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THE POTENTIAL ROLE OF IN SILICO APPROACHES TO IDENTIFY BERBERINE FROM NATURAL RESOURCES AS A NATURAL ANTIHYPERLIPIDEMIC AGENTS

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ABSTRACT

Objective: Computational chemistry method approach is very helpful in finding natural active compounds through the interaction of active compounds with target receptors. This study aims to determine the mechanism of berberine compounds derived from *Phellodendron amurense* plants known by traditional Chinese medicine has fat-reducing activity. This study used 3 target molecules related to lipid metabolism; Niemann Pick C1 Like1 protein (NPC1L1), Lanosterol 14α-Demethylase (LDM), and Squalene Synthase (SqS) are known to be implicated in the physiology of hyperlipidemia. The interactions of Berberine was compared with the respective co-crystallized native ligands at the active sites of these receptors. **Materials and Methods:** The molecular docking method begins by preparing the ligand and receptor. Ligand and receptors files could be obtained by downloading at Pubchem, and Protein Data Bank (PDB). The receptor and ligand setup was done with Pyrx, MgTool, followed by docking and visualization processes using AutoDock Vina and Discovery Studio Visualizer. **Results:** The berberine ligand binding activity score with the NPC1L1 receptor is -8.3 kcal/mol, with LDM -5.2 kcal/mol, and with SqS -9.2 kcal/mol. **Conclusions**. The results of the molecular docing method concluded that the berberine compound has activity as an antihyperlipidemia through inhibitory activity at the NPC1L1 and SqS receptors.

KEYWORDS: molecular docking, antihyperlipidemic, berberine, NPCL1, LDM, SqS.

INTRODUCTION

Hyperlipidemia is a clinical condition which is characterized by an increase in one or more plasma lipids, including triglycerides, cholesterol, cholesterol esters, phospholipids and or plasma including very lowdensity lipo protein and low-density lipoprotein, and reduced high-density lipoprotein level.[1,2] Hyperlipidemia is considered as an important risk factor that causes cardiovascular disease.[3] The treatment approach with natural compounds is considered to have good physiological effects with low toxicity, non-mutagenic, and avoidable side effects and drug resistance.[4]

Berberine in dosage form is known as Berberine hydrochloride, an isoquinoline alkaloid compound, which is the result of extraction from plants such as *Coptis chinensis* and *Phellodendron amurense*.[5] This compound is used because it has an advantage in regulating blood glucose, and lipid blood cells, and endothelial blood vessels.[6] Studies have shown that berberine has activity in treating type 2 diabetes mellitus, hypertension and hyperlipidemia.[7]

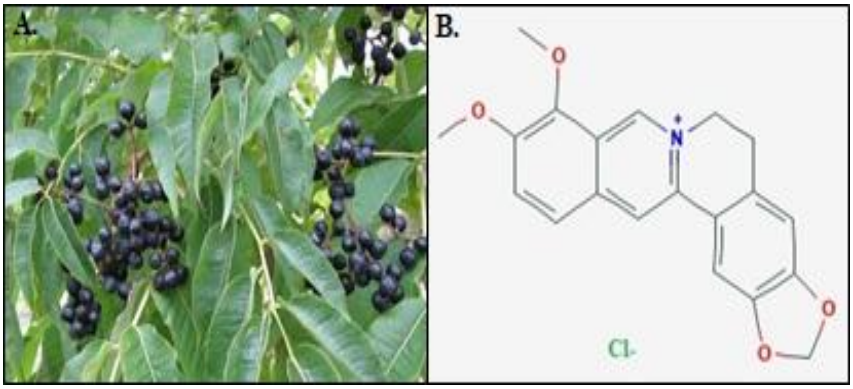

Figure 1: (A). *Phellodendron amurense*, (B). Structure of Berberine hydrochloride.

