COMBINATION EFFECTIVENESS OF GLUCOMANAN AND MORINGA OLEIFERA LEAF EXTRACT ON LIPID PROFIL OF HYPERCHOLESTEROLEMIA RATS

EFEKTIVITAS KOMBINASI GLUKOMANAN DAN EKSTRAK DAUN KELOR (MORINGA OLEIFERA) TERHADAP PROFIL LIPID TIKUS HIPERKOLESTEROLEMIA

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ABSTRACT

Hypercholesterolemia is a disorder in cholesterol metabolism that can cause an abnormal increase in total cholesterol levels in the blood. This study aims to determine the effectiveness of the combination of glucomannan (Amorphophallus muelleri Blume) and Moringa oleifera leaf extract on the lipid profile of hypercholesterolemic Wistar rats. This research design used True experimental with a pretest-posttest control group design. The animal used in this study was 24 male Wistar rats (Rattus norvegicus) aged 8 weeks divided into 2 control groups and 6 treatment groups of glucomannan and Moringa oleifera leaf extract. The results of this study showed that

Kata Kunci: Amorphophallus muelleri Blume; Moringa oleifera; Hypercholesterolemia; TC; TG; LDL-C; HDL-C.

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there were significant changes in TC, TG, LDL-C, and HDL-C levels (p<0.05). It can be concluded that the combination of glucomannan (Amorphophallus muelleri Blume) and Moringa oleifera leaf extract can change the lipid profile effectively in the P5 (80mg/kgBW GAmb: 120mg/kgBW MoEL) and P4 (120mg/kgBW GAmb:80mg/kgBW MoEL ) as the most influential group in reducing TG, TC, LDL-C levels and increasing HDL-C levels.

Keywords: Amorphophallus muelleri Blume; Moringa oleifera; Hypercholesterolemia; TC; TG; LDL-C; HDL-C.

INTRODUCTION

Unhealthy lifestyles, particularly the consumption of foods high in fat and low in fiber, contribute to the development of degenerative diseases, specifically hypercholesterolemia. Hypercholesterolemia is a medical condition characterized by abnormally high levels of cholesterol in the bloodstream due to disruptions in its metabolism [1]. Hypercholesterolemia is a medical disorder characterized by elevated levels of total cholesterol, triglycerides, and LDL-C (Low-Density Lipoprotein cholesterol), together with reduced levels of HDL-C (High-Density Lipoprotein cholesterol) as detected by a lipid profile test [2]. The standard lipid thresholds for humans are 200 mg/dL for Total Cholesterol, 130 mg/dL for LDL-C, over 40 mg/dL for HDL-C, and 150 mg/dL for Triglycerides [3], [4].

High cholesterol levels, especially LDL-C levels in the blood, will cause long-term risks such as narrowing of the blood vessels, or atherosclerosis, which can trigger Cardiovascular Disease (CVD) and stroke [5]. According to the 2018 National Basic Health Research, the incidence rate of heart disease in Indonesia, as determined by a medical professional, was 1.5%, with a cumulative count of 1,017,290 cases. In addition, the incidence rate of stroke was 10.9 per thousand, resulting in a total of 713,783 cases [6]. Therefore, there is a need for a solution to reduce the impact of hypercholesterolemia, which is to use traditional medication materials.

Indonesia is a country that has abundant biological wealth and is a mega-biodiversity country. About 40,000 species of flora worldwide, 30,000 of which grow in Indonesia, and 7,500 are herbal plants [7]. According to WHO records, around 4 billion or ± 80% of the world’s population uses medicines derived from plants, and there are 119 active ingredients for modern drugs obtained from medicinal plants [8]. Porang tubers (Amorphphallus muelleri Blume) and Moringa (Moringa oleifera) are plants with great potential in the food and medicine industry.

The Porang plant (Amorphophallus muelleri Blume) is one of Indonesia’s endemic plants originating from the Araceae family and has great potential in the cosmetic, food, and medicinal industries. However, the porang plant cannot be consumed or used directly because it contains oxalate crystals which can irritate and damage the liver and kidneys[9]. Hence, it needs purification to obtain glucomannan.

Glucomannan is a hemicellulose-type polysaccharide that absorbs water and functions as a source of dietary fiber [10]. The water-soluble nature of glucomannan can bind fat in the small intestine and bile salts while in the digestive tract, then excreted with feces, resulting in decreased cholesterol levels [11]. According to the study’s findings [12] High-Density Lipoprotein cholesterol (HDL-C) levels in the blood may increase if a diet containing 200 mg of porang flour is consumed. In addition, concentrations of 25 mg/kg BW, 50 mg/kg BW, and 100 mg/kg BW are highly effective at reducing total cholesterol levels [13].

