Analysis of Antidiabetic Potential of Palmitic Acid Compounds Through In Silico Activation

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Abstract

Diabetes Mellitus is a condition where the body's sugar level exceeds the blood's normal limit. Type 2 Diabetes Mellitus is the most common type of diabetes and is a chronic disease caused by insulin resistance and beta-cell dysfunction, resulting in decreased insulin sensitivity. The management of diabetes Mellitus is in the form of oral hypoglycemic drugs. Still, these drugs have side effects such as gastrointestinal disturbances, nausea, vomiting, hypoglycemia, macrovascular disorders, microvascular disorders, etc. So, it is necessary to seek alternative drugs to increase insulin sensitivity to lower blood sugar levels which are safer using herbal plants. One of the suspected plants that can be an alternative medicine to reduce excess blood sugar levels is the dandang gendis plant (Clinacanthus nutans). The dandang gendis plant (Clinacanthus nutans) contains palmitic acid, which can reduce excess blood sugar levels in the blood. This study aims to determine the potential of palmitic acid compounds from the Dandang gendis plant as an in silico AMPK protein activator. Qualitative descriptive method using molecular docking in silico method to determine the affinity and interaction with palmitic acid compounds in the dandang gendis plant in activating AMPK protein. Docking between palmitic acid and AMPK produces an energy value (∆Gbind) of -5.3 kcal/mol. docking of metformin resulted in the lowest energy value (∆Gbind) of 5.1 kcal/mol. The bond produced by palmitic acid docking has a fairly strong affinity for the AMPK protein of -5.3 kcal/mol. So palmitic acid, an ingredient in the dandang gendis plant (Clinacanthus nutans) has the potential as an antidiabetic.

Keywords: Palmitic acid, AMPK, Dandang gendis plant (Clinacanthus nutans), antidiabetic, in silico

Background

Diabetes mellitus (DM) is one of the focuses health problems in the world whose prevalence is increasing from year to year. The morbidity and mortality of diabetics in the world are also quite high. According to WHO (2016), there were about 1.6 million cases of death directly caused by diabetes and another 2.2 million deaths caused by high glucose levels in 2012. In Indonesia, there are 10.7 million cases of diabetic, of which Indonesia ranks the 7th most in Southeast Asia. Diabetes mellitus type 2 (DMT2) is the most common type of diabetes compared to other types (World Health Organization, 2016). DMT2 is a chronic metabolic disease characterized by
Hyperglycemia. This type of diabetes is often associated with insulin resistance and pancreatic beta-cell dysfunction (Bellou et al., 2018; Müller-Wieland et al., 2019). In type 2 diabetes, there is one enzyme molecule whose presence has a major effect on insulin resistance, namely adenosine monophosphate protein kinase (AMPK). AMPK is an enzyme involved in the regulation of metabolic homeostasis. It is known that AMPK activation in the liver can reduce gluconeogenesis and lipogenesis through the downregulation of certain genes. In muscle, AMPK activation increases glucose uptake, mitochondrial genes, and lipid oxidation. Therefore, AMPK plays an important role in the pathogenesis of T2DM. Treatment of DMT2 until now still needs attention. Currently, the treatment of diabetes uses oral hypoglycemic drugs (Joshi et al., 2019). It is known that metformin is one of the most commonly used DMT2 hypoglycemic drugs and works in increasing insulin sensitivity. This is related to the activation of AMPK (Janani & Ranjitha Kumari, 2015; Nandipati et al., 2017). Therapy for T2DM is generally effective, but many unexpected side effects arise such as gastrointestinal disturbances, nausea, vomiting, dizziness, tremors, and hypoglycemia and some patients may be intolerant of side effects (Putra et al., 2017).

In this study, palmitic acid (PA) compound from the plant Clinacanthus nutans was used which is thought to have the potential as an antidiabetic. Clinacanthus nutans (CN) is an herbal plant that is widely used to treat various diseases in the community (Alam et al., 2016). The antidiabetic potential of Clinacanthus nutans (CN) leaves is currently understudied and has not yet explained the mechanism of this plant in activating AMPK. Supported by the result of Imam’s research (2019) which was tested on mice showed that the CN plant has the potential as an antidiabetic. In this study, type 2 DM model rats given CN leaf extract showed better glycemic control results and their lipid profile significantly improved (Imam et al., 2019).

Based on this explanation, further research is needed on the potential of palmitic acid as a ligand and AMPK protein as a receptor. In silico is an information technology-based method for creating computational models or simulations that can be used to predict, and suggest hypotheses regarding the latest advances in treatment and therapeutic discoveries (Ekins et al., 2007).