A rational drug design approach based on structural modeling and rapid screening methods provides alternatives in the development of antihyperlipidemic molecules. The use of molecular docking methods that explain the interaction between ligand binding to proteins on the active side through a three-dimensional structure illustration. This docking method is able to screen molecules based on their free bond energies and hypothesize how molecules could inhibit targets.[8]

The molecular docking approach of berberine active molecules that will be used as hyperlipidemic agents uses 3 molecular targets, namely Niemann Pick C1 like1 protein (NPC1L1), lanosterol 14α-demethylase (LDM), and squalene synthase (SqS). Niemann-Pick C1 Like-1 (NPC1L1) has an important role in transporting cholesterol through the proximal

small usu membrane. NPC1L1 transfers intestinal sterols through a direct mechanism as a consequence of the identification of ezetimibe molecular targets. NPC1L1 (PDB code ID: 3QNT) is a molecular target for ezetimibe cholesterol-lowering drugs.[9, 10, 11]

Lanosterol 14α-demethylase (LDM) is an inhibiting enzyme as a mechanistic probes and helps improve hypercholesterolemia. Elimination of cholesterol compounds from the l4α-methyl lanosterol group through biosynthesis catalysis was also assisted by the cytochrome P450 enzyme.[12, 13]

Squalene synthase (SqS) is the main enzyme in regulating cholesterol biosynthesis through the formation of squalene which acts as the main cholesterol precursor. This process through the mechanism of the dimerization reaction between two molecules of arnesyl diphosphate (FPP), therefore SqS has always been the main focus in the study of hyperlipidemia.[14, 15]

MATERIALS AND METHODS

Software and Tools

Protein Data Bank (PDB), PubChem, Chem Draw Ultra 12.0, AutoDock Vina 1.1.2, MGL tools, Discovery Studio Visualizer, Pyrx.

Ligand Preparation

Berberine hydrochloride and various ligands (positive control) were used as ligands for docking studies were listed in Table 1.

Table 1: Ligands used in the study.

No	Ligand	Molecular Formula	References
1	Berberine	$C_{20}H_{18}NO_4^+$	[6]
2	Ezetimibe	$C_{24}H_{21}F_2NO_3$	[11]
3	Ketoconazole	$C_{26}H_{28}Cl_2N_4O_4$	[13]
4	INO	$C_{30}H_{31}ClN_2O_7$	[15]

Protein Preparation

Data on protein structure can be downloaded at Potein Data Bank (http://www.rcsb.org). The protein used is Niemann-Pick C1 Like-1 (NPC1L1; PDB ID: 3QNT), Lanosterol 14α-demethylase (LDM; PDB ID: 3LD6), Squalene synthase (SqS; PDB ID: 1EZF). The file for each receptor in the PDB file format will first be converted to PDBQT file format using the Open Babel.

Docking Studies Using AutoDock Vina

The berberine structure and energy-minimized ligand (positive control) bind to the target protein using AutoDock Vina 1.1.2. Modified receptor files containing atomic and ligand loads must first be changed from the pdb format to the PDBQT file format using the Open Babel program. The docking process was done with the receptor condition closed inside the box, with a box spacing of 1 Å, keeping the receptors stiff and ligands as flexible molecules. The interaction energy between the ligand and receptor was calculated for all binding sites and was expressed as affinity (kcal / mol).

Protein–Ligand Interactions

The Discovery studio visualizer program was used to explain the interactions between proteins and ligands that occured. Docking output files were visualized in 2D interactions. Receptor ligand interactions contain informative data from intermolecular interactions, including types of hydrogen bonds, bond distances, hydrophobics, and types of amino acids that interact at the active site.

RESULTS AND DISCUSSION

Ligand Preparation

The ligand structure was obtained from PubChem and converted to pdb format using Open Babel. The physicochemical properties of ligands can be seen in table 2. All ligand structures are then minimized and stored in PDBQT format by the Pyrx program (Figure 2).

Table 2: Physiochemical parameters of ligand.

