ORIGINAL ARTICLE

Antihyperlipidemic effects of apple peel extract in high-fat diet-induced hyperlipidemic rats

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ABSTRACT

Hyperlipidemia is generally managed with statin-based drugs. Simvastatin serves as a 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) inhibitor, with prolonged use proven to cause side effects. In the present study, antihyperlipidemic material is tested for its effect in lowering lipid in animals and its proven ability to bind to HMGR. Hyperlipidemia rats were divided into four groups, with different doses of 0, 57, and 114 mg/kg BW of apple peel extract (APE) and simvastatin (3.6 mg/kg BW). The total cholesterol (TC), total triglyceride (TG), low-density lipoprotein cholesterol (LDLc), and high-density lipoprotein cholesterol (HDLc) serum were measured. *In silico* inhibition test of HMGR activity was conducted by molecular docking using PyRx software. This process places HMGR as a receptor and active compound of apple peels as a ligand. APE treatment with a dose of 114 mg/kg BW could significantly reduce LDLc and increase serum HDLc levels. Docking tests confirmed that quercetin, chlorogenic acid, epicatechin, and catechins depicted HMGR inhibition. Quercetin could bind to HMGR at a similar location to amino acid residues as simvastatin. These material extracts have inhibited cholesterol synthesis through a stronger HMGR inhibition than simvastatin.

Key words: Antihyperlipidemic, apple peels, high-density lipoprotein cholesterol, hydroxy-3-methylglutaryl coenzyme A reductase, low-density lipoprotein cholesterol, triglyceride

INTRODUCTION

Hyperlipidemia is a medical condition characterized by an increase in plasma lipids, including triglycerides, cholesterol, cholesterol esters, phospholipids, and or plasma lipoproteins.[1] Hyperlipidemia is characterized by elevated levels of one or more of the plasma lipids, including triglycerides, total cholesterol (TC) and or very low-density lipoprotein (vLDL) cholesterol and low-density lipoprotein (LDL) followed by reduced high-density lipoprotein (HDL levels.[2] Improved dietary patterns are advised as the first treatment to reduce cholesterol.

A 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is a regulatory enzyme involved in the biosynthesis of cholesterol in the liver, which catalyzes the synthesis of mevalonate from HMG-CoA. Therefore, HMG-CoA reductase (HMGR) inhibition becomes the target of antihyperlipidemic drugs.[2] Statins act as inhibitors for cholesterol synthesis through inhibition of HMGR.[3] However, the use of statins has several side effects such as digestive disorder and myopathy and teratogenic agents.[4] Thus, the utilization of herbal materials, supported

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by empirical evidence, provides one of the proper alternatives besides the use of statins.

Apple peels discharged from industrial apple chips contain higher amount of flavonoids. The contents of flavonoids and polyphenols in apple peels are higher than in the apple pulp.[5] The ethanol extract of apple peel contains 52.26% flavonoids (quercetin and its derivates) and 16.14% catechin and its derivates.[6] Previous studies[7] indicated the role of apple reduced levels of TC and LDL and increased levels of HDL in hyperlipidemia patients. Ethanolic extract of apple flesh and peels had lowered TC, LDL, and weight of white adipose tissue in hyperlipidemic mice.[8] Anthocyanins and phenolics content of apple peels may contribute to its antihyperlipidemic activity.[9] The supplementation of apple peel phenol to hamster for 28 days decreased serum LDLc and HDLc.[10]

The present study aimed to examine the effect of apple peel extract (APE) on serum lipid profiles (TC, TG, LDL, and HDL) of high-fat diet-induced hyperlipidemic rats. Besides, whether apple peel active compound can bind to HMGR enzyme and inhibit enzyme activity in synthesizing cholesterol was examined through *in silico* study.

MATERIALS AND METHODS

In silico analysis

3-hydroxy-3-methylglutaryl coenzyme A reductase inhibition prediction

The SMILE of 11 active compounds of APE[11] and simvastatin are downloaded from http://pubchem.ncbi.nlm.nih.gov/[Table 1] and analyzed to obtain prediction with PASS software at http://www. pharmaexpert.ru/passonline.

