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Anti-atherogenic Effect of Mulberry Leaf Tea in Atherogenic Index on Rats Dyslipidemia

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A B S T R A C T

Dyslipidemia is a lipid metabolism disorder characterised by lipid fraction changes in the blood, such as an increase in total cholesterol, LDL, TG and a decrease in HDL, which is strongly associated with atherosclerosis. Mulberry leaves (*Morus alba L.*) contain quercetin and 1-deoxynojirimycin (DNJ), which help lowering cardiometabolic risk by reducing hyperlipidemia, oxidative stress and atherosclerosis. The objective of this study was to determine the effect of mulberry leaf tea (*Morus alba L.)* on atherogenic index (AI) in *Sprague Dawley* rats dyslipidemia. This study was true experimental research with randomised pre and post-test control group design. A total of 30 male *Sprague Dawley* (SD) rats were randomly divided into five groups. Calculation of AI value: log (TG/HDL-C). Statistical analysis that was used was the paired t-test and one-way ANOVA test. The intervention of mulberry leaf tea with the dosage of 36 mg/100 g BW/day (P1) and 72 mg/100 g BW/day (P2) were significantly reduced atherogenic index values. Simvastatin and P2 groups showed that there was no difference in atherogenic index values after the intervention. In conclusion, giving mulberry leaf tea was reduced atherogenic index values in SD rats dyslipidemia.

Kata kunci:

Teh daun mulberry
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A B S T R A K

Dislipidemia merupakan kelainan metabolisme lipid yang ditandai dengan perubahan fraksi lipid dalam darah seperti peningkatan kolesterol total, LDL, TG dan penurunan HDL yang berhubungan kuat pada kejadian aterosklerosis. Daun mulberry (*Morus alba L.*) mengandung kuersetin dan 1-deoxynojirimycin (DNJ) yang berguna dalam menurunkan risiko kardiometabolik dengan menurunkan keadaan hiperlipidemia, stress oksidatif dan aterosklerosis. Tujuan dari penelitian ini untuk mengetahui pengaruh teh daun mulberry (*Morus alba L.*) terhadap indeks aterogenik plasma (IAP) pada tikus *Sprague Dawley* dislipidemia. Penelitian ini merupakan penelitian *true experimental* dengan *randomized pre and post test control group design.* Sebanyak 30 ekor tikus *Sprague Dawley* (SD) jantan dirandomisasi menjadi 5 kelompok. Rumus perhitungan nilai IAP : log (TG/HDL-C). Analisis statistik yang digunakan adalah uji *paired t-test* dan uji *one-way* ANOVA. Intervensi teh daun mulberry dosis 36 mg/100 g BB/hari (P1) dan 72 mg/100 g BB/hari (P2) secara signifikan menurunkan nilai indeks aterogenik plasma. Pada kelompok S dan kelompok P2 menunjukkan bahwa tidak terdapat perbedaan nilai indeks aterogenik plasma setelah intervensi. Sehingga disimpulkan bahwa pemberian teh daun mulberry terbukti menurunkan nilai indeks aterogenik plasma pada tikus SD dislipidemia.

INTRODUCTION

Dyslipidemia is a lipid metabolism disorder characterized by the lipid fraction changes in the blood such as increasing in total cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides (TG) as well as decreasing high-density lipoprotein cholesterol (HDL-C), that is strongly associated with atherosclerosis. Consuming foods that contain cholesterol and high saturated fat is the leading cause of increased cholesterol levels in the blood(Kementrian Kesehatan RI, 2018). Elevated TG levels are always associated with increased LDL cholesterol particles and increased cardiovascular risk(Niroumand et al., 2015). Furthermore, LDL particles have very varied sizes, one of them is sdLDL(James et al., 2016).

Atherogenic index (AI) was well correlated with LDL particle size and was used to indicate atherogenic lipoprotein phenotype. This atherogenic index was positively related with the circulating fractional esterification rate of HDL (FER_{HDL}) and inversely related with circulating LDL. On the second hand, the plasma atherogenic index could also be used to mark the presence of sdLDL(Dobiášová et al., 2011; Khazaál, 2013; Niroumand et al., 2015). Small-dense low-density lipoprotein (sdLDL) was later known as the significant determinant of atherogenicity in LDL-C(James et al., 2016).