No	Ligand	Molecular Weight (Da)	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Log P	Minimize Energy
1	Berberine	336.36	0	4	3.096	497.77
2	Ezetimibe	409.43	2	3	4.888	1000.43
3	Ketoconazole	531.44	0	7	4.206	827.93
4	INO	567.04	0	8	4.795	1414.80

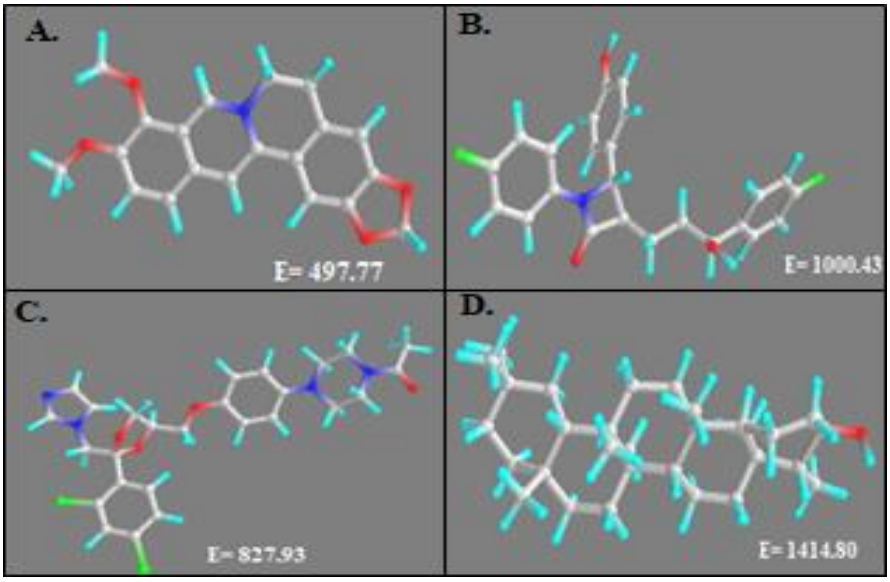

Figure 2. 3D ligand structure and energy minimized results. (A). Berberine, (B). Ezetamide, (C). Ketoconazole, (D). INO.

Protein Preparation

Protein as a molecular target for antihyperlipidemia is downloaded from the Protein Data Bank and (PDB), then converted to PDBQT format using the AutoDock tool (Figure 3). Proteins that were in PDBQT format were ready for docking with ligands.

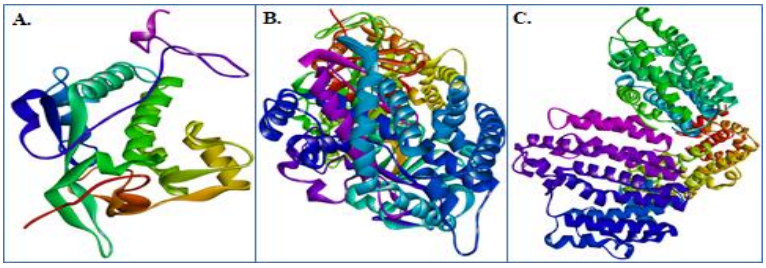

Figure 3: 3D structure of receptor, (A) Niemann-Pick C1 Like-1 (NPCL1), (B) Lanosterol 14α-Demethylase, (C) Squalene Synthase (SqS).

Docking Studies Using AutoDock Vina

Docking of Berberine into PDB structure of Niemann-Pick C1 Like-1 (PDB ID: 3QNT)

The results of molecular docking between ezetimibe and berberine against NPCL1 receptors had the same amino acid interactions: Arg 80, Asn 122, Glu 238, Gln 257. Where the interactions of ezetimide and NPC1L1 receptors were hydrogen bonds in Ala 241 amino acids. The NPC1L1 receptor forms hydrogen bonds on His amino acid 126, and carbon hydrogen bonds on Ser 130. Doking analysis was done by evaluating the binding affinity score which shown that berberine ligand had a value (-8.3 kcal/mol) better than ezetimibe

ligand (-4.9 kcal/mol). This could explain that berberine had antihyperlipidemic activity through inhibitory mechanisms at NPC1L1 receptors.