Ligand structure preparation

The three-dimensional structure of ligands from the active compound of APE was downloaded from PubChem (https://PubChem.ncbi.nlm.nih.gov) and saved in SDF format.[12]

Table 1: PubChem CID of simvastatin and active compound of apple peel extract

Compound	PubChem CID
Simvastatin	54454
Pectin	441476
Catechin	9064
Epicatechin	72276
Quercetin	5280343
Quercetin-3-O-rutinoside (Rutin)	5280805
Quercetin-3-O-rhamnoside	5280459
Quercetin-3-O-glucopside	15959354
Quercetin-3-O-galactoside	5281643
Chlorogenic acid	1794427
Phloridzin	6072
Procyanidin	107876

Protein receptors (3-hydroxy-3-methylglutaryl coenzyme A reductase) preparation

The reference protein used was HMGR (ID 1 hwk) obtained from the Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) and saved in PDB format. The separation of proteins from unnecessary molecules was conducted using PyMOL software, (Schrödinger, Inc., New York, USA)[13] and saved in PDB format.

Docking receptors with ligands

The docking process between HMGR receptors and ligands was conducted using AutoDock Vina in PyRx software[13] with the grid box position and Vina Search Space Center X: 2.834 Y: −11.461 Z: 7.220, Dimension (Angstrom) X: 47.334 Y: 50.696 Z: 49.618. Visualization of docking results was used BIOVIA Discovery Studio Software.

In vivo experiment

Materials

BR-1 (Broiler-1) diet was the product of PT Japfa Comfeed Indonesia Tbk. BR-1 diet consists of 21.5-22.5% crude protein; ≤12% moisture content; ≥5% fat; ≤5% crude fiber; ≤7% ash; 0.8%–1.1% calcium; ≥0.5% phosphorus, and 2950–3050 kcal/kg metabolic energy. Butter was from Kimia Farma Ltd. Indonesia. Propylthiouracil (PTU) was from PT Kimia Farma Indonesia. Rats were obtained from Rattus Breeding Centre, Malang, Indonesia.

Research design

Twenty-five *Rattus norvegicus* male rats (age = 60 days, body weight [BW]= 100–150 g) were randomly divided into five groups: control group (N), hyperlipidemia rat received 3.6 mg/kg BW of simvastatin (Simv), hyperlipidemia rat received APE at different doses: 0 mg/kg BW (APE-0), 57 mg/kg BW (APE-1), and 114 mg/kg BW (APE-2).[14] This research was approved by the Research Ethics Commission Institute of the State Islamic University (UIN) Malang, Indonesia, No. 010/EC/KEP-FST/2018.

Hyperlipidemia induction

The hyperlipidemia was induced by feeding animals with a high-fat diet, containing 3.0% duck egg yolk, 5.5% quail egg yolk, 15.5% butter, 6.0% used cooking oil, 70% BR1, and 0.01% PTU diluted in drinking water.[15] Animals were received PTU *ad libitum* for 30 days. The administration of APE through gavage was conducted for 30 days.

Preparation of extract

The apple used in this study was *Manalagi* apple (*Malus sylvestris* Mill.) variety. The simplicia powder of apple peels was obtained from Balai Materia Medica Batu Malang, East Java. The apple peels were dried using an oven at 45°C, then were milled and sieved using 60 mesh sieves.

Extraction was performed using a maceration method with 70% ethanol. The simplicia powder was immersed in ethanol (1:10 (w/v)) for 3 h × 24 h and then filtered using a Buchner funnel. Further, the filtrate was evaporated with a rotary evaporator at 40°C.

Preparation of rat's blood serum

At the end of the treatment, fasting rats were sacrificed by neck dislocation and blood was taken from the left ventricle. Rat blood was centrifuged at 3000 rpm for 15 min. The serum yielded was stored at −20°C for the measurement of lipoprotein level.

