

The effect of butterfly pea flower on total cholesterol level in male wistar strain rats induced by high fat diet

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ABSTRACT

Background

Benefits of butterfly pea flowers have been widely studied and proven to be able to reduce total cholesterol levels in male Wistar white rats which had previously been made hypercholesterolemic.

Aim

Butterfly pea flowers contain active compounds in the form of flavonoids and anthocyanins, both of which act as antioxidants in reducing total cholesterol levels. This study aims to determine the effect of butterfly pea flower water extract on total cholesterol levels in male white Wistar rats induced by a high cholesterol diet.

Methodology

This research uses a posttest only control group design method. There were 3 groups divided randomly from 36 mice, namely the normal group (P0), the sick group (P1), the treatment group (P2). Group P0 was given standard feed and distilled water, group P1 was given a high cholesterol diet in the form of quail egg yolk, and group P2 was given a high cholesterol diet and given butterfly pea flower water extract at a dose of 600mg/kg BB. Rat blood samples were taken from the orbital sinus at the end of the study to measure total cholesterol levels using the CHOD-PAP method. **Results:** The mean total cholesterol levels for groups P0, P1, and P2 were 89.15 ± 2.44 mg/dL, 194.37 ± 3.64 mg/dL, and 108.86 ± 2.84 mg/dL, respectively. Data analysis used the One-Way Anova test followed by the Post Hoc LSD test. The results of the One-Way Anova test showed that there was a significant difference with a p value <0.05 ($p=0.00$). Post Hoc LSD test results showed significant differences between all groups with a p value <0.05 ($p=0.00$). **Conclusion:** The conclusion of this research is that butterfly pea flower extract has an effect on total cholesterol levels in male white Wistar rats fed a high cholesterol diet.

Keywords

Butterfly pea flower water extract; total cholesterol levels

INTRODUCTION

Cholesterol has an important function for the body, namely as the most important component in the formation of cell membranes and external layers in plasma lipoproteins^{1,2}. Total cholesterol will be useful for the body if it is in a normal or stable state. High total cholesterol levels or hypercholesterolemia and not balanced with normal amounts of HDL (High Density Lipoprotein) cholesterol in the blood can cause cholesterol deposits in the artery walls or what is commonly called atherosclerosis. Atherosclerosis is an inflammatory disease caused by the accumulation of cholesterol and

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cholesterol esters from plasma lipoproteins in the artery walls^{3,4}. Someone who has lower HDL (High Density Lipoprotein) levels will have a higher risk of ischemic stroke^{5,6}. Utilizing traditional ingredients from nature is the right alternative that will help people with hypercholesterolemia in reducing total blood cholesterol levels. Butterfly pea flower is one of the alternative traditional medicines which specifically contains anthocyanins and flavonoids which can act as antioxidants in preventing diseases caused by free radicals which have been proven to be beneficial in lowering total cholesterol levels⁷. Previous studies processed butterfly pea flowers using ethanol extraction, research using butterfly pea flower water extract in lowering total cholesterol levels has not been widely studied.

The prevalence of hypercholesterolemia, especially in Indonesia, increases with age. Where the older the age, the higher the prevalence of hypercholesterolemia⁸. Factors related to this condition are high cholesterol food intake such as meat and processed meat products, offal, and eggs accompanied by lack of physical activity⁹. According to records conducted by the Ministry of Health in Indonesia in 2016, the percentage of visitors to the Integrated Development Post for Non-Communicable Diseases and the Health Centre with high cholesterol based on gender was 54.3% in women and 48% in men. Based on age, the percentage increases with age, namely over 60 years of age at 58.7%.

The role of butterfly pea flowers in reducing cholesterol levels is greatly needed by looking at the various properties contained in the flower petals, namely the content of phytanic acid which is useful for regulating triglyceride or cholesterol levels in skeletal muscles¹⁰. The higher the dose of extract used to induce mice (*Rattus norvegicus*), the lower the total cholesterol levels. Even with a dose of 400 mg/kgBW, cholesterol levels were reduced to almost the same as the positive control group receiving simvastatin 0.18 mg/kgBW¹¹. It was proven in a study Chayaratanasin 2019 that butterfly pea flower extract also inhibits the adipogenesis mechanism by suppressing cell proliferation and increasing lipolysis activity, this role is related to the phytochemical compounds contained in the extract^{12,13}. The use of butterfly pea flowers can also be processed into butterfly pea flower kombucha which can reduce LDL levels and increase HDL levels which are played by niacin or vitamin B3 as a content of the fermentation

results of kombucha. Niacin will control the decrease in cholesterol levels in broiler ducks that are given butterfly pea flower kombucha with varying concentrations of stevia sugar^{14,15}. The higher the concentration of stevia sugar kombucha tested on broiler ducks, the more it will reduce cholesterol levels. Niacin will also prevent atherosclerosis by inducing the release of the hormone prostaglandin I₂ which functions to prevent platelet aggregation⁷.