Docking of Berberine into PDB structure of Lanosterol 14α-Demethylase (PDB ID: 3LD6)

The results of molecular docking between berberine ligands and ketoconazole on the LDM receptor showed significant differences, there were no similarities in binding to the same amino acids. Ketoconazole had 2 hydrogen bonds on the amino acid Pro 376, and Met 487 and had a carbon hydrogen bond on the amino acid Ile 379. In the interaction of berberine ligands and LDM receptors, hydrogen bonds were not formed, only van der walls were formed which were bonds with low energy at the amino acid Phe 84, Asn 87, Ser 80, Glu 83, Asn 87, Tyr 92, Ser 80, Ala 76, His 73. Evaluation of binding affinity score obtained by berberine ligand score -5.2 kcal/mol, this score was lower compared to ketoconazole ligand (-10.0 kcal/mol) on the LDM receptor. It could be concluded that berberine did not have antihyperlipidemic activity through the mechanism of inhibition of LDM receptors.

Docking of Berberine into PDB structure of Squalene Synthase (PDB ID: 1EZF)

There were many similarities in amino acids that form bonds between ligands INO, berberine with SqS receptors, namely the amino acids Phe 54, Tyr 73, Asp 80, Ala 176, Ala 179, Gly 180, Leu 183, Leu 211, Phe 288, pro 292. In the interaction of INO ligands and SqS receptors, hydrogen bonds were formed at Thr 214, Asn 215, Arg 218, while berberine ligands with Sqs receptors form hydrogen bonds at Cys 289, and carbon-hydrogen bonds at Gln 212. Docking evaluation results by looking at binding activity scores showed that the berberine bond with the Sqs receptor had a binding activity score of -9.2 kcal/mol lower than the INO ligand with the SqS receptor of -10.0 kcal/mol. It can be concluded that berberine ligand has antihyperlipidemia activity through the mechanism of Sqs receptor inhibition. Comparison of binding activity scores between ligands and receptors is in table 4.

Table 4: Comparative binding affinity of different ligands with receptors.

No.	Receptor	Ligand	Binding Affinity (kcal/mol)
1	Niemann-Pick C1 Like-1 (NPC1L1)	Ezetimibe Berberine	-4.9 -8.3
2	Lanosterol 14α-Demethylase (LDM)	Ketoconazole Berberine	-10.0 -5.2
3	Squalene Synthase (SqS)	INO Berberine	-10.2 -9.2

The results of the visualization of ligand and receptor interactions with The Discovery studio visualizer program can be seen in Figure 4.

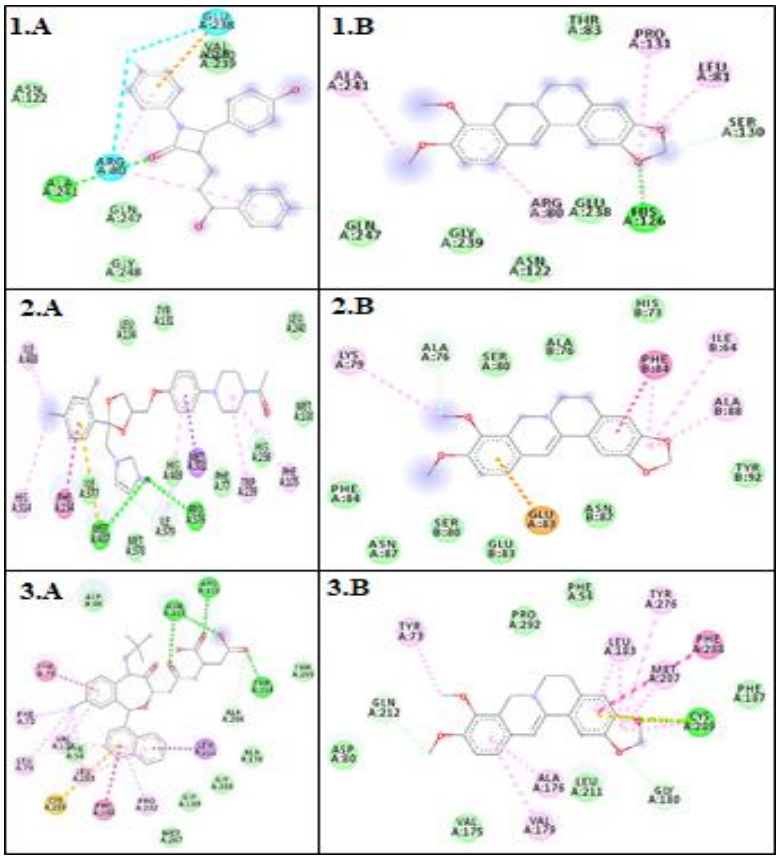

Figure 4: Interaction of ligands and target receptors. (1.A) Ezetemibe bound to NPCL1, (1.B) Berberine bound to NPCL1, (2.A) Ketoconazol bound to LDM, (2.B) Berberine bound to LDM, (3.A) INO bound to SqS, (3.B) Berberine bound to SqS.

