Original Research

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Effects of Metformin on High-Fat Diet-Induced Hyperlipidemic Rats

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Abstract

Metformin as insulin sensitizer is now often associated with a significant decrease in plasma triglycerides, plasma total cholesterol, LDL cholesterol, and a significant increase in plasma HDL cholesterol without causing hypoglycemia. Current research aim are to determine the effects of metformin on high-fat diet-induced hyperlipidemic rats. To this end, a total of 30 Sprague-Dawley rats were divided into six groups (N = 5). Normal control was not treated, the negative group was given 0.5% CMC, the positive group used simvastatin dose of 1.8 mg/kg BW/day orally and three variation dose groups of metformin were given at a dose of 225; 450; and 900 mg/kg BW rat/day orally. Induction was carried out for 28 days and continued for 14 days treated with an administration orally. Lipid profile was measured by the colorimetric enzymatic method. As a result, all doses of metformin significantly decreased total cholesterol and LDL ($p < 0.05$) compared to normal and negative controls. The highest dose significantly decreased triglyceride levels ($p < 0.05$) and increased HDL levels ($p < 0.05$) compared to normal and negative controls. Based on these results, metformin has a potential effect of antihyperlipidemic at a dose of 225 mg/kg BW rat.

Keywords: Cholesterol, High-Fat Diet, Hyperlipidemic, Metformin, Triglyceride

1. Introduction

Hyperlipidemia is a condition of lipid abnormality characterized by an increase one or more total cholesterol, LDL, or triglycerides, and or a decrease in HDL[1]. Data WHO show that an increase cholesterol levels is estimated to cause 2.6 million deaths or 4.5% of total and 29.7 million Disability Adjusted Life Years (DALYS) which is about 2% of the total DALYS[2]. Based on the results of Riskesdas the population of 15−24 years found that the proportion of total cholesterol was abnormal with the borderline category was 7.2% and the high category was 1.9%. The highest abnormal borderline category was found in the 55−64 years, which was 29.2% and the high category was 12.6%. Low HDL in the age group of 15−24 years was 24.8%. LDL abnormal borderline category at age 15−24 years was 11.5% and the high category was 2.6%, high borderline triglycerides are 8.7%, and 7.1% has high LDL[3].

Antihyperlipidemic drugs are often used to control plasma lipid levels. The first-line antihyperlipidemic drug is statin group. Statins are lipid-lowering drugs with a mechanism to inhibit HMG-CoA Reductase which plays a role in cholesterol synthesis. Simvastatin as an example of this group has the ability to reduce LDL levels by increasing the removal of LDL precursors, VLDL and IDL, and reducing the production of VLDL in the liver[4].

Analysis of data from several studies shows that intensive therapy with metformin significantly reduces diabetes-related endpoints and deaths, and meta-analysis of clinical trials found that the drug can also significantly reduce the total cholesterol and LDL levels significantly without causing hypoglycemia[5]. Research by Kashi, Mahrooz, Kianmehr, and Alizadeh also states that metformin can significantly reduce lipid profile levels[6]. There are other studies also showing that metformin can slightly increase or only slightly decrease total plasma cholesterol,

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LDL, and triglycerides[7]. That means it is possible to use metformin as another option for antihyperlipidemic drugs which has officially circulated. Therefore, this study was carried out non-clinically using Sprague Dawley (SD) rat which has been made into hyperlipidemic animal models by inducing high-fat diet and continued treatment with metformin variation doses.

2. Materials and Methods

2.1 Animals

Animals used in this study were 30 male rats from the Sprague Dawley strain of 2−3 months with a body weight from 150−200 grams obtained from Non-Ruminansia dan Satwa Harapan laboratory, Faculty of Animal Husbandry IPB. Rats used has been declared healthy and meets the requirements as a test animal. Rats used have obtained ethical approval issued by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia following the certificate passed the ethical review of KET-227/UN2.F1/ETIK/PPM.00.02/2019. Before treatment, the rats were acclimatized for 7 days at the Experimental Animal Laboratory of the Faculty of Pharmacy UI. Acclimatization aims to make the rats adapt to the new environment to minimize the effects of stress on mice that can interfere with the research process. During acclimatization, rats were kept on a standard diet and water ad libitum.