Prevention of atherosclerosis could be done by treating the condition of dyslipidemia through controlling cholesterol levels in the body by adjusting diet or food patterns(Zhong et al., 2006). The treatments that was used for dyslipidemia was by giving conventional drugs, such as simvastatin was widely used by most people nowadays, had several side effects such as muscle pain, gastrointestinal symptoms, rhabdomyolysis, myopathy and kidney failure(Hodel, 2002; Yi et al., 2021). Mulberry leaf tea was one of the plants that have compounds that are proven to protect the body from various diseases and do not have cytotoxic activity(Rodrigues et al., 2019). Mulberry leaves contain rutin, quercetin, alkaloids, gallic acid, essential oils and components of other bioactive compounds that are useful in lowering cardiometabolic risk by reducing hyperlipidemia, hyperglycemia, obesity, oxidative stress and atherosclerosis.

The content of quercetin in mulberry leaves is around 1.87 – 2.12 mg/g(Przygoński & Wojtowicz, 2019). Mulberry leaves are also rich in alkaloids, such as, the type 1-deoxynojirimycin (DNJ). Flavonoids can inhibit the formation of reactive oxygen species (ROS) and reduce levels of *3-hydroxy-3-methylglutaryl-CoA* (HMG-CoA) reductase, therefore, it can reduce cholesterol levels in a body. The mechanism of DNJ and quercetin, as the antihypercholesterolemic agent is activating the expression of AMP-activated protein kinase (AMP-K) and peroxisome proliferator-active receptor (PPAR)-α, which causes an increase in β-oxidation of free fatty acids and breakdown of lipids(Thaipitakwong et al., 2018).

A previous study showed that giving mulberry leaf tea was proven to reduce total cholesterol, LDL and triglyceride levels with the highest dose of 0.50% in type 2 DM rats(Wilson & Islam, 2015). This prompted researcher's interest in determining the effect of giving mulberry leaf tea (*Morus alba L.*) with a dose of 36 mg/100g BW/day and a dose of 72 mg/100g BW/day on atherogenic index (AI) in dyslipidemic rats.

METHOD

Animals

The animals used in this study were male *Sprague Dawley* rats aged 2-3 months with its weight around 150-200 grams. Experimental animals totalling 30 rats were obtained from the Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta. This research had been approved by the Health Research Ethics Commission, Faculty of Medicine, Diponegoro University, No. 53/EC/H/FK-UNDIP/V/2021.

Research design

This research was conducted by a true experimental approach with a randomized pre and post-test control group design. A total of 30 rats were randomly divided into five groups. The negative group (K-) : standard feed, the positive group (K+) : HFD, the simvastatin group (S): HFD + simvastatin 0.18 mg/200 g BW/day, treatment group 1 (P1) : HFD + leaf tea mulberry 36 mg/100g BW/day and treatment group 2 (P2) : HFD + mulberry leaf tea 72 mg/100 g BW/day.

Diet Composition

The standard diet that was used was an AD-II feed and high-fat diet induction was given by combining the standard feed with 0.2% cholic acid and 10% quail eggs for 2 weeks.

Mulberry Leaf Tea

Making mulberry leaf tea was began by washing and then separating the leaves from the stems. Furthermore, the leaves were withered by steaming for 90 seconds at a temperature of 100°C. The drying process was carried out using a cabinet dryer at 60°C for 90 minutes. The dried mulberry leaf tea was grounded in a blender and mashed with a 230 mesh filter until it became a fine powder.

Weighing

Rat body weight (BW) was measured once a week.

Biochemical Analysis

The examination of HDL cholesterol levels was carried out using the CHOD-PAP (Cholesterol Oxidase-Para Aminophenazone) method and triglyceride levels was used the GPO-PAP (Glycerol Phosphate Oxidase-Para Aminophenazone) method.

Atherogenic Index Calculation

Atherogenic index (AI) was obtained by calculating the formula = Log (serum TG/serum HDL-C).

