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Effectiveness gynura procumbens on blood cholesterol levels in a rat model of diet-induced hypercholesterolemia

Sri Wahyuni Gayatri[1]*, Aryanti Bamachry[1], Priyo Sasmito[2]

[1]Fakultas Kedokteran Universitas Muslim Indonesia
[2]Fakultas Kedokteran Universitas Padjadjaran
Corresponding author: *E-mail: sriwahyuni.gayatri@umi.ac.id

Abstract

Background: Gynura procumbens (Lour.) is a medicinal plant known for its potential in managing various metabolic disorders, including hypercholesterolemia. However, scientific data supporting its cholesterol-lowering effects remains limited.
Purpose: To evaluate the effect of ethanol extract of Gynura procumbens leaves on total cholesterol levels in male Wistar rats induced with a high-fat diet.
Method: A quasy experiment approach, specially focusing on post-test only group. The study took place from March-August 2023, in Research Laboratory of the Faculty of Medicine, Universitas Muslim Indonesia. The independent variable was gynura procumbens extract dependent variables is total cholesterol levels. Using purposive sampling and federer formula, 21 participants were selected which were divided into 3 groups. Group I (negative control) received a placebo, group II (treatment group) received gynura procumbens extract, and group III (positive control) received simvastatin, who met specific inclusion and exclusion criteria. Analysis proceeded with a univariate test to determine the frequency distribution of respondent's characteristics, followed by bivariate analysis using man whitney and anova test.
Results: After induction, all groups showed a marked increase in cholesterol levels. Following the treatment period, the group receiving Gynura procumbens extract demonstrated a significant reduction in cholesterol levels (123.43 ± 24.80 mg/dl, p = 0.018), which was statistically comparable to the simvastatin group (112.83 ± 8.52 mg/dl, p = 0.033). In contrast, the negative control group showed no significant change (231.33 ± 90.56 mg/dl, p = 0.075).
Conclusion: These findings indicate that Gynura procumbens leaf ethanol extract has a significant cholesterol-lowering effect in hypercholesterolemic rats, with efficacy comparable to simvastatin as standard pharmacological therapy.

Keywords: Cholesterol; Gynura Procumbens; Hypercholesterolemia; Rat Model.

INTRODUCTION

Hypercholesterolemia is recognized as one of the major risk factors in the development of cardiovascular diseases such as atherosclerosis, coronary heart disease, and strok (Kaptoge, Pennelis, De Bacquer, Cooney, Kavousi, Stevens, & Di Angelantonio, 2019). This condition is characterized by elevated total cholesterol levels in the blood, generally exceeding 200 mg/dl (Grundy, Stone, Bailey, Beam, Birtcher, Blumenthal, & Yeboah, 2019). According to the World Health Organization (WHO), more than 36 million people die each year due to non-communicable diseases, with a significant proportion attributed to cardiovascular condition (Mach, Baigent, Catapano, Koskinas,

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Casula, Badimon, & Wiklund, 2020). Risk factors such as hypertension, high glucose levels, and obesity, high-fat dietary patterns also substantially contribute to the increased risk. Managing hypercholesterolemia is of critical importance. One of the primary approaches is pharmacological treatment, particularly with statins. However, in the context of long-term risk and limited access to conventional therapies, lifestyle modifications and the use of functional foods or nutraceuticals are also recommended, especially for individuals with low-to-moderate cardiovascular risk (Poli, & Visioli, 2019).

Statin therapy has been associated with long-term side effects such as liver dysfunction and reversible memory impairment, particularly in elderly patient (Adhyaru, & Jacobson, 2018). These concerns have prompted the search for safer and natural-based alternatives. One medicinal plant that has gained attention is Gynura procumbens (Lour.), commonly known in Indonesia as "sambung nyawa," which contains bioactive compounds such as flavonoids and tannins with potential cholesterol-lowering effects (Manogaran, Vuanghao, & Mohamed, 2020). Gynura procumbens exhibits significant antioxidant and anti-inflammatory activities and is capable of modulating blood pressure and lipid profiles (Ahmad, Fauzi, Buang, Saad, Husain, Jantan, & Jubri, 2019). Additional studies have demonstrated that G. procumbens reduces total cholesterol, triglycerides, and Low-Density Lipoprotein (LDL) levels while increasing High-Density Lipoprotein (HDL) levels in hyperlipidemic mice, potentially through the enhancement of antioxidant enzyme activity, such as superoxide dismutase (SOD) and catalase (CAT) (Ai, Xiong, Chen, Song, Song, Xu, & Chen, 2024)