2.2 Drugs

Metformin tablets (Hexpharm Jaya, Indonesia) were used as drug treatment and simvastatin tablets (Dexa Medica, Indonesia) as positive drugs. Metformin and simvastatin were dissolved in CMC 0.5% and given orally. The doses were chosen for the drug based on doses used in previous research. Dose of metformin was 225; 450; and 900 mg/kg BW rat and dose of simvastatin was 1.8 mg/kg BW.

2.3 Induction of Hyperlipidemia

The hyperlipidemia in this model was made by combine using solid food and liquid food; solid food was given 20 grams/day/animal. The composition of solid food is standard rat food + quail egg yolk + butter (50 : 40 : 10) and 0.01% PTU drinking water. The composition of Liquid food are quail egg yolk + high fructose corn syrup + butter (80 : 15 : 5) and 0.1% PTU. This oral induction The study period was 28 days and high-fat diet was provided daily.

2.4 Chemical Materials

Aquadest (Brataco, Indonesia), aquabidest (Ikapharmindo, Indonesia), CMC (Brataco, Indonesia) were obtained. Chemical Reagents (Kit) used were total cholesterol kit (Dyasis, USA), triglyceride kit (Dyasis, USA), and HDL kit (Dyasis, USA).

2.5 Experimental Design

During the experiment, the normal group was not treated while the induction group was given a high-fat diet (20 g/day/rat) day[8] and treated orally (3 mL/200 g BW rat)[9] and PTU 0.01% as drinking water[10]. After that, thirty male rats were divided into six groups (n = 5) as follow: The first group as a normal group, second group as a positive control group given simvastatin 1.8 mg/kg BW rat, the third group as a negative control group given CMC 0.5%, and three other groups given metformin 225; 450; and 900 mg/kg BW rat. The drug or vehicle treatments were given for 14 consecutive days.

2.6 Weight Observation

The animals were also observed for their body weight weekly on day-0 or before treatment, day-7, day-14, day-21, day-28, day-35, and day-42.

2.7 Sample Plasma Preparation

The blood samples were taken on day-0 before treatment, day-28 after induction of a high-fat diet, and day-42 after drug administration. Blood samples taken from sinus orbital eyes rats were collected in a microtube. Next, the blood was centrifuged at 7000 rpm for 15 minutes. The supernatant was taken to obtain plasma samples. Then, plasma samples were analyzed to determine the levels of total cholesterol, triglyceride, HDL and LDL.

2.8 Lipid Profile Measurement

Lipid profile measurements used a total cholesterol kit, triglycerides kit and HDL kit by enzymatic colorimetry using UV-VIS spectrophotometry at a wavelength of 500 nm. The absorbance obtained is compared with the standard absorption then multiplied by the standard concentration. The LDL levels were measured indirectly

using the Friedewald formula: LDL cholesterol = total cholesterol – triglyceride/5.5 – HDL Cholesterol

2.9 Statistical Analysis

The data results were expressed as means of 5 rats ± Standard Deviation (SD). The data obtained were processed statistically using SPSS version 25. Firstly, it was done by a normality test (Saphiro-Wilk) and homogeneity test (Levene test). If the data is normally distributed and homogeneous, a parametric test is performed using one-way Analysis Of Variance (ANOVA) and followed by the Least Significant Difference test (LSD). If one of the requirements for ANOVA is not met, then the Kruskal-Willis nonparametric test is carried out and continued with the Mann Whitney test. To see the relationship between groups, an independent-t-test was used if the data were normal and homogeneous.

3. Results

The first stage in this research was to make rats models of hyperlipidemia. This stage was successfully because the total cholesterol level exceeds 200 mg/dL followed by significant increase of triglycerides, HDL, and LDL.

