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Effectiveness of Mulberry Leaf Extract Supplementation on C-Reactive Protein (CRP) Levels in Sprague Dawley Rats Hyperlipidemia Model

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Abstract

Introduction: Hyperlipidemia is a major risk factor for cardiovascular disease and is closely associated with chronic inflammation, reflected by elevated C-reactive protein (CRP). Although statins effectively reduce lipid levels, their long-term use may produce adverse effects, highlighting the need for evidence-based complementary herbal therapies. Mulberry leaf (*Morus alba* L.) contains flavonoids and other bioactive compounds with hypolipidemic, antioxidant, and anti-inflammatory properties.. This study evaluated the effect of mulberry leaf extract supplementation on CRP levels in Sprague Dawley rats with hyperlipidemia.

Methods: A true experimental pretest-posttest control group design was conducted using 24 male *Sprague Dawley* rats assigned to four groups (n = 6): normal, negative control, positive control (simvastatin), and treatment (*Morus alba* leaf extract, 600 mg/kg body weight/day). Hyperlipidemia was induced with a high-fat diet for eight weeks, followed by five weeks of intervention. Serum CRP levels were measured using a rat CRP ELISA kit. Data were analyzed using paired *t*-tests, Kruskal-Wallis, and Mann-Whitney post hoc tests ($p < 0.05$).

Results: CRP levels decreased significantly in both the simvastatin and mulberry leaf extract groups ($p < 0.001$). Delta CRP values differed significantly among groups ($p < 0.001$), confirming that mulberry leaf extract effectively reduced systemic inflammation.

Conclusion: Mulberry leaf extract shows promise as an evidence-based complementary herbal therapy for reducing inflammation and preventing cardiovascular complications, supporting its integration into complementary nursing interventions for cardiovascular disease prevention.

Keywords: C-reactive protein, cardiovascular disease, complementary nursing, hyperlipidemia, inflammation, mulberry leaf extract

Introduction

Hyperlipidemia is an increase in blood fat levels due to excessive fat consumption, resulting in an imbalance between fat intake and metabolism. Fat accumulation is caused by excess calorie intake, lack of exercise, and the interaction of various socioeconomic, environmental, and genetic factors (Lavanya et al., 2017). This increase is closely linked to atherosclerosis. Long-term hyperlipidemia can trigger inflammation, which then leads to vascular remodeling and plaque formation in blood vessels. Chronic vascular remodeling and plaque formation can lead to cardiovascular disease (Yousuf et al., 2013). The inflammatory process that occurs in chronic dyslipidemia is characterized by the presence of various

biomarkers, one of them is CRP (Kim et al., 2012).

C-Reactive Protein (CRP) is an acute-phase protein produced by the liver in response to inflammation. Inflammation plays a crucial role in the formation of atherosclerotic plaque. Among inflammatory markers, CRP is considered a marker of inflammation in coronary heart disease (Noviyanti & Setiawan L, 2019). Thakur et al. estimated CRP levels in coronary heart disease and found a significant increase in CRP levels (Kamath et al., 2015). Elevated CRP levels are associated with the severity and progression of coronary atherosclerosis (Dash et al., 2025)

Pharmacological management of hyperlipidemia involves administering normocholesterolemic drugs (*chemicals*). The most commonly prescribed and effective normocholesterolemic drugs are statins. This class of drugs is a group of chemicals whose long-term use can cause side effects (Samson, 2008). In addition to using medications to treat high blood cholesterol, people have also begun using natural ingredients to lower blood cholesterol levels. Knowledge of medicinal plants is based on experience and skills passed down from one generation to the next (Nuralifah et al., 2019). Vegetables and fruit are sources of dietary fiber and also contain antioxidants and anti-inflammatory properties (Knekt et al., 2004). However, insufficient dietary intake has increased interest in evidence-based complementary medicine.