Materials and Methods

The ligands used in this study were palmitic acid as a test ligand with code 985 (Ismail et al., 2020), metformin as a comparison ligand with code 4091, native ligand ADP 1327, ADP 1328, and AMP contained in the protein structure of AMPK with code 2Y8L and ATP from protein crystal 2V92 with code 5957.

Ligand preparation

Palmitic acid and metformin ligands are downloaded via the PubChem website in *.sdf format and can be accessed at http://pubchem.ncbi.nih.gov. The ligand file is opened using the PyMOL application or UCSF Chimera (software discovery studio visualizer) and saved in PDB format (*.pdb).

Receptor protein preparation

AMPK code 2Y8L is downloaded in the form of a PDB file (*.pdb) via the protein data bank (PDB) website in 3D. The downloaded file is then opened via PyMOL and Chimera software. Cleaning unnecessary chains and separating water molecules from AMPK protein so that it can be used in the molecular docking process. Addition of hydrogen atoms into AMPK to provide partial charges. The final result in cleaning and partial loading is stored in the format (*.pdb).

Human intestinal absorption (HIA) test

The test is used to predict the absorption of palmitic acid compounds. It can be accessed through the online pre-ADMET site http://preadme.bmdrc.org/. The uploaded ligand structure is in the form of a file format (*.mol).
**Lipinski rule of five test**
This test predicts whether palmitic acid compounds used as oral drugs can work actively and can enter cells. Accessible via the SCFBio Lipinski website.

**Molecular docking**
Change the receptor and ligand data format which previously had *.pdb format to *pdbqt. Doing molecular anchoring using AutoDock Vina online software at Mcule by setting the active receptor site grid which is then running. The docking results are saved in PDB format and the binding affinity value data is stored in Microsoft Excel.

**Docking result visualization**
The docking results are visualized in 2D and 3D using discovery studio visualizer software and Ligplot+1.4.5.

## Result and Discussion

**Prediction of compound absorption with human intestinal absorption (HIA) parameters**
The compound is said to have good absorption when the Human Intestinal Absorption (HIA) test value is more than 70%, moderate is 20-70%, and 0-20% low (Nerkar et al., 2012). In the HIA test, the percentage of the palmitic acid compound obtained is 98.29%. Based on these results, it can be shown that palmitic acid is a compound that can be well absorbed in the body. Meanwhile, metformin has a value percentage of 45.66%. This shows that metformin has the possibility of being a compound that is quite well absorbed in the body. Both palmitic acid and metformin are well absorbed and well enough in the intestines that they can be given orally.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>HIA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic Acid</td>
<td>98.297110</td>
</tr>
<tr>
<td>Metformin</td>
<td>45.666887</td>
</tr>
</tbody>
</table>

**Prediction of potential compounds passing cell membranes with Lipinski rule of five parameters**
If a drug has been absorbed in the intestine and then enters the blood circulation, the drug will be distributed throughout the body tissues by penetrating the cell membrane so that the drug can reach the target receptor to be targeted (Tian et al., 2015). Lipinski's Rule of five can be used to determine solubility and permeability of a ligand. It is said that a ligand can be well absorbed if it fulfills this rule (Ferdian et al., 2016). Lipinski Rule of five consists of 4 parameters, namely molecular weight <500, hydrogen bond donor (HBD) <5, hydrogen bond acceptor (HBA) <10, and logP coefficient <5. This analysis is called the Rule of five because the parameter criteria above have limitations with the number 5 or multiples thereof.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Molecular weight (Da)</th>
<th>Hydrogen Bond Donor (HBD)</th>
<th>Hydrogen Bond Acceptor (HBA)</th>
<th>logP</th>
<th>Molar Refractivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic Acid</td>
<td>556.00</td>
<td>1</td>
<td>2</td>
<td>5.55</td>
<td>77.94</td>
</tr>
<tr>
<td>Metformin</td>
<td>129.00</td>
<td>5</td>
<td>5</td>
<td>-1.24</td>
<td>37.22</td>
</tr>
</tbody>
</table>