Statistical Analysis

Statistical analysis was used SPSS 24 program. Paired t-test was used to determine the effect on each group before and after treatment. One-way ANOVA test was used to determine the differences in each group which was used followed by the Bonferroni post-hoc test. The statistical test was carried out at the 95% of confidence level ($p<0.05$).

RESULT AND DISCUSSION

The characteristics of rats used in this study had an average overall body weight of 175 grams during the acclimation period. The weighing was done once a week from the beginning to the end of the study. The average body weight of rats before and after HFD could be seen in table 1.

Table 1. Average body weight of rats before and after HFD

Groups	Body Weight (BW) (g)		
	BW Early HFD	BW Final HFD	Δ (%)
K-	179.17 ± 1.94	191.00 ± 2.10[a]	11.83 ± 0.75 (6.60%)
K+	180.83 ± 2.64	207.50 ± 2.43[a]	26.67 ± 0.82 (14.75%)
S	181.67 ± 3.27	208.83 ± 3.19[a]	27.17 ± 0.75 (14.96%)
P1	181.00 ± 3.29	207.00 ± 3.41[a]	26.00 ± 0.89 (14.37%)
P2	182.00 ± 5.22	208.17 ± 5.81[a]	26.17 ± 0.75 (14.38%)
p			0.000*

Note: [a]paired t-test (p<0.05), *One-way ANOVA test (p<0.05)

Based on the paired t-test results, there was a significant difference in the bodyweight of rats before and after administration of HFD in each group (p=0.000). In table 1, it can be seen that there are significant differences in changes in body weight in all groups (p=0.000). Further analysis using Bonferroni's post-hoc test showed a difference in weight gain in all groups compared to the K- group (p=0.000). The group was fed by the high-fat diet experienced a higher weight gain than the standard feed group (K-). This difference occur due to the different energy absorption of each feed. The high fat diet could stimulate weight gain by increasing its energy absorption. The HFD′s type of feed that contained more energy density derived from fat about 50-60% higher than standard feed(Lacerda et al., 2018; Liang Wang et al., 2020). Energy derived from fat would contribute more to the weight gain. Thermogenesis in fat was only 2-3%, while in carbohydrates was 6-8%, and protein was 25-30%. The thermogenesis which derived from the diet was the energy used to digest, absorb and store nutrients. Therefore, fat had much higher energy efficiency of 97-98%, while carbohydrates was 92-94% and protein was only 70-75%(Maulana & Ridwan, 2021).

Table 2. Average body weight of rats before and after intervention

Groups	Body Weight (BW) (g)		
	BW Early Intervention	BW Final Intervention	Δ (%)
K-	191.00 ± 2.10	209.00 ± 2.61[a]	18.00 ± 0.63 (9.42%)
K+	207.50 ± 2.43	244.50 ± 3.62[a]	37.00 ± 1.79 (17.83%)
S	208.83 ± 3.19	227.83 ± 3.54[a]	19.00 ± 1.26 (9.10%)
P1	207.00 ± 3.41	232.33 ± 3.44[a]	25.33 ± 1.75 (12.24%)
P2	208.17 ± 5.81	227.83 ± 5.19[a]	19.67 ± 1.03 (9.46%)
p			0.000*

Note: [a]paired t-test (p<0.05), *One-way ANOVA test (p<0.05)

The intervention period could increase food intake, hence, the energy balance or calorie storage would increase and affect the weight gain of the rats (p=0.000). Table 2 showed the results of the one-way ANOVA test, that there was a significant changes in body weight in all groups (p=0.000). Based on the average percentage change in weight of the rats, the highest occurred in K+ (17.83%), where only HFD was given to this group until the end of the intervention. Giving HFD could reduce levels of the hormone leptin, therefore, the appetite and food intake would increase. Hence, it causes the increasing body weight to be much heavier in the group that only received HFD compared to the group that received standard feed and additional intervention(Restuti & Yulianti, 2019).

