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Preventive effect of mulberry (*Morus alba*) leaf tea on low-density lipoprotein and malondialdehyde levels in dyslipidemic sprague-dawley rats

Pengaruh pemberian teh daun mulberry (Morus alba) terhadap kadar low-density lipoprotein dan malondialdehyde pada tikus sprague-dawley dislipidemia

Tia Rahmania Virdias[1*], Mohammad Sulchan[2], Ahmad Ni'matullah Al-baarri[3]

[1] Department of Nutrition Science, Faculty of Medicine, Diponegoro University, Semarang, Indonesia.
E-mail : virdiasrahma@gmail.com
[2] Department of Nutrition Science, Faculty of Medicine, Diponegoro University, Semarang, Indonesia.
E-mail : mohsulchan@gmail.com
[3] Department of Food Technology, Faculty of Animal and Agricultural, Diponegoro University, Semarang, Indonesia.
E-mail : albari@live.undip.ac.id

***Correspondence Author:**
Department of Nutrition Science, Faculty of Medicine, Diponegoro University, Semarang, Indonesia.
Email : virdiasrahma@gmail.com

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Abstract

A high-fat diet can lead to dyslipidemia, which is characterized by elevated levels of Low-Density Lipoprotein (LDL). LDL is rapidly oxidized, resulting in oxidative stress. This increase in oxidative stress can lead to lipid peroxidation, ultimately producing Malondialdehyde (MDA). One way to prevent increased LDL and MDA levels is to consume mulberry leaf tea (MLT). This study aimed to analyze the effects of MLT administration on LDL and MDA levels in dyslipidemic Sprague-Dawley rats fed a high-fat diet (HFD). This study was conducted in 2021 at the Laboratory of the Center for Food and Nutrition Studies at Gadjah Mada University, Yogyakarta. True experimental research with a randomized pre- and post-test control-group design was used in this study. Thirty male Sprague-Dawley rats were used in this study. After 21 days of intervention, LDL and MDA levels were analyzed using CHOD-PAP and ELISA, respectively. One-way ANOVA and Kruskal-Wallis tests were used for statistical analyses. The administration of 36 mg and 72 mg of MLT for 21 days was able to have a significant effect on reducing LDL (p= 0,000) and MDA (p= 0,000) levels. In conclusion, mulberry leaf tea decreased the LDL and MDA levels in Sprague-Dawley rats fed a high-fat diet.

Keywords: Cardiovascular disease, lipid profile, mulberry leaf, oxidative stress,

Abstrak

Pemberian diet tinggi kolesterol (HFD) dapat menyebabkan dislipidemia, dapat ditandai dengan kenaikan kadar Low-Density Lipoprotein (LDL). LDL mudah teroksidasi sehingga mampu menyebabkan keadaan stres oksidatif. Peningkatan stres oksidatif dapat menyebabkan peroksidasi lipid sehingga mampu membentuk Malondialdehyde (MDA). Salah satu upaya untuk mencegah terjadinya kenaikan kadar LDL dan MDA yaitu dengan pemberian teh daun mulberry (MLT). Tujuan penelitian ini untuk menganalisis pengaruh pemberian MLT terhadap kadar LDL dan MDA pada tikus Sprague-Dawley dislipidemia yang diberikan HFD. Penelitian ini dilakukan pada tahun 2021 di Laboratorium Pusat Studi Pangan dan Gizi Universitas Gadjah Mada, Yogyakarta. Penelitian true experimental dengan randomized pre dan post-test control group design digunakan dalam penelitian ini. Tikus Sprague-Dawley jantan sebanyak 30 ekor digunakan pada penelitian ini. Setelah intervensi selama 21 hari, dilakukan analisis kadar LDL dengan metode CHOD-PAP, sedangkan kadar MDA dianalisis menggunakan

ELISA. Analisis statistik yang digunakan yaitu one-way ANOVA dan Kruskal-Wallis. Pemberian MLT dosis 36mg dan 72mg selama 21 hari mampu memberikan pengaruh secara signifikan terhadap penurunan kadar LDL (p=0,000) dan berpengaruh secara signifikan pula pada penurunan kadar MDA (p=0,000). Kesimpulannya, teh daun mulberry terbukti mampu menurunkan kadar LDL dan MDA pada tikus Sprague-Dawley dislipidemia yang diberikan diet tinggi kolesterol.