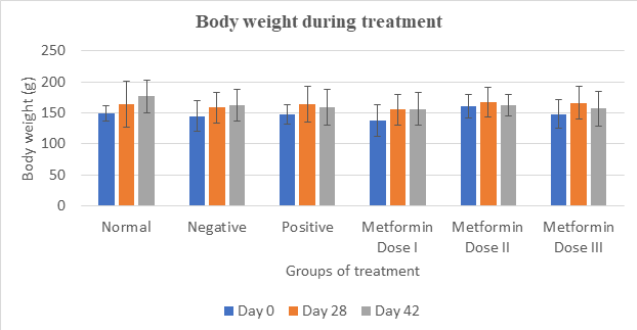
Table 1. Lipid profile of rat after induction with high-fat diet 28 day.

Group	The difference in average lipid profile ± SD (mg/dL)			
	Total Cholesterol	Triglyceride	HDL	LDL
	ΔD28-D0	ΔD28-D0	ΔD28-D0	ΔD28-D0
Normal	−4,43 ± 23,67	29,37 ± 27,99	8,16 ± 3,45	−11,70 ± 18,64
Induction	236,78 ± 77,15*	61,37 ± 38,81*	−14,90 ± 21,28*	234,17 ± 75,49*

Note: Normal (Not treated); Induction (High Fat Diet 20 g/day/rat and orally 3 mL/200 g BW rat), (*) = $p < 0.05$ succeeded in making animal models (there was a significant increase between the normal group and the induction group)

After making the rats model, it was continued to evaluate the effects of metformin in animal model. After being treated by metformin for 14 days, the average lipid profile showed a significant decrease.

Body weight rat after drug administration



Note: (*) $p < 0.05$, a significant weight loss occurred after 14 days of drug administration compared with the negative group

Figure 1. Average body weight diagram during treatment.

4. Discussion

4.1 Hyperlipidemic Animal Models

Based on statistical tests, there was a significant difference ($p < 0.05$) of total cholesterol ($p = 0.001$), LDL ($p = 0.001$) and triglyceride ($p = 0.041$) levels and a significant decrease in HDL levels ($p = 0.024$). This study similar with previous research by Li *et al.*, 2016 that a high-fat diet will increase total cholesterol and LDL levels[11]. The increase was due to increase consumption of saturated fatty acids. Fatty acids will be converted into acetyl CoA through beta-oxidation, while acetyl CoA is a precursor of cholesterol synthesis. An increase in the number of precursors will cause an increase in cholesterol synthesis resulting in an increase in VLDL, LDL levels, and a decrease in HDL[12].

Propylthiouracil (PTU) is a drug that is known to have hypothyroidism effects. So in the condition of hypothyroidism, a decrease in thyroid hormone results in slowing down the flow of bile acids which is marked by a decrease in the level of cholesterol secretion into the bile resulting in an increase in intrahepatic cholesterol[13].

Table 2. Lipid profile after metformin treatment.