One other plant that has the property of reducing the risk of hypercholesterolemia is Moringa oleifera. The Moringa oleifera plant is commonly referred to as “The Miracle Tree,” “The Tree of Life,” and “The Cure All Tree” due to its abundant potential. This plant’s various sections are highly valuable as they contain a wealth of nutrients, antioxidants, and phytochemicals [14]. Moringa oleifera leaf extract (MOLE) contains many high-antioxidant compounds, including flavonoids, sapo-nins, tannins, and alkaloids, which reduce the risk of hypercholesterolemia. The content of flavonoids in Moringa oleifera leaves (MOL)
can reduce blood cholesterol and TG levels and increase serum HDL-C levels [15]. Flavonoid compounds function as inhibitors of the HMG-CoA reductase enzyme, resulting in a decrease in cholesterol synthesis owing to an increase in LDL-C (Low-Density Lipoprotein cholesterol) receptor activity in the liver [16], [17].

Dried Moringa oleifera leaves contain flavonoid compounds consisting of quercetin (0.20 mg/g), myricetin (5.8 mg/g), and kaempferol (7.57 mg/g) [18]. Moreover, the chemical quercetin can impede the synthesis of cholesterol by promoting the uptake of LDL-C from peripheral tissues and decreasing the buildup of TG in the liver [19]. Studies have demonstrated that giving a dosage of 100 mg/kg of body weight of Moringa oleifera leaf extract can significantly reduce total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), while simultaneously boosting high-density lipoprotein cholesterol (HDL-C) [20].

Based on the explanation above, this research was conducted to determine the effectiveness of the combination of glucomannan (Amorphophallus muelleri Blume.) and Moringa oleifera leaf extract on changes in the lipid profile of wistar rats with hypercholesterolemia model induced by a high-fat diet.

METHOD
Materials
This study utilized a digital weighing scale, a porang cutter, an oven, a tray, sieves (60 and 80 mesh), a heated plate magnetic stirrer, a blender, a volumetric flask, filter paper, a rotary evaporator, a water bath, an ultraviolet spectrophotometer, a rat cage, an oral probe, and a syringe.


Amorphophallus muelleri Blume. Porang tubers (Amorphophallus muelleri Blume) were made into chips by drying them at 55°C for 14 hours and blended into a fine powder (500 g). The porang powder (Amorphophallus muelleri Blume) was purified using aqua dest water solvent at a ratio of 1:30 on a hot plate stirrer at 55°C and a speed of 1000 rpm - 1500 rpm. The filtrate was filtered and washed with 50% ethanol at a ratio of 1:1 to form a gel precipitate [23]. The gel precipitate was dried in a 40°C oven for 24 hours. A 60-mesh sieve mixed and sieved the dry precipitate.

The extraction of Moringa oleifera Leaves (MoL)
Moringa leaves were obtained from the Kartasura area in Sukoharjo with a plant identification key based on [21] namely 1b – 2b – 3b – 4b -6b – 7b – 9b – 10b – 11b – 12b – 13b – 14a – 15b – 197b – 208a – 209b – 210b – 211b – 214a belongs to the Moringaceae family and the Moringa species (Moringa oleifera Lam). The MoL was dehydrated by subjecting it to a temperature of 50°C in an oven for a duration of 12 hours until it reached a state of dryness [24]. Subsequently, the desiccated MoL leaves were pulverized to a fine consistency (800 g) and subjected to extraction through maceration, employing a 70% ethanol solution in a 1:1 ratio for a duration of 72 hours [25]. The maceration results were filtered through filter paper and concentrated using a rotary evaporator at a temperature of 40°C [26]. Subsequently, the extract was con-
Densed by exposing it to a water bath set at a temperature of 50°C until it attained a more viscous texture.

**Determination of Total Flavonoid Content of Moringa oleifera Leaf Extract (MoLE)**

The analysis of the total flavonoid content was conducted utilizing ultraviolet spectrophotometry [27]. MoLE was extracted from a sample containing 100 ml of 50% ethanol, which was then homogenized by centrifugation for 15 minutes and filtered. Then, 2 ml of 5% AlCl₃ was combined with 1 ml of the sample. Following the addition of 10 ml of ethanol to the MoLE sample, the absorbance at 420 nm was determined. A standard quercetin curve was constructed by employing different dilutions of 0.05 mg/ml, 0.015 mg/ml, 0.030 mg/ml, and 0.045 mg/ml.