Kata Kunci: Daun mulberry, penyakit kardiovaskular, profil lipid, stress oksidatif,

Introduction

Dyslipidemia is a condition in which lipid metabolism is disrupted, resulting in elevated total cholesterol and triglyceride levels. The consumption of high-fat diets can induce the development of this disorder. Dyslipidemia is a significant risk factor for the progression of cardiovascular disease, which remains the primary cause of mortality worldwide (Du & Qin, 2023; Thongtang et al., 2022). Non-communicable disease data submitted by the World Health Organization (WHO) in 2018 showed that 35% of Indonesia's population died from cardiovascular disease (World Health Organization, 2018).

Dyslipidemia also involves alterations in lipoprotein levels, including an increase in low-density lipoprotein (LDL) and decrease in high-density lipoprotein (HDL) levels. HDL plays an important role in CVD, because increasing HDL levels can reduce the risk of CVD. HDL can eliminate high amounts of cholesterol from the body through its function in reverse cholesterol transport (Ben-Aicha et al., 2020).

Low-Density Lipoprotein (LDL) is the main transport carrier of cholesterol between the liver and body cells, maintaining a constant cholesterol supply (Duan et al., 2022; Z. Yang et al., 2018). Elevated LDL levels are a significant contributor to the development and advancement of atherosclerosis, as well as a cause of cardiovascular disease. (Azemi et al., 2023; Nodeland et al., 2022; Simsek & Çakatay, 2019). Reducing LDL levels is a therapeutic target in a lipid reduction strategy that leads to preventing cardiovascular disease (Leibowitz et al., 2017; Morieri et al., 2020). LDL is very susceptible to oxidation, and oxidized LDL can directly increase oxidative stress in the blood vessels (Schluter et al., 2010; Schluter et al., 2017).

Oxidative stress occurs when the generation of reactive oxygen species (ROS) exceeds the capacity of the antioxidant defence mechanism (Alfarisi et al., 2020; Poznyak et al., 2021). Polyunsaturated fatty acids (PUFA) present in LDL are susceptible to oxidation when exposed to oxidative stress (Itabe, 2009). Oxidated PUFA, both enzymatically and non-enzymatically, are converted into lipid hydroperoxide and then broken down into highly reactive molecules, such as malondialdehyde (MDA) (Khatana et al., 2020). MDA's high reactivity and toxicity of MDA underlie its relevance in biomedical research (Ayala et al., 2014). Therefore, MDA is a reliable biomarker for oxidative stress.

The consumption of foods rich in antioxidants is considered an effective way to prevent oxidative stress. Antioxidants can prevent the detrimental effects of free radicals (Meng et al. 2020). The mulberry leaves contain antioxidants. Mulberry leaves contain flavonoids and phenolic compounds, which act as antioxidants (Hasim et al., 2020; Thabti et al., 2014). Additionally, mulberry leaves have a higher crude protein content than other verdant greens such as spinach (Yu et al., 2018).

Mulberry (Morus alba) is highly adaptable to a wide range of soil and climatic conditions (Rohela et al. 2020). Mulberry leaves have the potential to be developed into tea products. Tea is a common beverage consumed by Indonesians. Tea is typically derived from Camellia sinensis and brewed with water to produce a unique taste and aroma. However, it is also possible to produce tea from other plants using various plant components such as leaves, flowers, and roots (Poswal et al., 2019). Young mulberry leaves were utilized in this research as tea leaves owing to their elevated polyphenol content (27,45 mgGAE/ml), compared to the old mulberry leaves (14,85 mgGAE/ml) (Jurian et al., 2016).