Bonferroni's post-hoc follow-up test results showed no difference in body weight changes of rats in groups S (p=1.000) and P2 (p=0.448) compared to K-. The low-weight gain that occurred in the simvastatin group was in line with the results of research by Zhang et al., (2020) that administration of simvastatin significantly inhibited excessive weight gain in HFD-induced mice. Simvastatin could decrease leptin expression and secretion. Statins could affect leptin mRNA and leptin secretion via the ERK 1/2 and $PPAR_{\gamma}$ pathways. Statins would activate ERK 1/2 and then activate $PPAR_{\gamma}$, which by then decreasing leptin mRNA transcription(Paumelle & Staels, 2007). Based on research conducted by Singh et al.,(2018), it was shown that there was a reduction in leptin of about 20% due to simvastatin treatment.

Meanwhile, the low weight gain in the intervention group of mulberry leaf tea at a dose of 72 mg/100g BW/day was in line with research conducted by Lim et al., (2013) that the administration of mulberry leaf extract could reduce weight gain in rats given HFD. Herbal therapy alone could significantly limit weight gain(Yuniarti et al., 2019). The intervention of mulberry leaf tea at a dose of 72 mg/100g BW/day was able to reduce the weight gain of HFD-induced rats almost the same as simvastatin intervention.

HFD induction which was carried out for 2 weeks, affected the lipid profile levels of rats. The increase in blood lipid levels after consuming food is a physiological process of the digestive system and lipid transport(Zhukova et al., 2014). The composition of the HFD significantly affects the changes in the lipid profile that occured. This time, the HFD was a mixed by standard feed with 0.2% cholic acid and 10% quail eggs(Irmayanti & Ardiaria, 2016). Giving HFD would

cause an increase in blood lipid levels to reach the most significant concentration within 30 minutes after eating(Zhukova et al., 2014). The occurrence of an increase in LDL-C levels and a decrease in HDL-C, as well as an increase in TG, was always associated with an increased cardiovascular risk. CVD risk could be estimated by calculating using indicators such as atherogenic index(Niroumand et al., 2015; Nogay, 2017). The AI was comprehensive and was used as an appropriate marker for inflammation and disorders of lipid metabolism(Cho et al., 2020). In table 3, the results of one-way ANOVA test showed that there was a significant change in atherogenic index values in all groups (p=0.000). The paired t-test showed a significant difference in the atherogenic index value of rat plasma before and after the intervention in the K- (p=0.010) and K+, S, P1, P2 (p=0.000) groups.

Table 3. Average atherogenic index (AI) values before and after intervention

Groups	Atherogenic Index (AI)		
	AI Early Intervention	AI Final Intervention	Δ (%)
K-	(-0.04) ± 0.01	(-0.02) ± 0.01[a]	0.02 ± 0.01(-45.67%)
K+	0.76 ± 0.03	0.85 ± 0.03[a]	0.09 ± 0.02 (11.48%)
S	0.76 ± 0.05	0.06 ± 0.02[a]	(-0.70) ± 0.05 (-92.49%)
P1	0.76 ± 0.03	0.24 ± 0.02[a]	(-0.52) ± 0.03(-68.53%)
P2	0.75 ± 0.04	0.11 ± 0.01[a]	(-0.64) ± 0.03 (-84.73%)
p			0.000*

Note: [a]paired t-test (p<0.05), *One-way ANOVA test (p<0.05)

The highest change in AI values occurred in the simvastatin group, which was indicated by a decrease in the percentage of -92.49%, then the P2 group by -84.73%, followed by the P1 group -68.53% (see table 3). Simvastatin is a *3-hydroxy-3-methylglutaryl* Coenzyme A Reductase (HMGCR) inhibitor known to reduce serum lipid levels(Oikonomidis et al., 2016). The decrease in AI value on simvastatin administration occurred through inhibition of HMG-CoA reductase, a rate-limiting enzyme in the cholesterol biosynthetic pathway. Thus, its inhibition prevents the substrate from entering, therefore blocking the conversion of HMG-CoA to mevalonic acid, which is a key in cholesterol synthesis(Ward et al., 2019). HMGCR transcription is regulated by sterol regulatory element-binding protein-2 (SREBP-2), in which it is a major transcription factor. SREBP-2, affected by statin administration, could cause increasing the activity of other enzymes that would cancel the use of the substrate as a marker in the rate of cholesterol synthesis(Schonewille et al., 2016; Shi et al., 2019).