**Determination of dietary fiber content of Amorphophallus muelleri Blume glucomannan (GAmB) [28].**

A 0.5 g sample of material was mixed with 50 mL of pH 7 phosphate buffer and 0.1 mL of alpha-amylase enzyme using an Erlenmeyer flask. Subsequently, the mixture was subjected to boiling water at a temperature of 100°C for a duration of 30 minutes, with continuous stirring. Before adding 5 ml of 1 N HCL and 20 ml of distilled water, the sample was cooled. After a duration of thirty minutes, a volume of one centiliter of pepsin enzyme with a concentration of 1% was introduced. The solution was prepared by adding 0.1 ml of beta-amylase and 5 ml of 1 N NaOH. It was then cooked in a water bath for one hour. The substance was subsequently filtered via filter paper whose mass and weight remained constant. The introduction of 5 ml of 1 N HCL. After thirty minutes of immersion in a water bath, one centiliter of pepsin enzyme at 1% concentration was introduced. After adding 0.1 ml of beta-amylase enzyme and 5 ml of 1 N NaOH, the mixture was subjected to water bath cooking for a duration of one hour. In the end, the substance underwent filtration using constant filter paper with a pre-established weight.

The insoluble dietary fiber content was assessed in the sample that underwent cleaning using 2 x 10 ml of ethanol and acetone. Before being weighed, the glucomannan sample was dried in an oven at a temperature of 105°C for 12 hours. Afterwards, it was chilled in a desiccator. To produce soluble dietary fiber, 400 ml of 95% ethanol is mixed with 100 ml of filtrate collected from the sample, and then allowed to separate. Afterwards, the liquid that passed through the filter was filtered again using filter paper that does not contain any ash. Then, it was washed with two separate portions of 10 ml of ethanol and two separate portions of 10 ml of acetone. The final product was dried for 12 hours at 105°C in an oven and then weighed after being placed in the desiccator. All of the dietary fiber comes from sources that include both insoluble and soluble fiber.

**Research Design**

This study employed a genuine experimental design with pre- and post-testing control groups.

**Experimental animals**

The experimental animals used were male wistar strain white rats aged eight weeks with a body weight of 139-200g obtained from CV. Dunia Kaca received a Health Certificate from the Karanganyar Regency Livestock and Fisheries Agency with No. 696/SKKH/VII/2022. In addition, this research has been approved. It has obtained a letter of permission from the ethical review committee of the Faculty of Medicine of Muhammadiah University of Surakarta with No. 4139/A.1/KEPK-FKUMS/III/2022. The experimental rats were divided into eight groups, namely:
1) P1 (positive control group).
2) P2 (Hypercholesterolemia) HFD + negative control group.
3) P3 (Hypercholesterolemia) HFD + 100mg/kgBW GAmB[29]: 100 mg/kgBW MoLE.
4) P4 (Hypercholesterolemia) HFD + 120 mg/kgBW GAmB: 80 mg/kgBW MoLE.
5) P5 (Hypercholesterolemia) HFD + 80 mg/kgBW GAmB: 120 mg/kgBW MoLE.
6) P6 (Hypercholesterolemia) HFD + 50 mg/kgBW GAmB[13]: 50 mg/kgBW MoLE[30].
7) P7 (Hypercholesterolemia) HFD + 60 mg/kgBW GAmB: 40 mg/kgBW MoLE.
8) P8 (Hypercholesterolemia) HFD + 40 mg/kgBW GAmB: 60 mg/kgBW MoLE.

The blood sample was taken through the orbital sinus 2 cc for each mouse which had been fasted for 12 hours and stored in a microtube without anticoagulant. Lipid profile examination used the analysis kit reagent which was HDL-C Diasys reagent, LDL-C Diasys reagent, Triglyceride Diasys reagent, and Cholesterol Diasys reagent.

Data Analysis
The lipid level test results were analyzed using One Way Analysis of Variance (ANOVA) SPSS version 20 and the Paired-T test.

RESULTS AND DISCUSSION
The total flavonoid content of Moringa oleifera leaves extract (MoLE)

The determination of the total flavonoid content of MoLE was carried out using the aid of a standard AlCl₃ reagent solution and quercetin at the maximum absorption wavelength of 420 nm. Total flavonoid content was determined by entering the MoLE sample absorption value into the quercetin standard curve equation [32]. The result obtained from the calibration curve was the linear regression equation \( y = 3.579x + 0.0046 \) with \( r^2 = 0.9988 \) and \( r = 0.9993 \). The value of \( r \) approaching 1 indicates a relationship between absorption and concentration in the linear calibration curve [33].