Previous studies on rats fed a high-fat diet (HFD) showed that intervention with mulberry leaf extract had a hypolipidemic effect by improving blood lipid levels (Ann et al., 2015; Chang et al., 2016; Kobayashi et al., 2015). Research conducted by Wilson and Islam (2015) showed that mulberry leaf tea had a hypolipidemic effect(Wilson & Islam, 2015). Quercetin and kaempferol contents in mulberry leaves can have a hypocholesterolemic effect. Quercetin inhibits cholesterol synthesis by inhibiting 3 – hydroxy – 3 – methylglutaryl - CoA reductase (HMGCR) (Kobayashi et al., 2015). 1Deoxynojirimycin (DNJ), which functions as an anti-atherogenic agent and inhibits lipid accumulation in the liver, is another compound present in mulberry leaves (Tsuduki et al., 2013). Various bioactive compounds in mulberry leaves exert pharmacological effects that positively affect human health. This study aimed to investigate the effect of mulberry leaf tea (MLT) on LDL level reduction and its potential to reduce serum MDA levels in rats with high-fat diet-induced dyslipidemia.

Methods

This study used a true experimental design with pre- and post-test control groups. This study was conducted at the Laboratory of the Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta from May to July 2021.

The experimental subjects were 30 male Sprague-Dawley rats, ranging in age from 8 to 12 weeks, and weighing between 150 and 200 g. The sample size required for this study was determined using Federer's formula (1967) as follows:

Federer's formula: $(n-1)(t-1) \geq 15$

$$(n-1)(5-1) \geq 15$$
$$4n - 4 \geq 15$$
$$n \geq 5$$

Description :
n = number of animals for each treatment group
t = number of treatment groups

The calculation results showed that five rats were required; however, to anticipate dropouts, one rat was added to each group, resulting in a total of 30 rats. The room was maintained at a temperature between 28-32°C with a 12-hour light and 12-hour dark cycle. All the rats were housed in individual cages and cleaned regularly. Rats were acclimatized for one week by being given a standard diet of AD II Comfeed with ad libitum drinking. After the acclimatization period, a random divide of rats resulted in five groupings, and every group consisted of six rats, including K1 (normal control group, standard diet); K2 (negative group, HFD); P1 (positive group, HFD + 0,18 mg/200 gBW simvastatin intervention); P2 (first treatment group, HFD + 36 mg/100 gBW intervention dose of mulberry leaf tea) and P3 (second treatment group, HFD + 72 mg/100 gBW intervention dose of mulberry leaf tea). Normal control rats were provided standard feed throughout the study period. The negative control rats were fed a HFD until the end of the study, with the HFD composition consisting of a mixture of quail eggs and cholic acid. In addition to the normal control rats, K2, P1, P2, and P3 rats were induced with HFD for two weeks. Subsequently, the rats underwent a 21 days intervention period, with P1 administered simvastatin, while P2 and P3 received mulberry leaf tea at different intervention dosages.

Mulberry leaves were collected from the Biopharmaca Conservation and Cultivation Station (BCCS), TropBRC, IPB University, Bogor, Indonesia. In this study, young mulberry leaves were utilized, specifically those from the uppermost to sixth leaves, to produce mulberry leaf tea. The leaves that were picked were separated from the stems. To initiate this process, it was necessary to clean the acquired mulberry leaves to eliminate any dirt or dust particles adhered to the leaves. The leaves were subsequently drained. Subsequently, mulberry leaves were steamed at 100°C. After wilting, leaves were dried in a cabinet dryer at 60°C for 90 min. After drying, the leaves were blended into a powder and sieved through a 230mesh sieve. The refined powder was then brewed using hot water at 100°C and administered to experimental animals.

Blood samples were collected twice from the retroorbital plexus of the rats before and after the intervention to assess LDL and MDA levels. LDL levels were measured using the CHOD-PAP method, and MDA levels were measured using ELISA. Blood samples were maintained, treated, and collected at the Laboratory of the Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta. Ethical approval for this study was obtained from the Research and Health Ethics Commission (KEPK) of the Faculty of Medicine, Diponegoro University Semarang (number 54/EC/H/FK-UNDIP/V/2021).

