HESPERIDIN INTERACTION WITH HMG-COA-REDUCTASE ENZYME IN HYPERCHOLESTEROLEMIA: A STUDY IN SILICO

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Abstract: Dyslipidemia is* a degenerative disease occurred *with increased levels of fat and cholesterol levels in blood. *One of the proteins used as anti-*cholesterol *is* an HMG-*CoA-Reductase. Hesperidin in orange peel can reduce cholesterol levels by interacting with HMG-CoA-Reductase. To prove this, an in silico method was used by using swissdock.ch (http://swissdock.ch/docking#). The receptor protein in dyslipidemia was obtained from the RCSB Protein Data Bank (https://www.rcsb.org) namely HMG-CoA-reductase receptor with code PDB: 1HW9. The natural ligand, hesperidin, was obtained from PubChem with code: 10621 (https://pubchem.ncbi.nlm.nih.gov/). Protein was prepared by omitting the natural ligand residues present in the protein. Ligand and protein preparations were used by the chimera 1.15. The result of this study indicated that the interaction of hesperidin with several amino acid recidues was predicted to provide inhibitory activity on HMG-CoA reductase as the protein target. Inhibition of HMG-CoA reductase will reduce mevalonate synthesis so that cholesterol levels will decrease.*

Keywords: *hesperidin, HMG-CoA-Reductase, cholesterol, dyslipidemia*

INTRODUCTION

Hypercholesterolemia is a condition of increasing cholesterol levels in blood more than 240 mg/dL. The number of people with hypercholesterolemia increases along the age. Patients with hypercholesterolemia in the age of 20 is around 7% while other 44% patients are in the age of more than 60. Therefore, it is not surprising that an estimated 2.6 million deaths are caused by this disease.[1]

Statins are medicines that are usually used for people with hypercholesterolemia. Statins work by blocking HMG-CoA reductase to decrease cholesterol production. Long-term consumption of statins can cause side effects such as muscle pain, liver damage, rhabdomyolysis, and kidney failure. Due to these side effects, alternative medicines that are more secure and affordable are needed. One of those alternative medicines is lemon peel (Citrus lemon.[2] Lemon peel contains an active compound hesperidin (figure 1) which is called a flavonoid glycoside that has difenol structure. Hesperidin has an aglycone (Hesperetin or Methyleriodictyol) bond to rutinose (6-O-(α-l-Rhamnopyranosyl)-D-glucopyranose) and or (6-O-(α-l-Rhamnosyl)-D-glucose).[3]

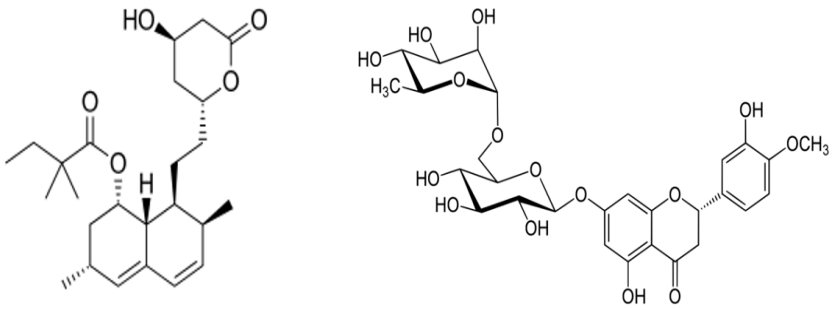


Figure 1. Structure of (a) Simvastatin and (b) Hesperidin

Hesperidin can be found in bergamot, banana, lemon, and lemon peel. Hesperidin is used as an anti-inflammatory, antioxidant, anti-cholesterol, anti-obesity, and also can lower blood pressure levels. Hesperidin is able to increase fat metabolism and regulate hepatic cholesterol synthesis by blocking HMG-CoA reductase activity.[3] HMG-CoA reductase is a key enzyme for lipid metabolism. Inhibition of this enzyme can reduce cholesterol biosynthesis in the liver, including HMG Coenzyme A reductase a (hmgcra), HMG Coenzyme A reductase b (HMGCRB), and 3-hydroxy-3-methylglutaryl-CoA synthase (hmgcs) which regulate cholesterol synthesis genes.[4]