Mulberry plants generally contain antioxidants beneficial for health in their fruit and leaves (Kasikorn et al., 2019; UĞUR, 2021). However, the bioactive components and bioactivity of mulberry leaves are influenced not only by the species but also by differences in geographical conditions and climate (Kintoko & Witasari, 2021; Megawati et al., 2019). The total antioxidant content in vegetables and fruits is almost 80% from flavonoids (Lestari, 2016). Previous research stated that mulberry leaf extract has a high total phenol and flavonoid content compared to the fruit (Hilwiyah et al., 2015). Flavonoids can reduce total cholesterol, triglycerides, FFA, VLDL, and K-LDL levels and increase K-HDL, lipoprotein lipase, and lecithin cholesterol acyltransferase levels in rats given 20% ethanol for two months (Ahmed, 2021). Therefore, flavonoids can reduce CRP levels (Hsieh et al., 2021). This prompted researchers to investigate the effect of administering mulberry leaf extract (*Morus alba* L.) at a dose of 600g/BW/day on CRP levels in hyperlipidemic rats.

Methods

This research is a true experimental type with a pre-post test with a control group design conducted with a completely randomized design. The purpose of using this design is to compare two or more groups by randomization and reduce confounding in the study (Campbell

& Stanley, 1966). Changes will be seen in the treatment group compared to the control group. This research design is intended to study the effects of *Morus alba* leaf extract through initial observations/measurements (before being treated) and final measurements (after being treated) by placing a control group. The sample used in this study was 30 rats. All rats will be divided into 5 groups and each group consists of 6 rats (Purnama et al., 2024a).

To determine the effect of mulberry leaf extract, the experimental animals were fed a high-fat diet using a mixture of standard comfeed PARs 60% rat feed mixed with 27.8% flour, 2% cholesterol, 0.2% cholic acid, and 10% lard for 8 weeks (Purnama et al., 2024a). The mulberry leaf extract given to Sprague Dawley rats was a dose of 600 g/kgBW via a tube for 5 weeks. CRP levels were measured using the CRP ELISA Kit for Rat (©Elabsience). The stages of this study included taking retro-orbital sinus vein blood samples from the experimental animals before and after treatment with mulberry leaf extract and positive controls.

The researchers have obtained ethical clearance from the ethics committee of the Faculty of Medicine, Universitas Diponegoro (FK UNDIP Semarang) under the code 23/EC-H/KEPK/FK-UNDIP/II/2023. Therefore, this study only required analyzing serum/blood samples previously stored in the UGM Inter-University Center for Food and Nutrition (PAU) laboratory.

Results

Table 1. Results of CRP level examination in all groups

Group	Mean (n=24)			p-value*
	Pre	Post	Delta	
N	0.72 ± 0.16	0.76 ± 0.15	0.38 ± 0.004	< 0.001
K-	2.26 ± 0.18	2.28 ± 0.13	0.02 ± 0.01	0.02
K+	2.30 ± 0.82	1.00 ± 0.15	1.30 ± 0.80	< 0.001
P	2.27 ± 0.45	0.90 ± 0.008	1.37 ± 0.39	< 0.001
p-value**	0.04	< 0.001	< 0.001	

Notes: N = Normal group, K- = Negative control, K+ = Positive control, and P = *Morus alba* leaf extract treatment group. *Paired t-test, Kruskal-Wallis test ($p < 0.05$: Significant).

Table 1 shows, CRP levels were significantly different ($p < 0.05$) in all treatment groups both before and after treatment (t-test). Delta CRP levels were also significantly different ($p < 0.05$) using the *Kruskall-Wallis* test because the data distribution on delta CRP was not normal, so it was continued with the Mann-Whitney Post-hoc Test.