Measurement of lipid serum

Lipid serum measurement was performed using an enzymatic colorimetric method.[16] Serum TC and TG were measured according to the kit protocol (Assay Kit from Elabscience, China). Serum LDL cholesterol (LDLc) measurement used the LDLc Reagent DiaSys, Germany, and HDL cholesterol (HDLc) Reagent from Glory Diagnostic, Spanyol.

Data analysis

Data about the levels of TC, TG, LDLc, and HDLc were analyzed by one-way ANOVA using SPSS software (ver. 16.0). Values of $P < 0.05$ were considered significantly different.

RESULTS

In silico analysis

Lipinski Ro5 test showed that three of eight active compounds in APE could pass the membrane such as pectin, phlorizin, and procyanidin [Table 2]. The activity prediction test of the APE phenolic compound confirmed that all compounds play as antihypercholesterol [Pa > Pi, Table 3] which were necessary to inhibit HMG-CoA synthase and HMGR enzymes [Pa > Pi, Table 4]. The docking results indicated that all APE compounds had a lower binding affinity than statin (ΔG-4.5 kcal/mol), emphasizing that quercetin had the most negative affinity value (ΔG-7.4 kcal/mol) [Figure 1].

The level of serum total cholesterol, triglyceride, low-density lipoprotein, and high-density lipoprotein

The results of this research indicated that the administration of ethanol extract of apple peels did not significantly ($P > 0.05$) affect serum TC and TGs. The administration of 114 mg/kg BW of APE significantly decreased ($P < 0.05$) serum LDL level and increased serum HDL level, which is equal to the effect of simvastatin administered for 30 days to reach the N control level [Table 5].

DISCUSSION

The administration of APE at a dose of 114 mg/kg BW for 30 days reduced LDLc levels and increased HDLc of hyperlipidemia rats to normal level [Table 2]. The effectiveness of APE in amelioration of both lipoprotein levels is the same as simvastatin treatment. This finding is in line with Poblete *et al*.'s study[8] who reported a decrease in LDLc and an increase in HDLc after the administration of ethanolic extract of apple peel at a dose of 400 mg/kg BW. Rutin can reduce activities of the acyl-CoA cholesterol transferase enzyme.[2,9] Besides, the administration of APE at a dose of 114 mg/kg BW for 30 days in this study did not significantly reduce TG levels. Thilakarathna *et al*.[10] explained that the administration of ethanolic extract of apple peels for 30 days could not reduce serum TG levels of hamsters with an atherogenic diet.

HMGR enzyme is attached to the reticulum endoplasmic membrane, so a particular compound must be able to pass the membrane.[17] To pass a membrane and penetrate the

Table 2: The prerequisites of Lipinski Ro5 tested compound

Compound	Lipinski requirement					Result
	Mass <500 Dalton	Hydrogen bond donor ≤5	Hydrogen bond acceptors ≤10	Lipophilicity (LogP) ≤5	Molar refractivity 40-130	
Simvastatin	1584.0000	0*	24	0*	0	No
Quercetin-3-O-rutinoside	610.0000	10	16	−1.8788*	137.4954	No
Quercetin-3-O-rhamnoside	448.0000*	7	11	0.29700*	104.862045*	Yes
quercetin-3-O-galactoside	464.0000*	8	12	−0.73060*	106.273842*	Yes
quercetin-3-O-glucoside	462.0000*	8	11	0.06700*	109.507835*	Yes
Quercetin	302.0000*	5*	7*	2.0109*	74.0505*	Yes
Catechin	290.0000*	5*	6*	1.5461*	72.6230*	Yes
Epicatechin	290.0000*	5*	6*	1.5461*	72.6230*	Yes
Procyanidin	594.0000	10	13	2.7327*	144.3050	No
Phloridzin	436.0000*	7	10*	−0.2024*	104.9250*	Yes
Chlorogenic Acid	353.0000*	5*	9*	−1.9806*	79.8900*	Yes
Pectin	193.0000*	4*	7*	−4.4638*	33.9072	Yes