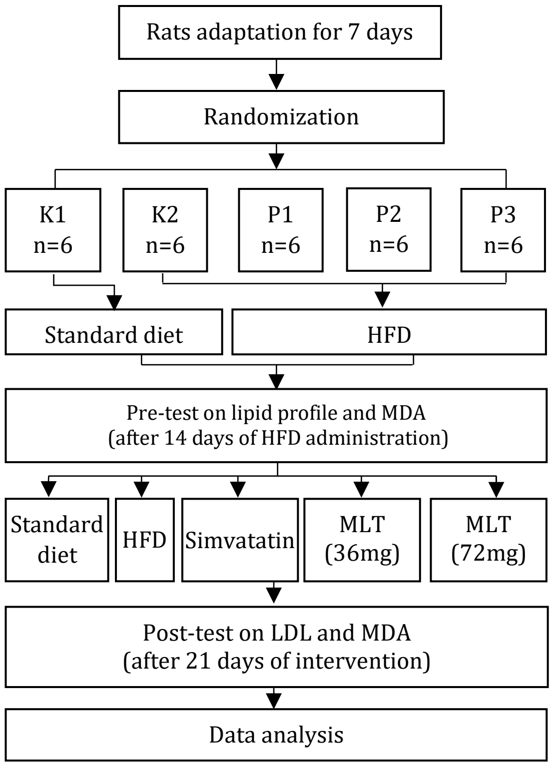

Figure 1. Research Flow

Data analysis was conducted using IBM SPSS 25. Owing to its normal distribution (p>0,05), a paired t-test was used to determine the mean differences in LDL and MDA levels between each group before and after treatment. One-way ANOVA was used to analyze the differences in the effects on LDL levels between the groups. Furthermore, the Kruskal-Wallis test was used to determine differences in the effects on MDA levels between the groups.

Result and Discussion

A high-fat diet (HFD) can significantly elevate the LDL levels in the bloodstream. LDL is known to play a significant role in atherogenesis, making it crucial to reduce LDL levels to prevent cardiovascular disease (Vekic et al., 2022). One of the treatments used to lower cholesterol levels is simvastatin. However, the use of simvastatin, a statin-class drug, is limited because of its unwanted side effects (Zhao et al., 2023). Therefore, it is necessary to develop another potentially effective option for correcting abnormal lipid levels, such as administration of mulberry leaf tea.

In this study, LDL levels were measured twice, before and after the administration of MLT. Preliminary measurements of LDL levels showed that LDL levels increased by 242,55% in rats fed a high-cholesterol diet compared with those fed a standard diet. The mean changes in the LDL levels before and after the intervention are shown in Table 1.

Table 1 . Mean LDL Levels of Rats Before and After the MLT Intervention

Intervention Group	LDL (mg/dL) Before Mean ± SD	LDL (mg/dL) After Mean ± SD	Δ LDL (mg/dL) Mean ± SD	%	p	p¹
P3	81,91±1,72	34,82±2,48	47,08±2,62	57,47±2,91	0,000	
P2	81,79±2,17	46,14±1,72	35,65±2,74	45,33±2,56	0,000	
P1	80,32±2,18	32,71±1,72	47,61±2,04	59,27±1,87	0,000	0,000
K2	79,97±1,32	81,21±1,59	1,24±0,89	1,55±1,11	0,019	
K1	23,55±1,65	26,61±1,61	3,06±0,93	13,14±4,11	0,000	

p = paired t-test
p¹ = one-way ANOVA

Table 1 shows the significant difference in LDL levels in each group both before and after treatment (*p*=0,000 for group K1, *p*=0,019 for group K2, and *p*=0,000 for groups P1, P2, and P3). The one-way ANOVA test results showed a *p*-value of 0,000, indicating a significant difference in LDL levels between the groups. Administration of 36 mg MLT dose was able reduced LDL levels by 45,33%, whereas administration of 72 mg MLT reduced LDL levels by 57,47%. The percentage value resulting from administration of MLT at a dose of 72 mg was similar to that of simvastatin, which reduced LDL levels by 59,27%. Welty *et al.* (2016) found that after six weeks of statin therapy in hypercholesterolemic patients, there was a decrease in LDL levels among study participants (Welty et al., 2016). Simvastatin can inhibit cholesterol synthesis by suppressing HMG-CoA reductase enzyme activity and accelerating circulating LDL clearance in the bloodstream by increasing LDL receptor expression (LDLr) (Gu et al., 2019).