Based on the table above, the data on palmitic acid compounds needed in the Lipinski Rule of five tests are obtained, namely the value of molecular weight 256, Hydrogen Bond Donor (HBD) 1, Hydrogen Bond Acceptors (HBA) 2, logP value 5.55 and molar refractivity 77.94, while The metformin compound data obtained were the molecular weight value 129, HBD 5, HBA 4, logP value -1.24 and molar refractivity 37.22.
Molecular weight describes the size of a molecule, the larger the size of the molecule, the more it affects the compound in penetrating the cell membrane. It is said that absorption in the intestines and blood vessels of the brain takes place well when the molecular weight of the ligand is less than 500. The palmitic acid compound obtained a molecular weight of 256 which indicates that this compound complies with the Lipinski molecular weight criteria, while the molecular weight of the drug metformin also meets the molecular weight criteria, that is equal to 129. It is known that hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) correlates with intermolecular bonds. The value of hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) can affect the compound in reaching its target cell, if the value of HBD and HBA is greater than more hydrogen bonds are formed and slow the compound to reaching its target so that the Lipinski Rule of five rules, the values of HBD and HBA are limited (Ekins et al., 2005; Abad-Zapatero, 2007; Lipinski et al., 2012). The values of HBD and HBA on palmitic acid compounds obtained values of 1 and 2 which indicate that the palmitic acid compound meets the criteria for HBD less than 5 and HBA less than 10. In metformin, the HBD value is 5 which means it does not meet the criteria because the value is not below five or equal to five, while the HBA value is 5 which means it is by the HBA criteria less than 10.

Lipophilicity or LogP describes the lipophilicity of a compound. A positive LogP value indicates the non-polar nature of the compound while a negative value indicates the polarity of the compound. The higher the lipophilicity of a compound, the better the ability of the ligand to penetrate the lipid bilayer of the cell membrane (Ekins et al., 2005; Abad-Zapatero, 2007; Lipinski et al., 2012). Based on the data obtained, the LogP value of the palmitic acid compound is 5.55, which means that this compound does not meet the criteria because the LogP value is more than 5 while the LogP value for metformin is -1.24, which means it is in accordance with the criteria because the value is below 5. A drug is declared capable of penetrating cell membranes if it meets at least 2 of the Lipinski Rule of five (Tian et al., 2015). Research conducted by Ismail et al, by using the same ligand, namely palmitic acid, three of the four criteria obtained have the same values as those obtained in this study, using different software, namely SwissADME, including molecular weights 256 Da, HBD 1, HBA 2 and LogP 4.19 (Ismail et al., 2020).

Based on the data obtained above, the compound palmitic acid violates 1 of the 4 Lipinski Rule of five criteria, namely the value of LogP equal to 5.55 which should be less than 5. The same is the case with the comparison compound, namely metformin which also violates 1 of 4 criteria, namely the HBD value of 5 which should have an HBD value of <5. However, this can still be tolerated and it can be said that the compound can penetrate the cell membrane so that the ligand can bind to the target receptor because it has fulfilled at least 2 of the Lipinski Rule of five rules. Both palmitic acid and metformin are safe for consumption and used as oral drugs.

**Optimization and preparation of three-dimensional structures of palmitic acid compounds and AMPK receptors**

The three-dimensional structure of the palmitic acid test compound obtained from the [https://pubchem.ncbi.nlm.nih.gov/](https://pubchem.ncbi.nlm.nih.gov/) page with code 985 was downloaded and the storage format changed from *.sdf to *.pdb format by using the Discovery Studio Visualizer software. Furthermore, assisted with AutoDock software to do geometry optimization to get the lowest energy of the test compound. The macromolecular structure used in the docking process was downloaded from the Protein Data Bank (PDB) page [https://www.rcsb.org/](https://www.rcsb.org/) with code 2Y8L (Figure 1). The macromolecular structure is bound in the form of a water molecule and its native ligand. Water molecules and native ligands were removed from the macromolecules because they could interfere with the docking process (Figure 2). After that, optimization is carried out in the form of adding a partial load so that it can be used in the docking process (Figure 2).
Ligand-receptor molecular docking test results

The Molecular Docking test was carried out using several software including PyMOL, Chimera, AutoDock, and Discovery Studio Visualizer. The lowest root means score deviation (RMSD) obtained through the docking process between AMPK and native ligands ADP 1327 and ADP 1328, was 1.078 and 1.136, respectively. for native ligands, ADP 1327 and ADP 1328 have the lowest binding affinity (ΔGbind) values of -7.9 kcal/mol and -8.3 kcal/mol. The molecular docking test is said to be valid if the RMSD value is below 2.5 Å (Baber et al., 2009). This indicates that the molecular docking method is valid and meets the validation parameter requirements because the RMSD value is less than 2.5 Å.