*Fulfilling Lipinski Ro5 Requirements. LogP: Lipophilicity

Table 3: Antihypercholesterolemic, 3-Hydroxy-3-methylglutaryl coenzyme A synthase, and 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibition activity prediction using Pass program

Compound	Antihypercholesterol		HMGS-Inh		HMGR-Inh	
	Pa	Pi	Pa	Pi	Pa	Pi
Simvastatine	0.533	0.027	0.356	0.040	0.881	0.001
Quercetin-3-O-rutinoside	0.9	0.003	-	-	-	-
Quercetin-3-O-rhamnoside	0.804	0.005	0.225	0.041	-	-
Quercetin-3-O-galactoside	0.871	0.004	0.125	0.112	-	-
Quercetin-3-O-glucoside	0.444	0.030	0.198	0.032	-	-
Quercetin	0.516	0.020	-	-	0.516	0.020
Catechin	0.631	0.012	-	-	0.039	0.037
Epicatechin	0.631	0.012	-	-	0.039	0.037
Procyanidin	0.357	0.045	-	-	-	-
Phloridzin	0.722	0.007	0.163	0.067	-	-
Chlorogenic acid	0.423	0.033	0.198	0.032	0.042	0.026
Pectin	0.627	0.012	0.186	0.038	-	-

HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A, HMGS-Inh: HMG-CoA synthase inhibition, HMGR-Inh: HMG-CoA reductase inhibition, Pa: probable activity, Pi: probable inactivity, -: Pa, Pi value is 0

Table 4: Binding affinity value of active compound apple peel with 3-hydroxy-3-methylglutaryl coenzyme A reductase

Compound	ΔG	Hydrophobic bond	Hydrogen bond
Simvastatin	−7.3	**His752, Ser565, Ala856, Cys561, Val683, Leu853, Glu559, Leu857**	**Asn755, Lys691, Asp690, Arg590**
Quercetin 3-O-Rutinoside*	−9.5	Asn755, Met657, Lys691, Leu562, **Cys561, Ser565, His752,** Glu559, **Val683, Leu853, Leu857,** Ser684	Lys735, **Arg590**, Lys692, Ala751, **Asp690,** Asn658, Ala856, Gly860
Quercetin-3-O-rhamnoside*	−8.2	Ser684, **Val683**, Arg590, Ser661, Asp690, Lys691, Leu853, **Glu559**, Asn658, **Cys561**	Lys753, Lys692, Ala751, Glu665
Quercetin-3-O-galactoside*	−7.6	Ser684, Lys691, **Leu853**, Asp690, Gly560, **Glu559**, Cys561, Asn658, Ser661, Arg590, **Val683.**	Lys735, Lys692, Ala751, Glu665
Quercetin-3-O-glucoside*	−8.5	Ala783, Ile729, Asn788, Glu782, Glu726, Thr725, Ile729, Ile733	Glu789, Glu730, Glu726, Asn788
Quercetin	−7.4	**His752, Ser565, Ala856, Cys561, Val683, Leu853, Leu857, Glu 559**	**Asn755, Arg590, Asp690, Lys691**
Catechin	−8.1	Leu562, **Cys561,** Gly560, **Ser565, His752, Glu559,** Asp690, **Leu853,** Ala751	**Arg590,** Lys692, Ser684, Lys735
Epicatechin	−8.1	Lys633, Glu610, Leu584, His635, Glu700, Ser637, Ile699, Pro798, Ser705, Ala585.	Lys606
Procyanidin*	−9.5	Gly860, **Leu857**, **Cys561**, Asn658, Gly560, **Glu559**, Arg590, **His752, Leu853, Val683**, Ser684, Asp690	Ala856, Glu665, Lys692, Ala751, Lys735
Phloridzin*	−8.9	Gln632, Pro798, Ile699, Ala585, Lys633, Asp586, Leu584, Met782, Ser637, His635, Glu610	Glu700, Gln648, Ile638, Lys606, Lys633, Leu634, Thr636
Chlorogenic acid	−8,3	Glu700, Glu 610, Ser 705, Lys 606, Ser 637, Ile 699, Ala 585, Leu 584	Lys 633, Leu 634, His 635, Ile638
Pectin*	−6.5	His762, Lys691, **Leu857**	**Arg590**, Ser684, **Asp690**, Ala751, Lys692, Lys753