CONCLUSIONS

Molecular docking studies of berberine compounds from the *Phellodendron amurense* plant have potential antihyperlipidemic activity through the mechanism of inhibition of NPC1L1 and SqS receptors.

REFERENCES

1. Mishra, P. R., Panda, P. K., Apanna, K.C., Panigrahi, S. Evaluation of acute hypolipidemic activity of different plant extracts in Triton WR-1339 induced hyperlipidemia in albino rats. *Pharmacologyonline*, 2011; 3: 925-934.
2. Jeyabalan, S., Palayan, M. Antihyperlipidemic activity of Sapindusemarginatus in Triton WR-1339 induced albino rats. *Res. J. Pharm. Tech*, 2009; 2(2): 319-323.

3. Jorgensen, T., Capewell, S., Prescott, E., Allender, S., Sans, S., Zdrojewski, T. Population-level changes to promote cardiovascular health. *Eur. J. Prev. Cardiol*, 2013; 20(3): 409-21.
4. Kong W, Wei J, Abidi P, Lin M, Inaba S, Li C, et al. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nat Med, 2004; 10: 1344–51.
5. Sirtori CR, Pavanello C, Calabresi L and Ruscica M: Nutraceutical approaches to metabolic syndrome. Ann Med, 2017; 49: 678-697.
6. Guarino G, Strollo F, Carbone L, Della Corte T, Letizia M, Marino G and Gentile S: Bioimpedance analysis, metabolic effects and safety of the association *Berberis aristata/Bilybum marianum*: A 52-week double-blind, placebo-controlled study in obese patients with type 2 diabetes. J Biol Regul Homeost Agents, 2017; 31: 495-502.
7. Tabeshpour J, Imenshahidi M and Hosseinzadeh H: A review of the effects of Berberis vulgaris and its major component, berberine, in metabolic syndrome. Iran J Basic Med Sci, 2017; 20: 557-568.
8. DiMasi, J.A.; Hansen, R.W.; Grabowski, H.G. The price of innovation: New estimates of drug development costs. J. Health Econ, 2002; 22: 151–185.
9. Kwon HJ, Palnitkar M, Deisenhofer J. The structure of the NPC1L1 N-terminal domain in a closed conformation. PLoS ONE, 2011; 6: e18722.
10. Ge L, Wang J, Qi W, Miao HH, Cao J, Qu YX, Li BL, Song BL. The cholesterol absorption inhibitor ezetimibe acts by blocking the sterol-induced internalizationof NPC1L1. Cell Metab, 2008; 7: 508–519.
11. Arya N, Kharjul MD, Shishoo CJ, Thakare VN, Jain KS. Eur J Med Chem. . Some molecular targets for antihyperlipidemic drug research, 2014.
12. Strushkevich N, Usanov SA, Park HW. Structural basis of human CYP51 inhibition by antifungal azoles. J Mol Biol, 2010; 397: 1067–1078.
13. Gibbons GF. The role of cytochrome P450 in the regulation of cholesterol biosynthesis. Lipids, 2002; 37: 1163–1170.
14. Pandit J, Danley DE, Schulte GK, Mazzalupo S, Pauly TA, Hayward CM, Hamanaka ES, Thompson JF, Harwood HJ Jr. Crystal structure of human squalene synthase. A key enzyme in cholesterol biosynthesis. J Biol Chem, 2000; 275: 30610–30617.
15. Nikitakis A, Kourounakis AP. QSAR of substituted morpholines with antioxidant and squalene synthase inhibitory activity. Med Chem Res, 2011; 20: 566–575.