In the previous research stated that an ethanolic extract of lemon peel in the form of hesperidin which can lower cholesterol level in rats by increasing LDL receptors and decreasing HMG-CoA reductase[2]. However, the mechanism of hesperidin in inhibiting HMG-CoA reductase has not yet known. Therefore, this study will examine the interaction of hesperidin HMG-CoA reductase in lowering cholesterol levels by using in silico method.

RESEARCH METHOD

HMG Co-A reductase enzyme was obtained from RCSB Protein Data Bank (https://www.rcsb.org.search) with code PDB: 1HW9.[5] Protein was prepared by omitting the natural ligand residues present in it using Chimera 1.15 program.

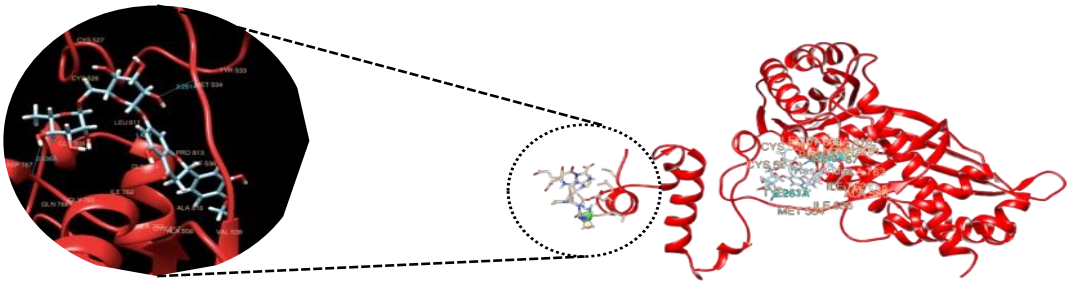
Hesperidin ligands were obtained from PubChem National Center for Biotechnology Information (https://pubchem.ncbi.nlm.nih.gov) with PubChem code: 10621 and simvastatin with PubChem code: 54454. Ligands were

prepared by reducing the ligand structure. Ligand preparation was also used in the Chimera 1.15 program. Protein docking with ligands (docking) was used by the SwissDock program (http://www.swissdock.ch/docking).

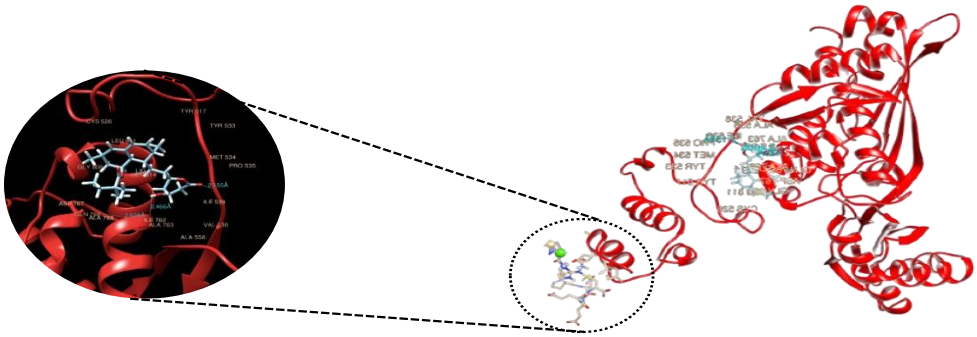
Analysis and visualization of docking results using the Chimera 1.15 program. Visualization is used to explain the interaction between the ligand and the receptor protein residues, namely in the form of amino acids bound to the ligands, hydrogen bonds that exist between the ligands and amino acids, and hydrophobic interactions.