There remains a gap in understanding how its efficacy compares to conventional therapies such as simvastatin, especially in animal models of diet-induced hypercholesterolemia. Prior in vivo research primarily focused on female rats in the context of postmenopausal condition (Ahmad, Haji, Fauzi, Buang, Jantan, & Jubri, 2021).

RESEARCH METHOD

A quasy experiment approach, specially focusing on post-test only group. The study took place from March-August 2023, in Research Laboratory of the Faculty of Medicine, Universitas Muslim Indonesia. The independent variable was gynura procumbens extract dependent variables is total cholesterol levels. Using purposive sampling and federer formula, 21 participants were selected which were divided into 3 groups. Group I (negative control) received a placebo, group II (treatment group) received gynura procumbens extract, and group III (positive control) received simvastatin, who met specific inclusion and exclusion criteria.

The inclusion criteria required male rats (Wistar or Sprague-Dawley strain) in a healthy condition, aged 6-8 weeks at the start of the study and weighing 150-200 grams. Exclusion criteria included rats with congenital abnormalities or illness during the acclimatization period, rats that did not show an increase in cholesterol levels after being fed a high-cholesterol diet (failed model), rats that were intolerant to the intervention (Gynura procumbens), for example, showing signs of acute toxicity or drastic weight loss.

Gynura procumbens extract was prepared using the maceration method with ethanol as the solvent. Dried Gynura procumbens foliage was ground into a smooth powder, then placed in ethanol for three days at room temperature. After that, the solution was filtered and evaporated using a rotary evaporator until a concentrated extract was obtained. The dosage of the extract was calculated based on the conversion of the human equivalent dose to the test animal using a conversion factor of 0.018. Based on this calculation, the extract was administered at a dose of 2.7 mg/day for every 200 grams of body weight of the rat. As a positive control, simvastatin was used at a dose of 3.6 mg/day per 200 grams of body weight, also calculated using the human-to-animal dose conversion method.

Sri Wahyuni Gayatri[1]*, Aryanti Bamachry[1], Priyo Sasmito[2]

[1]Fakultas Kedokteran Universitas Muslim Indonesia
[2]Fakultas Kedokteran Universitas Padjadjaran
Corresponding author: *E-mail: sriwahyuni.gayatri@umi.ac.id

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Before treatment, all rats were first given a high-fat diet for seven days to induce hypercholesterolemia. The diet consisted of egg yolks, corn flour, fish meal, mung beans, beef fat, and butter. After the induction period was complete, total blood cholesterol levels were measured via the caudal vein using a NESCO Multi-Function Monitoring System.

Treatment was conducted for seven days according to group assignment. Group I received a placebo, Group II received Gynura procumbens extract, and Group III received simvastatin. The treatment was given orally using the oral gavage technique. At the end of the treatment period, on day 14, blood samples were collected to measure total cholesterol levels using the same method as before.

The analysis proceeded with a univariate test to determine the frequency distribution of respondent's characteristics, followed by bivariate analysis using man whitney and anova test. This research has obtained permission from the Research Ethics Committee Muslim Indonesia University with number No. 039/A.1/KEKP-UMI/2023.

RESEARCH RESULTS

Table 1. Total Cholesterol Levels of Male Wistar Rats in Each Experimental Group (N=21)

Group	Total Cholesterol Level (mg/dl) (Mean ± SD)			
	Day 0 (n=7) (Pre-induction)	Day 7 (n=7) (Post-induction)	Day 14 (n=7) (Post-treatment)	p-value (Day 7 vs 14)
Negative Control	134.17 ± 12.04	237.50 ± 89.77	231.33 ± 90.56	0.075
Gynura Procumbens Extract	138.43 ± 14.51	305.43 ± 105.08	123.43 ± 24.80	0.018*
Simvastatin	114.50 ± 9.72	228.50 ± 97.77	112.83 ± 8.52	0.033*