Group	Average ± SD (mg/dL)															
	Total Cholesterol				Triglycerides				HDL				LDL			
	Day-28	Day-42	ΔD28 – D0	ΔD42 –D28	Day-28	Day-42	ΔD28 – D0	ΔD42 –D28	Day-28	Day-42	ΔD28 – D0	ΔD42 –D28	Day-28	Day-42	ΔD28 – D0	ΔD42 –D28
Normal	91,44 ± 11,57	82,51 ± 7,24	-4,43 ± 23,67	-8,93 ± 8,62	103,87 ± 33,60	68,99 ± 15,35a	29,37 ± 27,99	-34,89 ± 23,96	63,21 ± 6,58	62,79 ± 12,16	8,16 ± 3,45	-0,42 ± 15,87	15,56 ± 5,83	12,28 ± 11,13	-11,70 ± 18,64	-3,29 ± 13,54
Positive	319,57 ± 63,13	133,91 ± 9,61 a	224,48 ± 63,57	-185,66 ± 67,35#*	146,09 ± 21,44	91,76 ± 17,21	52,35 ± 31,89	-54,33 ± 23,41	31,56 ± 4,72	76,17 ± 9,15a	-34,06 ± 17,88	44,62 ± 5,89	261,45 ± 64,53	41,05 ± 15,20a	249,03 ± 61,44	-220,40 ± 68,84#*
Negative	254,69 ± 86,13	235,26 ± 77,89 a	174,07 ± 102,05	-19,43 ± 54,85	136,86 ± 14,36	177,32 ± 45,78	55,32 ± 37,79	40,46 ± 37,00	50,47 ± 30,55	47,67 ± 24,47a	-5,94 ± 35,03	-2,79 ± 8,86	179,34 ± 71,23	151,24 ± 53,26a	169,94 ± 74,90	-28,10 ± 53,55
Metformin Dose 1	310,98 ± 54,82	195,70 ± 41,44 ab	228,02 ± 53,26	-115,28 ± 72,65#*	137,03 ± 12,55	111,64 ± 35,10 a	56,45 ± 19,07	-25,38 ± 38,02	53,17 ± 8,81	66,88 ± 21,23	-3,82 ± 9,45	13,70 ± 29,19	219,43 ± 57,73	108,53 ± 60,69ab	208,12 ± 57,12	-110,90 ± 84,14#*
Metformin Dose 2	334,13 ± 71,73	150,99 ± 59,77	256,25 ± 70,43	-183,14 ± 63,99#*	176,27 ± 55,64	87,34 ± 47,26	92,70 ± 54,74	-88,93 ± 71,18*	38,26 ± 13,42	87,12 ± 30,70	-19,61 ± 14,15	48,86 ± 41,12*	251,33 ± 80,47	47,98 ± 48,09	246,52 ± 81,31	-203,35 ± 47,49#*
Metformin Dose 3	388,92 ± 51,16	117,82 ± 38,03	301,10 ± 51,12	-271,11 ± 74,27#*	156,67 ± 31,62	73,91 ± 18,97	82,03 ± 33,33	-82,76 ± 42,77#*	52,96 ± 15,38	81,80 ± 24,03	-11,08 ± 11,05	28,84 ± 23,92#*	307,47 ± 63,98	26,88 ± 26,70	297,26 ± 59,12	-280,59 ± 83,99#*

Note: Normal (Not treated); Positive (Simvastatin 1.8 mg/kg BW rat); Negative (High Fat Diet 20g/day/rat and Orally 3 mL/200 g BW rat); Metformin Dose 1 (225 mg /kg BW rat); Metformin Dose 2 (450 mg/kg BW rat); Metformin Dose 3 (900 mg /kg BW rat); (#) = $p < 0.05$ compared to the normal group; (*) = $p < 0.05$ compared to the negative group; (a) = $p < 0.05$ compared to the normal group (back to normal or not); (b) = $p < 0.05$ compared to the positive group

Based on Table 1, the results of this study showed the large standard deviation which means an increase in total cholesterol, triglyceride, LDL and a decrease in HDL had more variation. The variation from this study may be caused by differences in consumption of high-fat diet feeds in each rat, there were rats whose food was depleted in a day and some were not.

There were a significant increase (p = 0.041) in triglyceride levels after given an induction, however, the increase that occurred was still within the normal threshold. These results are same with research conducted by Kusuma, Asarina, Rahmawati, and Susanti, that the increase occurred still within the normal threshold is possibility due to the movement of rats, so the triglycerides formed are used as energy[12]. The decrease in HDL that occurred in rats that had been induced in this study was also significant (p = 0.024) but also within normal threshold too. The same results also occurred in a study conducted by Li *et al.*, that in animal models of hyperlipidemia, HDL levels were at least much lower than normal animals because neutralization by HDL also requires a long duration[11].