Table 3. Results of native ligand docking validation

<table>
<thead>
<tr>
<th>No.</th>
<th>Native ligand</th>
<th>Binding affinity (Kcal/mol)</th>
<th>RMSD (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ADP 1327</td>
<td>-7.6</td>
<td>1.078 A</td>
</tr>
<tr>
<td>2</td>
<td>ADP 1328</td>
<td>-8.0</td>
<td>1.136 Å</td>
</tr>
</tbody>
</table>

Docking simulations were carried out between the test compound and the comparison compound against AMPK, using the region and the anchoring center (grid box) adjusted to the complex
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ligand-binding region (native ligand) at the 2Y8L receptor and have been validated. All ADP, AMP, and ATP ligands produce negative ΔGbind values, in the sense that binding of these ligands occurs spontaneously and results in non-covalent binding interactions. The smallest value of ΔG ATP is -8.6 kcal/mol, lower than the value of ΔG AMP of -8.3 kcal/mol and ΔG ADP of -7.6 kcal/mol and -8.3 kcal/mol. The results of docking between palmitic acid and AMPK resulted in a ΔGbind value of -5.3 kcal/mol (Table 4). Meanwhile, metformin has a ΔGbind value of -5.1 kcal/mol. Based on these data, the two compounds above have binding affinity values that are greater and closer to the value of ΔG AMP or at least have a value of around ΔG ADP which is predicted to trigger AMPK activation and prevent AMPK dephosphorylation. On the other hand, if the value of the two compounds is smaller or closer to the value of ΔG ATP, it is predicted that it will inhibit AMPK activation and trigger AMPK dephosphorylation. This prediction leads to the natural state of AMPK regulation of the subunit where attachment of AMP to this subunit will trigger the phosphorylation of Thr172 residues on the subunit so that AMPK is active. ADP attachment is known to only play a role in preventing AMPK dephosphorylation. Unlike the case with ATP, if ATP attaches to the y subunit, it will inhibit the phosphorylation of the Thr172 residue so that AMPK is inactive. AMP has a role as an allosteric activator of AMPK, ADP as an active structural defense so that AMPK remains active while ATP acts as an allosteric inhibitor of AMPK (Carling, 2005; Hardie, 2008; 2015; Lim et al., 2010; Xiao et al., 2013; Calabrese et al., 2014). Based on data on ΔGbind values, palmitic acid and metformin compounds are predicted to potentially trigger AMPK activation and prevent AMPK dephosphorylation because their ΔGbind values are closer to AMP values or at least around ΔG ADP and greater than ΔG ATP.

Table 4. Validation results of the test compound docking with comparison

<table>
<thead>
<tr>
<th>No.</th>
<th>Ligand</th>
<th>Binding affinity (Kcal/mol)</th>
<th>RMSD (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ATP</td>
<td>-8.5</td>
<td>1.158 Å</td>
</tr>
<tr>
<td>2</td>
<td>AMP</td>
<td>-8.0</td>
<td>0.464 Å</td>
</tr>
<tr>
<td>3</td>
<td>Palmitic acid</td>
<td>-5.0</td>
<td>1.708 Å</td>
</tr>
<tr>
<td>4</td>
<td>Metformin</td>
<td>-4.6</td>
<td>1.504 Å</td>
</tr>
</tbody>
</table>

The binding formed at the AMPK receptor and all ligands used were analyzed and visualized in 2D using LigPlot+ 1.4.5 software. The bond interactions analyzed are in the form of hydrogen bonds and hydrophobic bonds. It can be seen in the results of the binding of ATP (Figure 3) that hydrogen bonds and hydrophobic bonds are produced more than ADP, which is more than AMP. One of these conditions can occur because ATP has more phosphate groups than ADP more than AMP. The phosphate group has an O atom that can form hydrogen bonds, as well as hydrophobic bonds. The more bonds formed, the more negative ΔG values are produced (de Beer et al., 2010). This can be seen in Table 4 which shows that ATP does have a more negative ΔG value than ADP than AMP.

The similarity of the interaction between the receptor and the ligand is carried out because it can be a mimetic indicator between the test ligand and the known role of the ligand. The high similarity presentation shows the similarity of properties so that it can be predicted to have the same role as the comparison ligand (Ferdian et al., 2016). The interaction similarity data in Table 5 shows that metformin has residues that tend to have a greater similarity to AMP than to ATP. It was characterized by the presence of the same residue which was only found in AMP ligands, namely, Ser225 and Ser315. Meanwhile, the palmitic acid residues formed tend to have a greater similarity to ADP and ATP. However, most of these residues tend to be closer to ADP than to ATP because the hydrogen bonds present in this compound are also found in ADP hydrogen bonds, including His297, Ser241, and Arg298. Both metformin and palmitic acid have residues with greater similarity to AMP and ADP than ATP, which means that both compounds are predicted to be able to act as allosteric activators of AMPK by direct binding. Based on the greater similarity of metformin to AMP compared to the similarity of palmitic acid, which is greater than ADP is greater, ATP is greater than AMP, then palmitic acid compounds can be said to be able to activate
AMPK *in silico* but not as good and effective as metformin which has the potential to be an anti-diabetic compound.