Based on Table 1, On day 0, total cholesterol levels were within the normal range in all groups: 134.17 ± 12.04 mg/dl in the negative control group, 138.43 ± 14.51 mg/dl in the Gynura procumbens extract group, and 114.50 ± 9.72 mg/dl in the simvastatin group. After seven days of a high-fat diet, there was a significant increase in cholesterol levels across all groups: 237.50 ± 89.77 mg/dl (negative control), 305.43 ± 105.08 mg/dl (extract), and 228.50 ± 97.77 mg/dl (simvastatin). On day 14, after seven days of treatment, the negative control group showed a decrease in cholesterol levels to 231.33 ± 90.56 mg/dl, but this decrease was not statistically significant (p = 0.075). In contrast, the group administered G. procumbens extract showed a significant decrease to 123.43 ± 24.80 mg/dl (p = 0.018), while the simvastatin group also experienced a significant decrease to 112.83 ± 8.52 mg/dl (p = 0.033).

Table 2. One-Way ANOVA Analysis Results

Group Comparison	Mean Difference (mg/dl)	p-value
Extract vs Simvastatin	66.33	0.253
Extract vs Negative Control	175.83	0.004*
Simvastatin vs Negative Control	109.5	0.006*

Based on Table 2, the One-way ANOVA analysis showed statistically significant differences in cholesterol levels between groups on day 14. The Least Significant Difference (LSD) post hoc test showed that there was no significant difference between the group given Gynura procumbens leaf extract and the simvastatin group (p=0.253). However, both groups differed significantly from the negative control group (p = 0.004 for extract vs. control, and p = 0.006 for simvastatin vs. control). These findings indicate that Gynura procumbens leaf ethanol extract has a significant cholesterol-lowering effect in hypercholesterolemic rats, with efficacy comparable to simvastatin as standard pharmacological therapy.

Sri Wahyuni Gayatri[1]*, Aryanti Bamachry[1], Priyo Sasmito[2]

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Corresponding author: *E-mail: sriwahyuni.gayatri@umi.ac.id

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DISCUSSION

This study demonstrated that the administration of G. procumbens ethanolic leaf extract significantly reduced total cholesterol levels in male Wistar rats induced with a high-fat diet. The observed cholesterol-lowering effect after seven days of treatment was comparable to that of simvastatin, while the negative control group (placebo) did not show significant changes. These findings suggest that the pharmacological activity of G. procumbens is responsible for the observed effects.

The results are consistent with previous studies that reported the hypolipidemic activity of G. procumbens. The plant is rich in bioactive compounds, particularly flavonoids such as quercetin, which are known to act as antioxidants by inhibiting LDL oxidation and enhancing lipoprotein lipase activity involved in triglyceride and cholesterol metabolis (Zhou, Ryan, Ernst, Zoungas, Tonkin, Woods, & Aspree, 2021). Tannins in the extract are suggested to inhibit lipid absorption in the gastrointestinal tract, further contributing to its cholesterol-lowering effect (Murugaiyah, Saeed, Kuong, Murugesu, Parasuraman, Asmawi, & Sadikun, 2018). Tannins may influence bile acid metabolism by enhancing their excretion, which in turn stimulates the liver to utilize more cholesterol for bile acid synthesis, effectively lowering circulating cholesterol levels. The presence of tannins, alongside other bioactive compounds such as flavonoids and saponins in Gynura procumbens, likely acts synergistically to improve lipid profiles (Chen, Chen, & Huang, 2024).

The comparable efficacy of G. procumbens to simvastatin highlights its potential as a natural alternative for managing hypercholesterolemia. Simvastatin exerts its effect through inhibition of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which plays a critical role in hepatic cholesterol biosynthesi (Setyowati, Ikawati, Hertiani, & Pramantara, 2021). Flavonoids from G. procumbens may similarly modulate lipid metabolism-related gene expression and exert anti-inflammatory and antioxidant effects that support cardiovascular health (Balansag, Limocon, Medina, & Paulo, 2016).