Based on the results of Tamhane's post-hoc test, it showed that there was no difference in the AI values of rats in S and P2 groups (p=0.260). This means that the decrease in AI values in the P2 group did not differ much from the S group. The highly decreasing in the AI value in the intervention group of mulberry leaf tea (*Morus alba L*) at a dose of 72 mg/100g BW/day occurred because mulberry leaves contained various bioactive compounds, such as flavonoids type quercetin and alkaloids type 1-deoxynojirimycin (DNJ). In line with the research conducted by Metwally et al., (2019) that administration of *Morus alba* leaf ethanol extract significantly reduced weight gain and reduced the increase in cholesterol, TG, atherogenic index and coronary artery index in hyperlipidemic rats fed in a high cholesterol diet (HCD).

The low atherogenic value would potentially reduce atherosclerosis and cardiovascular disease. Flavonoids could reduce the value of AI by increasing HDL-C levels. The higher of HDL-C level, the lower of atherogenic value, hence, the risk of atherosclerosis would be smaller. The antioxidant activity of flavonoids could increase levels of HDL-C(Sa′ Adah et al., 2017). Flavonoids increased HDL-C levels by rising the production of Apo A1, which acted as an enzyme cofactor for lecithin-cholesterol acyltransferase (LCAT) as well as a ligand to interact with lipoprotein receptors in tissues. LCAT was an enzyme that was responsible for the formation of most cholesterol esters and was also important for the maturation of HDL metabolism. Increased LCAT activity induced by increased production of Apo A1 will increase HDL-C levels so that it can fight atherosclerosis(Srinivasan & Pari, 2013; Tenda & Toyo, 2021). The content of quercetin in mulberry leaves was known to reduce the activity of HMG-CoA reductase which affects cholesterol synthesis(Deng et al., 2020). In addition to quercetin, the DNJ content of mulberry leaves could also reduce TG and cholesterol levels by inhibiting the accumulation of lipids in the liver by increasing β-oxidation of fatty acids caused by the activation of AMPK and PPAR-α(Kojima et al., 2010; Thaipitakwong et al., 2018; Tond et al., 2016). In accordance with the research of Tond et al., (2016), that the content of DNJ and quercetin in mulberry leaf extract and mulberry leaf powder could significantly reduce triglyceride levels. Wilson & Islam, (2015) research also showed that the administration of 0.5% mulberry leaf tea for 4 weeks was able to significantly reduce triglyceride levels in rats.

IAP reflects the true relationship between protective and atherogenic lipoproteins associated with the particle size of preatherogenic and antiatherogenic lipoproteins(Niroumand et al., 2015). It was previously known that the atherogenic index correlated well with LDL particle size could be used as an indicator of atherogenic lipoprotein phenotype. The decrease in AI values in this study was caused by the administration of mulberry leaf tea could indirectly reflect a decrease in sdLDL particles. These particles were more atherogenic than larger-density LDL particles(Liang et al., 2015). Although this study did not examine plasma lipoprotein levels based on particle size, a study by Kojima et al (2010) had elaborated that high DNJ content in mulberry leaf extract could reduce LDL fraction very small(Kojima et al., 2010). In addition, the logarithmic transformation of the low molar concentrations of TG and HDL-C in this study was closely related to the particle size of HDL and VLDL cholesterol, indicating a lower cardiovascular risk(Luzhao Wang et al., 2020).

CONCLUSION AND SUGGESTIONS

The administration of mulberry leaf tea (*Morus alba L.*) at a dose of 36 mg/100 g BW/day and 72 mg/100 g BW/day was significantly reduced the atherogenic index value in dyslipidemic *Sprague Dawley* rats. The decrease in AI values in the mulberry leaf tea intervention at a dose of 72 mg/100 g BW/day was almost equivalent to the simvastatin intervention.

Suggestions for further research is necessary to conduct further examination of the toxicity of mulberry leaf tea.

ETHICAL CONSIDERATIONS

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Conflict of Interest Statement

The author(s) declared no conflict of interest in this research

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