Similar to statins, the phytochemical compound apigenin in mulberry leaves can reduce the expression of HMG-CoA reductase, a rate-limiting enzyme involved in cholesterol formation in the liver (Wong et al., 2017). Apigenin demonstrates its ability reduced the levels of cholesterol, triglycerides, and LDL in rats fed a high-fat diet. This leads to an improvement in hyperlipidemia status, which in turn lowers the risk of atherosclerosis (K. Zhang et al., 2017). According to a study by Lee et al. (2021), LDL levels can be decreased by administering mulberry leaf extract for four weeks (Lee et al., 2021).

Quercetin compounds in mulberry leaves have been shown to prevent the development of atherosclerosis and protect against cardiovascular diseases (Jia et al., 2019). Quercetin is a flavonoid that increases cholesterol 7α - hydroxylase (CYP7A1) expression via the activation of Liver X Receptor Alpha (LXRα). LXRα regulates the expression of CYP7A1, a rate-limiting enzyme in bile acid production, causing a reduction in plasma cholesterol levels (Zhang et al., 2016).

Quercetin increased mRNA expression of ATP-binding cassette transporter G1 (ABCG1). ABCG1 is one of the main transport proteins that carries cholesterol across cell membranes (M. Zhang et al., 2016). This is consistent with the findings of Harumi et al. (2015), LDL levels in Rattus norvegicus rats fed an HFD for four weeks were reduced by the administration of quercetin (Harumi et al., 2015).

In this study, administration of MLT reduced blood serum LDL levels. This finding indicates that MLT can effectively lower blood cholesterol levels. Additionally, various phytochemical compounds present in mulberry leaves can exert hypolipidemic effects.

MDA Levels

Elevated cholesterol levels can lead to various pathological conditions. This condition can lead to oxidative stress, which can damage proteins, nucleic acids, and lipid molecules, whereby LDL can be modified into oxidized LDL (oxLDL) by Reactive Oxygen Species (ROS) (Khatana et al., 2020). In addition, ROS can attack lipid membranes such as phospholipids containing PUFA by initiating lipid peroxidation (Papac-Milicevic et al., 2016). The hydroxyl radical (OH) group is the most reactive and dangerous ROS and plays an important role in lipid peroxidation reactions because of its ability to attack PUFA (Ito et al., 2019). PUFA, particularly arachidonic acid and linoleic acid, found in LDL, are highly prone to oxidation (Levitan et al. 2010). These oxidized PUFAs are then converted into lipid hydroperoxides (Ayala et al., 2014; Khatana et al., 2020). Furthermore, the compounds are broken down, generating more reactive aldehyde products, such as malondialdehyde (MDA) (Khatana et al., 2020; Papac-Milicevic et al., 2016). Table 2 shows the mean MDA levels in the experimental rats before and after the MLT intervention.

Table 2. Mean MDA levels in experimental rats before and after the MLT intervention.

Intervention Group	MDA (ng/mL)		Δ MDA (ng/mL)	(%)	p	p¹
	Before Mean ± SD	After Mean ± SD	Mean ± SD			
P3	9,11±0,27	2,06±0,13	7,05±0,19	77,41±1,08	0,000	
P2	9,43±0,24	3,85±0,35	5,58±0,50	59,09±4,29	0,000	
P1	9,27±0,31	1,84±0,42	7,42±0,28	80,07±0,38	0,000	0,000
K2	9,60±0,16	9,76±0,11	0,16±0,12	1,71±1,27	0,021	
K1	1,03±0,01	1,11±0,21	0,08±0,01	8,22±1,13	0,000	

p = paired t-test
p¹= Kruskal-Wallis

There were significant differences in the MDA levels in each group, as shown in Table 2, in the K1 (p= 0,000), K2 (p= 0,021), P1 (p= 0,000), P2 (p= 0,000), and P3 (p= 0,000) groups. The group that received MLT intervention at a dose of 72 mg experienced a decrease in MDA levels by 7,05 ng/mL, whereas at a dose of 36 mg, MDA levels by 5,58 ng/mL. The results of the Kruskal-Wallis test for MDA levels showed a significant difference in MDA levels between the groups (p=0,000). This indicated that mulberry leaf tea reduced MDA levels in dyslipidemic rats.