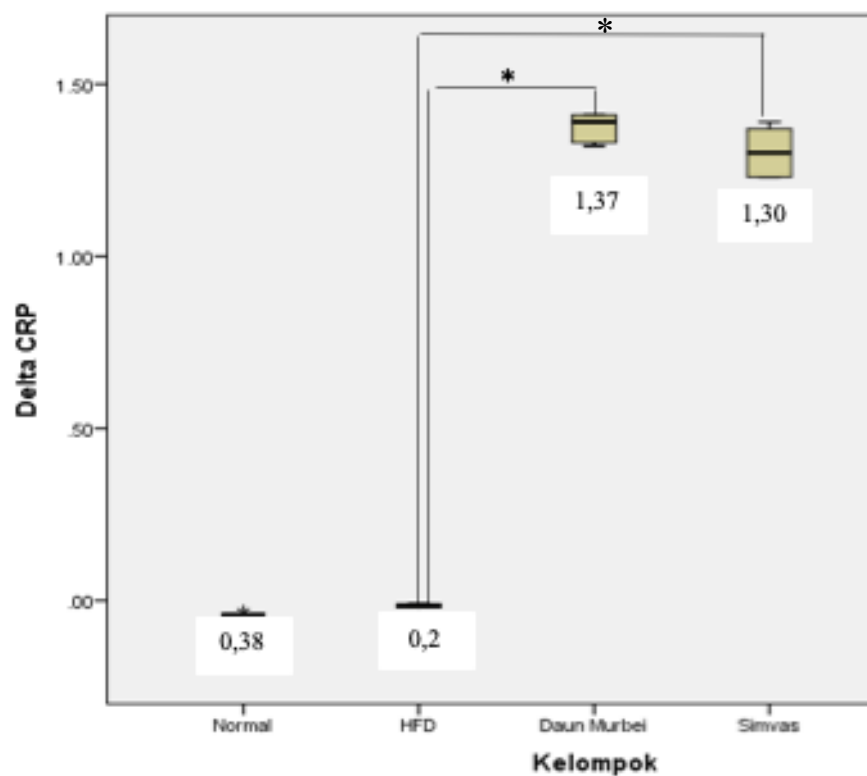


Figure 1. Boxplot of the Mann-Whitney post hoc test. CRP levels * $\alpha < 0.05$

Figure 1 shows that almost all treatment groups differed compared to the normal group. Furthermore, the group of rats with dyslipidemia differed significantly from the group mulberry leaves and the group simvastatin.

Discussion

This study demonstrates that mulberry leaf extract (*Morus alba*) supplementation significantly impacts C-Reactive Protein (CRP) levels in Sprague Dawley rats (a model of hyperlipidemia). Analysis using a paired t-test revealed a significant difference in CRP levels before and after treatment across all groups ($p < 0.05$). Furthermore, analysis of delta CRP using the *Kruskal-Wallis* test also revealed a significant difference between groups ($p < 0.05$), which was further confirmed by a Mann-Whitney test. These findings indicate that the interventions administered to each group resulted in different changes in the inflammatory response.

CRP is an acute-phase protein produced by the liver in response to systemic inflammation. In hyperlipidemia, increased lipid levels, particularly LDL cholesterol, can trigger oxidative stress and endothelial dysfunction, which in turn activates inflammatory mediators such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α). Activation of

these cytokines will stimulate CRP synthesis in the liver so that CRP levels increase and become an important biomarker in the development of atherosclerosis and cardiovascular disease (Virdias et al., 2024).

In the K- group (high-fat diet without therapy), changes in CRP levels suggest that the high-fat diet plays a role in increasing the inflammatory response. High-fat diets are known to cause lipid accumulation, free radical formation, and increased LDL oxidation, which contribute to chronic low-grade inflammation. This condition accelerates the activation of the Nuclear Factor-kappa B (NF- κ B) pathway, which increases proinflammatory cytokine production and CRP levels (Virdias et al., 2024). The researchers suggest that the absence of therapeutic intervention allowed persistent lipid-induced oxidative stress and inflammation, thereby sustaining CRP production.

In the K+ group (high-fat diet + simvastatin), there was a significant change in CRP levels. This effect is thought to stem not only from simvastatin's ability to lower cholesterol levels but also from its pleiotropic effects as an anti-inflammatory agent. Simvastatin is known to inhibit mevalonate synthesis, which plays a role in the activation of various inflammatory mediators, thereby suppressing the production of pro-inflammatory cytokines and CRP. Therefore, statin is often used as a positive control in studies evaluating the anti-inflammatory effects of natural products on hyperlipidemia (Lavanya et al., 2017).