RESULTS AND DISCUSSION

Based on the docking results obtained, the interaction of hesperidin with HMG-CoA reductase was greater than with simvastatin (figure 2).



(a)



(b)

Figure 2. HMG-CoA reductase interaction with (a) Hesperidin and (b) Simvastatin

Based on figure 2, it is known that there are hydrogen bonds between residues and ligands, which are shown in table 1.

Tabel 1. Binding free energy, hydrogen bonds, and hydrophobic interactions of HMG-CoA reductase interaction with ligand

Ligand	Binding Free Energy ΔG (kCal/mol)	Hydrogen bonds	Hydrophobic interactions
Hesperidin (Pubchem CID 10621)	-9,42	ASP 767, MET 534	ASP 767, GLN 766, GLY 765, GLY 808, LEU 811, CYS 526, CYS 527, ILE 762, ALA 763, CYS 817, GLN 814, ALA 556, VAL 538, ALA 816, ILE 536, PRO 813, MET 534, TYR 533
Simvastatin (Pubchem CID 54454)	-8,74	ILE 536, ILE 762	TYR 517, GLY 808, LEU 811, GLN 814, GLN 766, ALA 768, VAL 538, ILE 762, ILE 536, ALA 556, ASR 767, PRO 535, TYR 533, CYS 526, MET 534

This study showed that hesperidin has a lower binding free energy than simvastatin. This indicates that hesperidin has a higher affinity for the binding site of the HMG-CoA reductase enzyme than simvastatin. Based on the results of this study, hesperidin has the potential to reduce cholesterol levels better than simvastatin. The binding free energy value is the contribution of various binding effects, other electronic effects, and regression values that occur during the binding process. Experimentally the binding free energy (ΔG) is directly related to the inhibition constant. Therefore, the determination of the value of the binding free energy can predict the ability of the compound to inhibit the action of the enzyme.

Amino acid residues in HMG-CoA Reductase, that are ASP 767, GLN 766, GLY 765, GLY 808, LEU 811, CYS 526, CYS 527, ILE 762, ALA 763, CYS 817, GLN 814, ALA 556, VAL 538, ALA 816, ILE 536, PRO 813, MET 534, TYR 533 have the potential as a binding side, specifically the active site of interaction between ligand and protein that are to be expected to have a role in the process of inhibiting activity on the active site. Therefore, the inhibition on the protein target could be predicted from hesperidin which has interactions with several amino acid residues. The inhibition of HMG-CoA will reduce the synthesis of mevalonate to that cholesterol will be reduced.

HMG-CoA Reductase (HMGR) is the statins primary target enzyme to reduce cholesterol. HMG-CoA Reductase is an enzyme of the eukaryotic mevalonate pathway for isoprenoid biosynthesis[6]. Biosynthesis mechanism of cholesterol goes by four steps (figure 2) and starts with acetyl-CoA. The first step is the mevalonate synthesis from acetyl-CoA; the second stage is converts mevalonate to activated isoprenes; the third stage is squalene synthesis; fourth stage is cholesterol synthesis. HMG-CoA as a controller of the levels of cholesterol synthesis. The inhibition of HMG-CoA will cause mevalonate synthesis to be reduced so that it will decrease cholesterol.[1]

Hesperidin was first isolated from orange peel as an effective obesity therapy. Hesperidin can affects the AMP, PPAR signaling pathways and regulates the NF-kB inflammatory signaling pathway and reduces inflammation and apoptosis. Hesperidin can also directly regulate the oxidation index, inhibit apoptosis, thereby protecting against damage that caused by oxidative stress, and increasing lipid peroxidation. Research by Xiong et al[3] that shows hypoglycemic and hypolipidemic effects. Research by Xiong et al[3] states that hesperidin can change the expression of genes encoding PPAR, 3-hydroxy-3-methyl-glutatil coenzyme A(HMG-CoA) reductase and LDL receptors in Goto-Kakizaki rats with type 2 diabetes.[7]