Previous investigations have also supported the multifaceted lipid-lowering mechanisms of G. procumbens. Gynura procumbens extracts significantly reduced total cholesterol, triglycerides, and LDL levels while increasing HDL levels in hyperlipidemic animal models, with efficacy comparable to statins. The plant inhibits foam cell formation, a key step in atherogenesis, by reducing lipid accumulation and Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) gene expression, and upregulating ATP-binding cassette transporter A1 (ABCA-1) expression, which facilitates cholesterol efflux from macrophages (Sahin, & Ilgun, 2022).

Additional mechanisms involve inhibition of HMG-CoA reductase activity and pancreatic lipase, reduction of lipid absorption in the intestines, and enhancement of endogenous antioxidant enzyme activities such as SOD, CAT, and glutathione peroxidase (GPx), while simultaneously reducing levels of malondialdehyde (MDA), a marker of lipid peroxidation and oxidative damage (Jabbar, Alamri, Abdulla, Salehen, Al-Sinawi, & Alfaifi, 2023).

In high-cholesterol diet-induced animal models, G. procumbens supplementation has been shown to lower not only total cholesterol, triglycerides, and LDL, but also improve oxidative status by enhancing the body's antioxidant defense and decreasing lipid accumulation in blood vessels (Jobaer, Ashrafi, Ahsan, Hasan, Rashid, Islam, & Masud, 2023). bioactive compounds present in G. procumbens, such as flavonoids and phenolic acids, are believed to play a crucial role in reducing oxidative stress and inflammation, which are key contributors to the development of atherosclerosis. The combined lipid-lowering and antioxidant properties make G. procumbens a promising natural therapeutic agent for managing hypercholesterolemia and preventing cardiovascular complications associated with elevated cholesterol levels (Nedkoff, Briffa, Zemedikun, Herrington, & Wright, 2023). These effects are closely related to its bioactive constituents, particularly flavonoids and phenolic compounds, which act as free radical scavengers and mitigate oxidative lipid damage, thereby preventing cellular membrane disruption (Zubaidah, Siregar, Islamatasya, Nisa, Wibowo, & Manuhara, 2024).

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Corresponding author: *E-mail: sriwahyuni.gayatri@umi.ac.id

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Gynura procumbens has been reported to downregulate the expression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), while upregulating the expression of genes involved in antioxidant defense systems. This dual action of modulating both oxidative stress and inflammation supports the hypothesis that G. procumbens provides multifaceted protection against hypercholesterolemia-induced damage (Amin, Afrin, Meghla, Nur, Rahman, & Uddin, 2021). Gynura procumbens helps prevent lipid peroxidation and the subsequent formation of atherosclerotic plaques, which are critical factors in cardiovascular disease progression. Simultaneously, its anti-inflammatory properties mitigate the chronic low-grade inflammation often associated with elevated cholesterol levels, thereby preserving vascular integrity and organ function (Olmastroni, Molari, De Beni, Colpani, Galimberti, Gazzotti, & Casul, 2022).

The therapeutic potential of Gynura procumbens in cholesterol metabolism is not solely limited to its lipid-lowering capacity, but also includes a crucial role in restoring oxidative and inflammatory balance in the body. Gynura procumbens exhibits substantial hypocholesterolemic and antioxidative potential through multiple cellular and molecular pathways, highlighting its role as a promising candidate for the development of natural lipid-lowering agents. However, inter-individual variations in response among the animals also highlight the influence of biological factors such as metabolic rate, body weight, and immune response on treatment outcomes (Halim, Ghafar, Das, Zainalabidin, & Jubri, 2021).

CONCLUSION

Gynura procumbens ethanolic leaf extract significantly lowers total cholesterol in high-fat diet-induced male Wistar rats, with effects comparable to Simvastatin. The hypocholesterolemic action is attributed to flavonoids and tannins, which regulate lipid metabolism and enhance antioxidant activity. These findings support its potential as a natural alternative to statins and highlight the need for further clinical research to assess its safety and efficacy in humans.

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[1]Fakultas Kedokteran Universitas Muslim Indonesia
[2]Fakultas Kedokteran Universitas Padjadjaran
Corresponding author: *E-mail: sriwahyuni.gayatri@umi.ac.id

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[1]Fakultas Kedokteran Universitas Muslim Indonesia
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Corresponding author: *E-mail: sriwahyuni.gayatri@umi.ac.id

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