Bold prints indicate the same amino acid as simvastatin. *HMG-CoA reductase inhibition activity prediction using Pass program negative. HMG-CoA: 3-Hydroxy-3-methylglutaryl coenzyme A, HMGR: HMG-CoA reductase

cell, a compound must fulfill Lipinski Ro5.[18] The results indicated that 11 flavonoid derivate molecules, besides statin, extracted from apple peels fulfilled Lipinski Ro5 requirements except for procyanidin and rutin [Table 2]. Simvastatin also does not meet the Lipinski Ro5 requirement, due to a large molecule, and more than ten hydrogen bond acceptors made simvastatin a lipophilic compound.[19]

Table 3 demonstrates the activity prediction test of APE content as antihypercholesterol, indicating that all extract

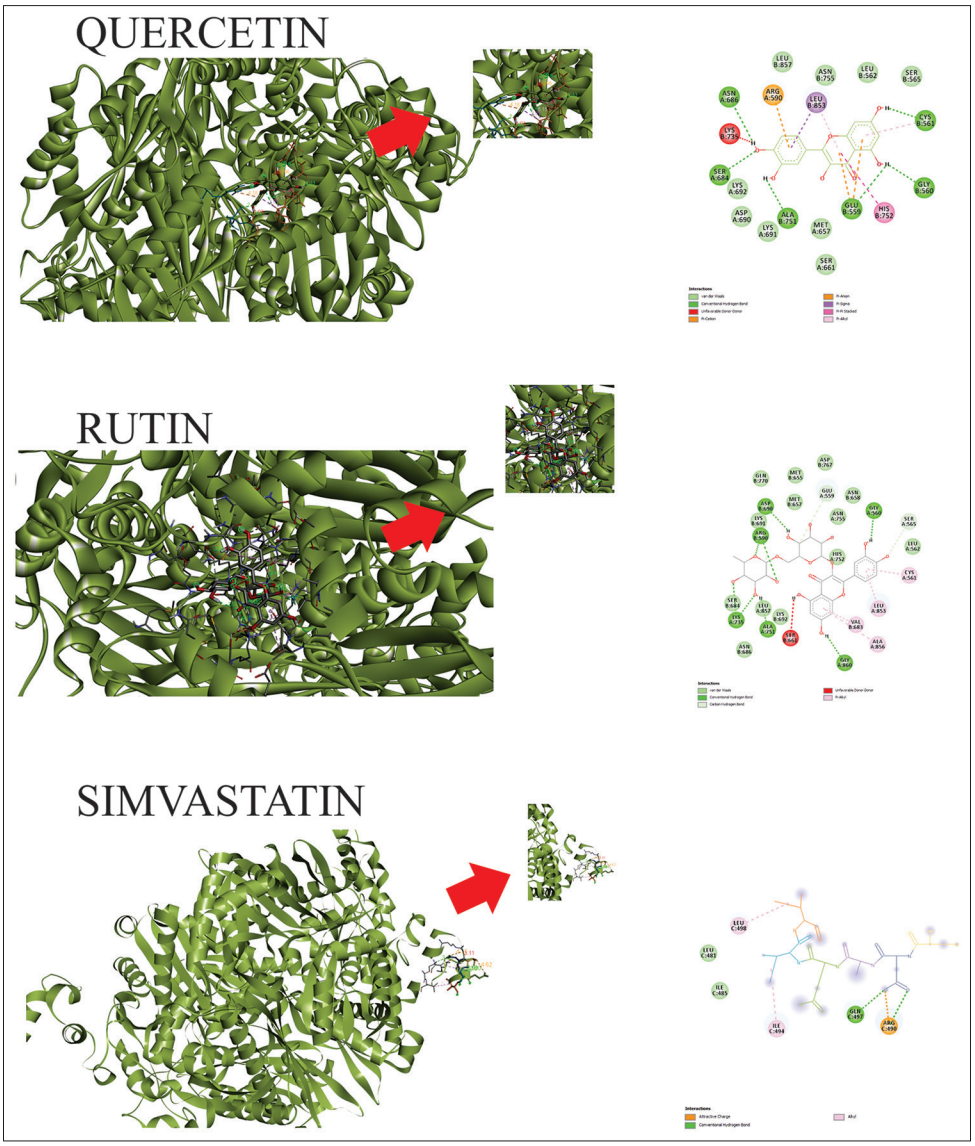

Figure 1: Interaction between ligand and receptor was visualized by BIOVIA Discovery Studio software. Three-dimensional and two-dimensional images of the binding position of active compounds and binding ligand on 3-hdroxy-3-methylglutaryl coenzyme A reductase

Table 5: Serum lipid profile of rats treated with apple peel extract

Parameter (mg/dl)	Treatment					ANOVA (*P*)
	N	APE-0	APE-I	APE-2	Simv	
TC	50.2±8.76	51.8±8.11	50.8±8.35	42.8±4.87	46.8±4.21	0.289[ns]
TG	76.4±22.57	130±36.57	137.4±30.59	62.6±29.36	64.6±20.01	0.064[ŧkns]
LDL	11.0±1.67[ab]	13.8±1.17[c]	11.8±1.72[bc]	8.4±2.42[a]	9.8±1.17[ab]	0.003**
HDL	41.2±5.53[b]	32.2±1.95[a]	35±2.00[a]	40.6±3.37[b]	45.8±3.37[b]	0.000**