Interestingly, a significant decrease in CRP levels was also found in group P (high-fat diet + mulberry leaf extract). These results indicate that mulberry leaf extract has anti-inflammatory potential in hyperlipidemia. Mulberry leaves are known to contain various bioactive compounds such as flavonoids, quercetin, rutin, chlorogenic acid, and 1-deoxynojirimycin (DNJ), which have antioxidant and anti-inflammatory activities. These compounds can suppress the formation of reactive oxygen species (ROS), inhibit LDL oxidation, and reduce the activation of the NF- κ B inflammatory pathway, thereby reducing the production of pro-inflammatory cytokines and CRP (Purnama et al., 2024b).

In addition, several previous studies have demonstrated that mulberry leaves possess significant hypolipidemic properties that contribute to the improvement of lipid profiles and the reduction of vascular inflammation. Mulberry leaf extract has been reported to decrease total cholesterol, triglyceride, and low-density lipoprotein (LDL) concentrations through multiple mechanisms, including inhibition of intestinal cholesterol absorption, enhancement of cholesterol excretion via bile acids, modulation of hepatic lipid metabolism, and upregulation of LDL receptor expression, thereby facilitating the clearance of circulating LDL particles. These improvements in lipid metabolism reduce the accumulation and oxidation of LDL within

the vascular endothelium, limiting foam cell formation and slowing the progression of atherosclerotic plaque development (Lee et al., 2021). As a consequence, endothelial dysfunction and oxidative stress are attenuated, leading to reduced activation of inflammatory signaling pathways such as nuclear factor-kappa B (NF- κ B) and decreased production of pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α). Since IL-6 is the primary inducer of hepatic C-reactive protein (CRP) synthesis, suppression of these inflammatory mediators subsequently lowers circulating CRP concentrations. Therefore, the hypolipidemic effect of mulberry leaf extract not only improves lipid homeostasis but also indirectly attenuates systemic inflammation, as reflected by reduced CRP levels, highlighting its potential role in preventing the progression of cardiovascular disease (Dash et al., 2025).

The results of this study also align with clinical studies reporting that mulberry leaf consumption can reduce CRP levels in individuals with mild dyslipidemia. This reduction in CRP indicates that mulberry leaves not only act as a hypolipidemic agent but also have anti-inflammatory activity that has the potential to prevent the progression of atherosclerosis and cardiovascular disease.(Aramwit et al., 2013). The significant difference in delta CRP values between groups indicates that the response to each intervention was not equal. The Kruskal-Wallis test was chosen because the delta CRP data were not normally distributed, making non-parametric analysis more appropriate. The results of the Mann-Whitney test, which further showed differences between groups, confirm that mulberry leaf extract supplementation has a biological effect on reducing inflammation in the hyperlipidemia model. From a nursing perspective, these findings support the integration of evidence-based complementary interventions into nursing care for individuals at risk of cardiovascular disease. As a functional herbal therapy, mulberry leaf extract may serve as a complementary approach to conventional treatment by helping reduce systemic inflammation, thereby enhancing preventive and promotive nursing strategies aimed at improving cardiovascular health and reducing the burden of chronic non-communicable diseases.

Conclusion

This study demonstrates that mulberry leaf extract supplementation has the potential to reduce CRP levels in hyperlipidemia-model Sprague Dawley rats. This effect is thought to be due to a combination of hypolipidemic, antioxidant, and anti-inflammatory mechanisms possessed by mulberry leaf phytochemicals. Therefore, mulberry leaf extract has the potential to be developed as a complementary herbal therapy to prevent inflammatory complications

from hyperlipidemia and cardiovascular disease. These findings provide evidence supporting the integration of mulberry leaf extract into evidence-based complementary nursing interventions aimed at promoting cardiovascular health, reducing systemic inflammation, and enhancing preventive care for individuals at risk of chronic cardiovascular diseases. Future clinical studies are warranted to evaluate its effectiveness and safety in human populations and to strengthen its application in complementary nursing practice.

Author Contributions

First Author (1st Author): Conceptualized the study, executed the research methodology/experiments, prepared the initial draft, and performed critical editing and revisions of the manuscript.

Second Author (2nd Author): Responsible for translating the manuscript into English, as well as proofreading, linguistic editing, and general manuscript refinement.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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