In addition to the administration of mulberry leaf tea to the P2 and P3 groups, the administration of simvastatin to the P1 group reduced MDA levels in experimental rats. In line with the results of Muntafiah et al. (2022), this study discovered the same thing, where hypercholesterolemic rats given a 0,018 mg dose of simvastatin showed a decrease in MDA levels (Muntafiah et al., 2022). Although statins can reduce MDA levels, several studies have reported their toxic effects. Therefore, alternative options for statins should be explored. A promising alternative is mulberry leaf tea, which has demonstrated its ability to decrease MDA levels.

A study investigated by Taghizadeh et al. (2022) demonstrated that patients diagnosed with type 2 diabetes mellitus experienced a reduction in MDA levels after 12 weeks of treatment with 300 mg of mulberry leaf extract (Taghizadeh et al., 2022). Treatment with mulberry leaf extract decreased MDA levels in rats with Non-Alcoholic Steatohepatitis (NASH) induced by a HFD for 21 days, according to a study by Sari (2019)(Sari, 2019).

In addition, an HFD stimulates oxidative stress. Oxidative stress occurs when the antioxidant system is unable to cope with the excess production of free radicals. Cell membrane damage due to ROS-induced oxidation can produce aldehyde compounds, known as MDA (Feng et al., 2022; Wahjuni, 2014; X. Yang et al., 2010).

Consistent with this study, the Sprague-Dawley rat group fed an HFD in the study by Feng et al. (2022) also showed an increase in MDA levels (Feng et al., 2022). Antioxidants can play a role in inhibit oxidation by blocking free radicals (Atta et al., 2017). Fauza et al. (2019) demonstrated that foods rich in antioxidants can enhance the total antioxidant capacity of HFD-fed rats induced by an HFD (Fauza et al., 2019).

Mulberry leaves contain microminerals, such as copper, zinc, manganese, and iron. These copper, manganese, and iron compounds contain prosthetic groups comprising the superoxide dismutase (SOD) enzyme, which plays a role in SOD enzyme activity. In addition, zinc stabilizes the SOD structure (X. Yang et al., 2010). SOD is an enzyme that protects cells from the damage caused by free radicals (Adnan et al. 2022). Mulberry leaves contain kaempferol compounds that can increase the production of ROS-removing enzymes such as SOD. A significant increase in SOD activity can decrease oxidative products including MDA (Wang et al., 2015).

Furthermore, the rutin component of the mulberry leaves functions as an agent to eliminate free radicals. In rutin, hydrogen atoms are transferred from the phenolic hydroxyl groups to free radicals. This results in the increased stability of free radicals, thus causing antioxidant effects (Guo et al., 2023).

The findings of this study indicate that MLT intervention can protect dyslipidemic rats from HFD-induced oxidative stress caused by HFD. A limitation of this study is the absence of an analysis of the bioactive constituents present in mulberry leaf tea, such as 1-deoxynojirimycin (DNJ).

Conclusion

Intervention with mulberry leaf tea at doses of 36 mg and 72 mg significantly reduced LDL and MDA levels in Sprague-Dawley rats with dyslipidemia induced by a high-fat diet (HFD). Mulberry leaf tea has the potential to improve lipid abnormalities and exhibits anti-atherosclerotic properties. Further studies are required to investigate the effects of mulberry leaf tea on other markers of oxidative stress such as ox-LDL. Further studies are required to evaluate the safety and efficacy of mulberry leaf tea in humans.

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