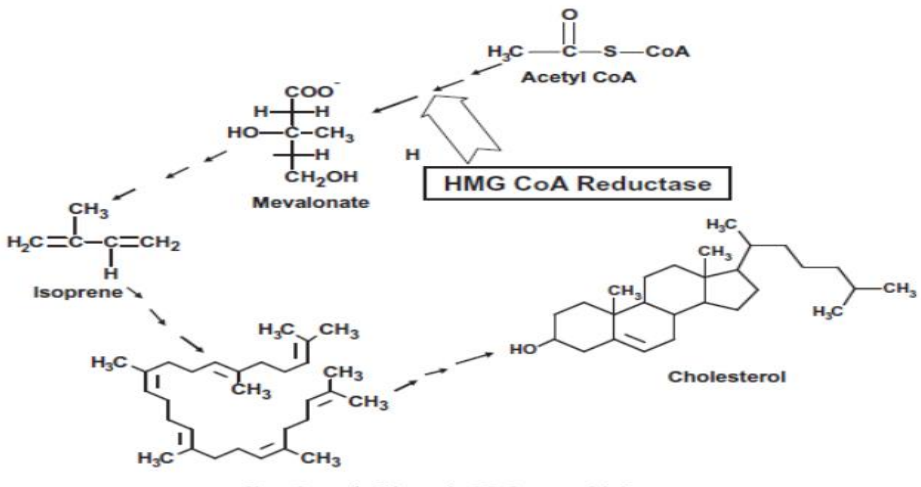


Figure 2. The synthesis of cholesterol

CONCLUSION

This study shows that the interaction between hesperidin compounds with certain amino acid residues is predicted to provide inhibitory activity on the target protein, namely HMG-CoA reductase. The potential of hesperidin inhibition in lowering cholesterol levels will be better than simvastatin. Inhibition of HMG-CoA reductase will reduce mevalonate synthesis so that cholesterol levels will decrease.

REFERENCES

1. Adelina, R., Kurniatri, A.A. Mekanisme Katekin sebagai obat antidislipidemia uji in silico. Buletin penelitian kesehatan. 2018; 46(3): 147-54
2. Hartanto, D.T., Kurniasari, E.L., Maria, R.A., Dewi, P.S. Septiani, V. Potensi ekstrak etanol kulit jeruk lemon (Citrus limon L.) sebagai obat alternatif hiperkolesterolemia pada tikus wistar hiperglikemik. Jurnal Ilmiah Farmasi. 2018; 6(2): 81-85
3. Xiong, H., Wang, J., Ran, Q., Lou, G., Peng, C., Gan, Q., Hu, J., Sun, J., Yao, R., Huang, Q. Hesperidin : a therapeutic agent for obesity. Drug design development and therapy. 2019; 13:3855-66
4. Zhao, Z., Zhong, W., Lin, H., Huang, P., Ma, N., Zhang, Y., Zhou, C., Lai, Y., Huang, S., Huang, S., Gao, L., Lv, Z. 2017. Hesperidin Protects against Acute Alcoholic Injury through Improving Lipid Metabolism and Cell Damage in Zebrafish Larvae. Evidence based complementary and alternative medicine. 2017: 1-9
5. Singgih, M., Permana, B., Maulidya, S. A. I., Yuliana, A. Studi In silico Metabolit Sekunder Kapang Monascus sp. Sebagai kandidat obat antikolesterol dan antikanker. 2019; 15(1):104-23
6. Burg, J. S., Espenshade PJ. Regulation of HMG-CoA reductase in mammals and yeast. Progress in lipid research. 2011; 50(4): 403-10.
7. Aziz, S., Adliani, N., Sukrasno, S. Penambatan molekul senyawa turunan orizanol terhadap enzim 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reduktase. Journal of Science and Applicative Technology. 2020; 4(1):43-8.