Based on these problems, researchers have the aim to explore the healing properties of natural herbal ingredients, especially butterfly pea flower extract (*Clitoria ternatea*) which will be tested for its potential in lowering total cholesterol levels. In previous studies, butterfly pea flower extract has shown all kinds of benefits for human health.

MATERIALS AND METHODS

The study was conducted by selecting an experimental design and using a post-test only control group design. This study was conducted in the nutrition laboratory of the Food and Nutrition Study Centre (PSPG) of Gadjah Mada University. The subjects used in this study were 21 male white Wistar rats aged 3-4 months with a body weight of 180-200 grams. The rats were adapted for 7 days and then randomized and grouped into 3 groups, namely: Group P0, P1, and P2.

The rats that had been adapted and divided into 3 groups were then given a high-cholesterol diet induction using quail egg yolks, for 28 days for group P1 and 14 days for group P2. Treatment was given for 14 days after being given a high-cholesterol diet. Group P0 was only given standard feed in the form of HBS pellets, group P1 was given a high-cholesterol diet in the form of quail egg yolks, and group P2 was given a high-cholesterol diet for 7 days and continued with the administration of butterfly pea flower water extract at a dose of 600 mg/kgBW for 14 days. At the end of the treatment, blood samples were taken from the orbital sinus of mice and total cholesterol levels were measured using the CHOD-PAP (Cholesterol Oxidase Phenol Aminophenazone) method. This research has obtained ethical clearance from the Bioethics Commission of the Faculty of Medicine, Sultan Agung Islamic University with number 378/XI/2023/Bioethics Commission.

The data obtained were then analyzed using the One-Way Anova statistical test, if the results obtained were significantly different ($p < 0.05$), then it could

be continued with the LSD post hoc test to determine which groups were significantly different.

RESULTS AND DISCUSSION

The results of measuring total cholesterol levels using the CHOD-PAP method produced the following average results:

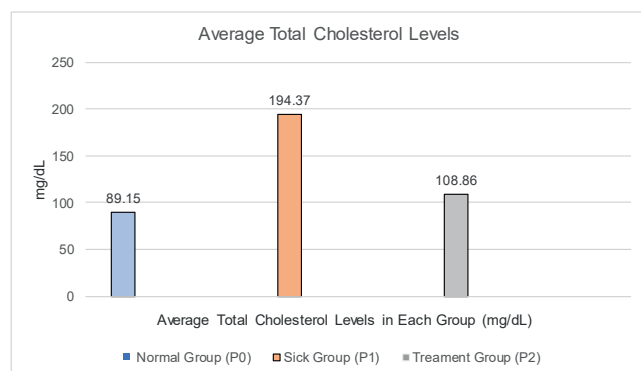


Figure 1. Graph of Average Total Cholesterol Levels in Mice

The average total cholesterol level in the normal group (P0) was the lowest level among the sick group (P1) and the treatment group (P2), which was 89.15 mg/dL. It can be seen in Figure 1 if sorted from the largest to the smallest average cholesterol levels respectively, namely 194.37 mg/dL (P0), 108.86 mg/dL (P1), and 89.15 mg/dL (P2).

Table 1 shows that there are differences in the average total cholesterol levels in each group.

Table 1. Results of normality and homogeneity tests

Group	Average Cholesterol Levels (mg/dL) $\pm$ SD	Shapiro-Wilk	Levene Test
Normal Group (P0)	89,15 $\pm$ 2,44	0,853	0,649
Sick Group (P1)	194,37 $\pm$ 3,64	0,194	
Treatment Group (P2)	108,86 $\pm$ 2,84	0,194	

The Shapiro-Wilk test obtained data results from the three groups that were normally distributed with p values of 0.853; 0.194; and 0.194 ($p > 0.05$). The results of the Levene Test on the three groups obtained homogeneous results with a p value of 0.0649 ($p > 0.05$).