[ŧ]Kruskal-Wallis, **ANOVA test $P<0.01$, Different letters in the same line showed significant difference ($P<0.05$). N: Normal rats, APE-0: Hyperlipidemic rats without treatment, APE-1 and APE-2: Hyperlipidemic rats received 57 mg/kg BW APE and 114 mg/kg BW, Simv: Hyperlipidemic rats received 3.6 mg/kg BW simvastatin, APE: Apple peel extract, BW: Body weight, NS: Not significant, TC: Total cholesterol, TG: Triglyceride, LDL: Low-density lipoprotein, HDL: High-density lipoprotein

compounds exhibit antihypercholesterol activity (Pa > Pi), unlike in simvastatin. Some inhibitors include HMGS activity or HMGR inhibitors or both. Only chlorogenic acid can inhibit the enzyme similar to simvastatin. In addition, the activity prediction test indicated that HMGR inhibitors such as simvastatin, catechin, epicatechin, quercetin, and chlorogenic acid have the highest Pa than Pi, emphasizing HMGR inhibition activity.[19,20] Our results demonstrated that all compounds had better docking scores than simvastatin, except for pectin. Quercetin has similar hydrophobic and hydrogen bonds (eight hydrophobic bonds and six hydrogen bonds) as simvastatin [Table 4].

The eight hydrophobic bonds formed between HMGR and quercetin are likely to occur with C atoms without hydroxyl groups in the cyclic chains A and B quercetin with four bonds.

CONCLUSION

The APE could act as an antihyperlipidemic agent by reducing the LDL level and elevating the HDL level in the hyperlipidemic rat model. The ability of apple peel extract in lowering LDL level was optimal at a dose of 114 mg/kg BW. The molecular docking results clarified the potential of quercetin-3-O-rutinoside, quercetin-3-O-rhamnoside, quercetin-3-O-galactoside, and procyanidin as HMGR inhibitors.

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Conflicts of interest

There are no conflicts of interest.

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