![Figure 3. Hydrophobic and hydrogen bonds between AMPK receptors and ligands a) ADP 1237; b) ADP 1238; c) metformin; d) palmitic acid (PA); e) AMP; and f) ATP](image1)

**Table 5. Amino acid residues that form hydrogen bonds and hydrophobic bonds**

<table>
<thead>
<tr>
<th>Ligand Model</th>
<th>Hydrogen Bonds</th>
<th>Interacting Residual</th>
<th>Hydrophobic Bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP 1237</td>
<td>His150; Thr88; Arg151</td>
<td>Lys148; Ile149; Lys126; Thr86</td>
<td></td>
</tr>
<tr>
<td>ADP 1238</td>
<td>Arg151; Arg69; Arg298; His297; Lys169; Ser241; Leu276; Asp244</td>
<td>Val296; Ile239; Gly274; Val275; Phe243</td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>Arg151; Arg268; Arg298; Phe243; Ser225; Leu276; Asp244; His297</td>
<td>His150; Lys242; Ser241; Ile239; Val296; Val275; Gly224</td>
<td></td>
</tr>
<tr>
<td>AMP</td>
<td>Ala204; Ala226; Arg298; Ser313</td>
<td>Thr199; Ser225; Ile203; Ile311; Val224; His150; Ser315; His297</td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>Ser225; Ser313; Ser315</td>
<td>Arg298; Val224; Ile311; Ala226</td>
<td></td>
</tr>
<tr>
<td>Palmitic Acid</td>
<td>His297; Ser241; Arg298</td>
<td>Phe243; Ile239; Arg268; Asp244; Val296; Phe272; Leu276; Gly274</td>
<td></td>
</tr>
</tbody>
</table>

Apart from the binding energy produced by palmitic acid to AMPK protein, palmitic acid is one of the ingredients found in the dandang gendis plant (*Clinacanthus nutans*). This can be proven in a previous study by Murugesu et al. in 2019 which proved that palmitic acid compounds were contained in the dandang gendis plant (Murugesu et al., 2019). In the research of Ismail et al. in 2020, it was proven that the palmitic acid content in the dandang gendis plant (*Clinacanthus nutans*) had a fairly high level compared to other types of plants which is 23.84% (Ismail et al., 2020).

Previous research by Dewinta et al. in 2020 that the administration of ethanol extract of dandang gendis leaves (*Clinacanthus nutans*) has potential as an antidiabetic by lowering blood sugar levels in diabetic white Wistar rats. This is because the leaves of dandang gendis (*Clinacanthus nutans*) contain flavonoids and fatty acids as antioxidants that can lower blood sugar levels (Dewinta et al., 2020). Another research conducted by Panggabean et al. in 2014 examined the "Activity Test of Increasing Insulin Sensitivity of Black Cumin Seed Extract (*Nigella sativa*) Through Measurement of Tyrosine Concentration Phosphorylated Insulin Receptor Substrate-1 (IRS-1)" that the administration of black cumin extract which has various contents, one of which is palmitic...
Acid can increase insulin sensitivity and lower blood glucose levels. In this study, it was found that giving black cumin extract to diabetic rats for 30 days was able to increase phosphorylated tyrosine levels on insulin receptor substrate-1 (IRS-1), insulin sensitivity and significantly reduce blood glucose levels at a dose of 96 mg/kg BW (Panggabean et al., 2014). The possible mechanism of action of palmitic acid in Clinacanthus nutans is as an AMPK agonist but needs to be proven in further studies in vivo. Further research is needed on the mechanism of action of other compounds in Clinacanthus nutans against AMPK.

**Conclusion**

Based on the results of the study, it can be concluded that the compound palmitic acid from the plant extract of Clinacanthus nutans has antidiabetic potential through in silico binding of the receptor-ligand to the AMPK active site. Palmitic acid compounds from plant extracts of Clinacanthus nutans can also be well absorbed in the body and can be used as oral drugs through the Human Intestinal Absorption (HIA) test and the Lipinski Rule of five tests.

**Acknowledgment**

The author expresses his gratitude along with the prayers and hopes of Jazakumullah Ahsanal Jaza to all those who have helped the completion of this thesis. The author expresses his gratitude to dr. Nurlaili Susanti, M. Biomed and dr. Zulvikar Syambani Ulhaq, M. Biomed, Ph.D as the thesis supervisor who has provided direction and guidance so that the author can arrange this research properly. The author's love goes to both parents who always provide prayers, material and non-material support, and their endless blessings to the author.

**References**


