74 μM (Table 2) The 3D result of the bond between (-) epicathen are shown in Figure 2. (-) Epicathen has been investigated to have various medical benefit such as antioxidant, antiangiogenic, antiproliferative effect on cancer cells, and lower blood glucose levels in diabetics [11]. Epicathecin content can minimize the degeneration process and increase the ability of pancreatic β islet cells [19].

Table 1 Molecular docking of ligands with INSR protein

Compound	Binding Energy (kcal/mol)	Inhibitory Constant (μM)	Van Der Waals Bond	Cluster RMSD
(-) Epicathecin	-6.97	7.74		0.0

(source : research data)

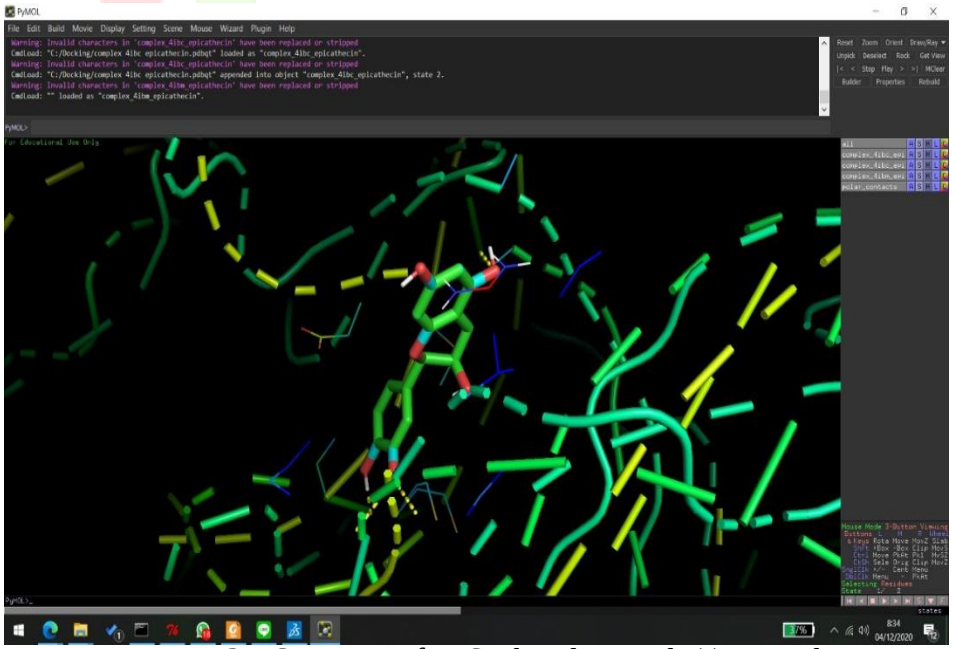

Figure 1. 3D Structure of INSR bonding with (-) Epicathecin

(Source : research data)

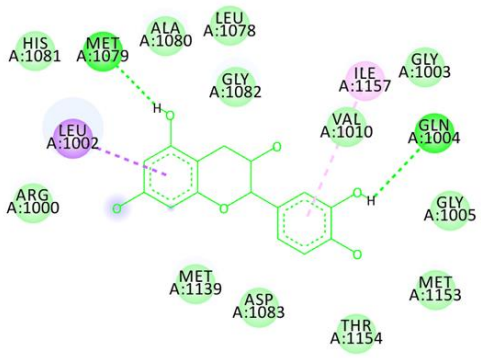

Figure 3. Docking Ligand Bond Interaction (source : research data)

IV. CONCLUSIONS AND SUGGESTIONS

The number of people with diabetes mellitus is increasing, accompanied by an increase in other metabolic diseases. Until now, existing therapies for type 2 DM have not been able to restore glucose levels to normal. Therefore, the development of new therapies is needed, one alternative therapy that has the potential to reduce blood glucose levels is the induction of INSR to increase its sensitivity. Cinnamon is a traditional medicinal plant that contains several active ingredients and has medicinal properties. In this study, it was found that compound (-) epicathecin has good potential to induce INSR activation. More in vivo and in vitro studies are expected to further examine the impact of (-) epicathecin on type 2 DM

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