<table>
<thead>
<tr>
<th>test</th>
<th>Abs.</th>
<th>total flavonoid levels (mgQE/g extract)</th>
<th>Average total flavonoid content (mgQE/g extract)</th>
<th>percentage of total flavonoid levels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.276</td>
<td>3.75</td>
<td>3.71</td>
<td>3.71</td>
</tr>
<tr>
<td>2</td>
<td>0.270</td>
<td>3.67</td>
<td>3.71</td>
<td>3.71</td>
</tr>
</tbody>
</table>

The determination of total flavonoid content in MoLE raw material was carried out before administering the dose of the drug to the test animal with the result of quersetin total flavonoid content of 3.71 mgQE/g (Table 1.). The result of total flavonoid content in
this study is less than the study [34] of 25.36 mgQE/g with the same maceration time of 48 hours. This is due to the difference in solvent evaporation methods and the use of a freeze dryer in the study [34] is more optimal for evaporating residual solvent with the cold temperature method.

**Glucomannan dietary fiber content (Amorphophallus muelleri Blume)**

Measurement of dietary fiber on glucomannan derived from *Amorphophallus muelleri* Blume. Produce 10.32% soluble food fiber, 9.53% insoluble food fiber and 18.85% total food fiber (Table 2). The content of water-soluble food fiber in glucomannan in this study was higher and lower compared to research [35] of 15.49% with the species *Amorphophallus konjac* K. Koch. However, the glucomannan content in this study was higher than the type of porang reported in the book [36], namely *Amorphophallus rivieri*, as much as 6.45%.

<table>
<thead>
<tr>
<th>Content test</th>
<th>Result (%)</th>
<th>Average (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble fiber</td>
<td>10.59</td>
<td>10.04</td>
</tr>
<tr>
<td>Insoluble fiber</td>
<td>9.51</td>
<td>9.55</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>20.11</td>
<td>19.59</td>
</tr>
</tbody>
</table>

Source: Author’s analysis (2022)

Differences can influence variations in the content of glucomannan water-soluble fiber in species, plant age and processing [37].

The research utilized male Wistar strain rats as experimental animals due to their more consistent biological conditions compared to female rat models [38]. The lipid profile of the experimental rats was assessed on two occasions: before administering a high-fat diet (pretest) and after administering a combined dose of GaMB and MoLE (posttest). The study’s findings indicated a decrease in TC levels in the P3, P4, and P5 treatment groups. This reduction was determined to be statistically significant (p < 0.05) through a paired-T test, demonstrating a notable difference in TC levels before and after the administration of the combined dose of MoLE and GaMB. The P4 treatment group experienced the most significant decrease in TC levels, with a reduction of -43.00 ± 5.57 mg/dl (Figure 2).
The average decrease in TG rate occurred in the P3, P4, and P5 treatment groups. However, the group that experienced a significant decrease was the P4 and P5 groups. In addition, the group with the most TG rate decrease was the P4 group by -43.00 ± 9.00 mg/dL, followed by the P5 treatment group with a decrease in TG of -33.00 ± 16.70 mg/dL (Figure 3).

The paired T-test result indicated a notable decrease in LDL-C levels for the P3, P5, and P7 groups, with a significance level of P>0.05. The P4 group demonstrated the most notable decrease in LDL-C values, at -17.67 ± 7.37 mg/dl. Nevertheless, the reduction in LDL-C levels did not reach statistical significance (P>0.05) (Figure 4). In addition, the P5 group exhibited the most substantial decrease in LDL-C levels compared to the P4 group, with a reduction of -8.00 ± 2.00 mg/dl (figure 4).

The study identified a statistically significant rise in HDL-C levels (p<0.05) in treatment groups P3, P4, P5, P6, P7, and P8. The P4 group exhibited the most significant elevation in HDL-C levels, reaching 37.67 ± 6.42 mg/dl (Figure 5).
The preliminary analysis of the lipid profile of rats that were given a high-fat diet orally for two weeks showed that the rats in the P2, P3, P4, P5, P6, P7, and P8 treatment groups had high levels of cholesterol in their blood, as indicated by total cholesterol levels above 240 mg/dL, blood pressure levels above 145 mg/dL, LDL cholesterol levels equal to or greater than 27.5 mg/dL, and low levels of HDL cholesterol below 35 mg/dL [3], [39], [40]. Furthermore, the P4 group exhibited the highest dosage that effectively lowered TC, TG, and LDL-C levels while increasing HDL-C levels. However, the decrease in LDL-C levels in the P4 group did not reach statistical significance (P>0.05). The P5 group exhibited the most significant reduction in levels of TC, TG, and LDL-C, and the greatest increase in levels of HDL-C compared to the P4 group. The overall statistical analysis, both before and after the intervention, demonstrated a significant change at a p-value of 0.05 (Figure 2 until 5).