4.2 Effects of Metformin on Total Cholesterol Levels

Total cholesterol levels shown in Table 2, shows that there was a significant decrease in total cholesterol levels after and before administration of the drug in all groups (p = 0.000). When compared to the normal group, simvastatin as a positive group was able to significantly reduce total cholesterol levels (p = 0.000), but did not return to normal (p = 0.000). Analysis using the Least Significant Difference (LSD) resulted that the normal group had no significant decrease in cholesterol level (p = 0.789) while the positive group and metformin group with variation dose had a significant decrease after and before drug administration when compared to the negative group. After being tested statistically using independent-t-test, it showed that only the metformin dose group 2 (p = 0.062) and metformin dose group 3 (p = 0.106) returned to normal levels. Metformin will increase activation of AMPK which will reduce cholesterol synthesis in the liver[14].

4.3 Effects of Metformin on Triglyceride Levels

In Table 2, shows that there was a significant decrease in triglyceride levels after and before drug administration in all groups (p = 0.005). Based on Mann-Whitney test when compared to the normal group, a significant decrease only occurred in the metformin group dose 3 (p = 0.028). When compared with the negative group there was also a significant decrease in triglyceride levels in the metformin group doses 2 and 3 (p = 0.009).

Based on the independent-t-test statistical test, it showed that the metformin dose 2 (p = 0.433) and metformin dose 3 (p = 0.664) had triglyceride levels that returned to normal. The positive control effect with the metformin group was also not significantly different, indicating that both had the same effect in reducing triglyceride levels. Independent t-test had the results (p = 0.289; p = 0.849; p = 0.158) after the positive group was compared to metformin group at dose 1, 2 and 3. Metformin also reduced regulation of the fatty acid synthase gene and SREBP-1C indicating that the lipogenesis pathway can be affected by metformin resulting in a decrease of fatty acids and triglycerides[15]. The mechanism of metformin through the AMPK pathway, which is activated AMPK, will phosphorylate Acetyl-CoA carboxylase, which inhibits fat synthesis and promotes fat oxidation instead, causing a reduced amount of triglycerides, reducing fatty liver stores and increasing liver insulin sensitivity[16].

4.4 Effects of Metformin on HDL Levels

Based on the results of Kruskal-Wallis statistical analysis, HDL levels shown in Table 2, shows that metformin was able to significantly increase HDL levels (p = 0.017). An increase in HDL levels that returned to normal occurred in all metformin groups (p = 0.453; 0.091; 0.094). When compared to the positive group, all dose of metformin groups showed results (p = 0.395; 0.467; 0.638) that there were no significant differences, which means that the effect of increasing HDL levels in the positive control group was same as the variation dose of metformin group.

4.5 Effects of Metformin on LDL Levels

Based on ANOVA test showed that there was a significant decrease in LDL levels after and before drug administration (p = 0.000). Based on Table 2, when compared to the normal group all treatments both giving simvastatin and metformin were able to significantly reduce LDL levels (p = 0.000). The pathway that induces a decrease in LDL cholesterol is the absorption of intestinal bile acids by metformin causes an increase in the synthesis of bile acids in the liver, and cholesterol is used for this process, thereby

causing a decrease in the amount of cholesterol in hepatocytes. Increased regulation of LDL receptors can increase lipoprotein absorption and restore adequate cholesterol levels in the liver. With the result that, LDL concentrations and total plasma cholesterol concentrations can be indirectly reduced by the action of metformin.[15]

4.6 Effect of Metformin on Body Weight

Metformin can play a role in losing weight by reducing food intake. Decreased food intake was seen during initial metformin treatment until it was continued over time[17]. Based on Figure 1, it shows that metformin caused weight loss in the metformin dose 2 ($p = 0.039$) and metformin dose 3 ($p = 0.003$) when compared to the negative group. After metformin treatment, weight loss is generally simple and is caused by fat loss rather than energy output[14].

5. Conclusion

Based on the analysis that has been done, it can be concluded that the induction method using a high-fat diet in the form of feed and orally can form hyperlipidemic rats. After given the drug administration of metformin at a dose of 45 mg/200 g BW, the rats have been able to provide an antihyperlipidemic effect.

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7. Declaration of Interest

The authors report no conflict of interest.

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