The administration of MoLE and GaMB doses in this study resulted in reduced levels of TC, TG, and LDL-C, as well as increased levels of HDL-C in the hypercholesterolemia rat model. The findings of this study are in opposition to the research [13] that suggests the administration of glucomannan (Amorphophallus oncophillus Prain ex Hook. f.) at a dosage of 50 mg/kg BW can more effectively decrease total cholesterol levels. By comparison, in this study, the application of glucomannan in the P4 group at a dosage of 120 mg/kgBW proved to be more efficacious in lowering TC levels than the P6 group at a dosage of 50 mg/kgBW. Furthermore, the disparity in outcomes between this study and the previous research [13] can be attributed to the origin of the glucomannan used. The study employed glucomannan derived from Amorphophallus muelleri Blume. The research focuses on Amorphophallus oncophyllus Prain ex Hook. f. [13].

The glucomannan found in the Amorphophallus muelleri Blume tuber functions as a water-soluble dietary fiber which has an impact on lipid metabolism, caloric intake and glucose homeostasis [10], [41]. Dietary fiber is a component found in plants, such as the Amorphophallus muelleri Blume tuber, that can be consumed. It is made up of carbohydrates and serves as a barrier against the digestive and absorption processes in the small intestine. It is partially or completely broken down through fermentation in the large intestine. This process has the potential to reduce cholesterol levels by binding with fats in the small intestine and binding bile acids [11], [42]. One of the key features of glucomannan is its ability to absorb water and form a thick gel in the digestive system. This gel-like substance can promote feelings of fullness, reduce appetite, and aid in weight loss efforts. By expanding in the stomach, glucomannan can help reduce calorie intake and prevent overeating.

MoLE at a dose of 100 mg/kg BW can effectively lower TC and LDL-C levels in the P3 group, which is consistent with previous research [20]. Nonetheless, a MoLE dose of 100 mg/kg BW did not result in a significant reduction in TG levels in the P3 group. As a result, the findings on TG levels in P3 contradicted the research [20]. Moreover, within the third-order groups, the P3 group exhibits the most significant decrease in TC, TG, LDL-C, and HDL-C levels. The hypercholesterolemic group exhibited reduced levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), while displaying elevated levels of high-density lipoprotein cholesterol (HDL-C). These findings indicate that the inclusion of flavonoids, specifically the component quercetin, in MoLE is extremely advantageous for individuals with hypercholesterolemia [43]. Quercetin inhibits the synthesis of cholesterol, leading to improved absorption of LDL-C from peripheral tissues and reduced buildup of TG in the liver [19].

The findings of this study demonstrate that the combined administration of GaMB and MoLE has a significant impact on altering the lipid profile of rats with elevated cholesterol levels. To be more precise, it leads to a reduction in total cholesterol levels (TC),
triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), while simultaneously increasing high-density lipoprotein cholesterol (HDL-C).

CONCLUSION

The combination of glucomannan (Amorphophallus muelleri Blume) and Moringa oleifera leaf extract can change the lipid profile effectively in a hypercholesterolemic male Wistar rat model. Statistically, the treatment group that was most significant in reducing lipid profile was the P5 group (HFD + 80mg/kgBW GAmB: 120 mg/kgBW MoLE) with a change of TC (-30.33 ± 11.59 mg/dl), TG (-33.00 ± 16, 70 mg/dl), LDL-C (-30.33 ± 11.59 mg/dl) and increased levels of HDL-C (31.00 ± 3.00 mg/dl). In comparison, the P4 group (HFD + 120mg/kgBW GAmB: 80 mg/kgBW MoLE) was the group that had the most lipid profile changes, namely TC (-43.00 ± 5.57 mg/dl), TG (-43.00 ± 9.00 mg/dl), LDL-C (-17.67 ± 7.37 mg/dl) and increased HDL-C (37.67 ± 6.42 mg/dl). Suggestions from this study for further research are that in processing moringa leaf extract, other methods, such as freeze dryers, can be used so that the extract can last longer.

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