Table 2. One Way ANOVA Test Results

Group	Average Cholesterol Levels (mg/dL) $\pm$ SD	P-value
Normal Group (P0)	89,15 $\pm$ 2,44	
Sick Group (P1)	194,37 $\pm$ 3,64	0,000
Treatment Group (P2)	108,86 $\pm$ 2,84	

The One-Way Anova parametric test in the three groups obtained a p value of 0.000 ($p < 0.05$), which means that H_0 is rejected and H_1 is accepted and there is a significant difference in the average total cholesterol levels in the three groups. After the One-Way Anova test, a Post Hoc LSD test was carried out with the aim of determining the difference in the average total cholesterol levels between groups.

Table 3. Results of the LSD Post Hoc Test

Group	Average Cholesterol Levels (mg/dL)	P-value		
		P0	P1	P2
Normal Group(P0)	89,1543	-	0,000	0,000
Sick Group (P1)	194,3686	-	-	0,000
Treatment Group(P2)	108,8643	-	-	-

In table 3, it can be concluded that the difference in the average total cholesterol levels between each group has a meaningful or significant difference. The normal group (P0) has a lower average total cholesterol level compared to the sick group (P1) and has a significant difference with a p value of 0.000 ($p < 0.05$). The normal group (P0) has a lower average total cholesterol level compared to the treatment group (P2) and has a significant difference with a p value of 0.000 ($p < 0.05$). The treatment group (P2) has a lower average total cholesterol level compared to the sick group (P1) and has a significant difference with a p value of 0.000 ($p < 0.05$).

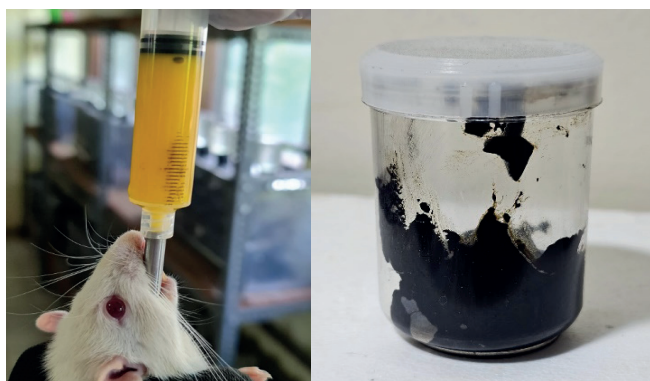


Figure 1. How to administer a high cholesterol diet and butterfly pea flower water extract

DISCUSSION

The One-Way Anova test on the three groups of mice to determine the difference in the average total cholesterol levels showed significantly different results with a *p* value of 0.000. Continued with the Post Hoc LSD test with the aim of finding out which group had a significant or meaningful difference in the average cholesterol levels, the results showed that all groups showed a significant difference in the average total cholesterol levels with a *p* value of 0.000. The sick group (P1) had a higher average cholesterol level compared to the normal group (P0). This is in accordance with the statement⁹ that food intake containing cholesterol such as meat and its processed products, offal, and eggs can cause increased blood cholesterol levels or hypercholesterolemia. In the study¹¹, egg yolk induction also succeeded in increasing the average cholesterol levels of the sick group in male white rats of the Wistar strain. The cholesterol contained in quail egg yolk is 2138.17 mg/100 g and can increase total cholesterol levels 0.5 times that of the normal group given standard feed and distilled water^{2,16}. The results of this study are in line with the statement that the average total cholesterol levels in the sick group (P1) were higher when compared to the normal group (P0) and statistically had a significant difference.

The administration of butterfly pea flower water extract successfully reduced or had an effect on reducing cholesterol levels in male white Wistar rats that had been given a high-cholesterol diet. This was stated by the results of the average total cholesterol levels in the treatment group that received butterfly pea flower extract (P2) which was lower when compared to the sick group (P1) which was only given a high-cholesterol

diet and had a significant difference in the average total cholesterol levels. This is in line with previous studies that butterfly pea flower extract can reduce total cholesterol levels in both experimental mice and meat ducks⁷. In previous studies using three doses of butterfly pea flower extract, namely 100 mg/dL, 200 mg/dL, and 400 mg/dL with the lowest average cholesterol levels in the group receiving 400 mg/dL butterfly pea flower extract with an average result of 112.62 mg/dL, the results are almost the same as the group given simvastatin as much as 0.18 mg/kgBW, namely with an average total cholesterol level of 108.19 mg/dL¹¹. The ability of butterfly pea flowers has indeed been proven to be efficacious in lowering total cholesterol levels and its effects have been tested on humans, 1 gram of dried butterfly pea flowers processed into tea by boiling using 250 ml of water and consumed once a day for seven consecutive days can reduce the average total cholesterol levels in 15 respondents¹⁷. The average total cholesterol level before consuming butterfly pea flower tea was 258.06 mg/dL and after consuming butterfly pea flower tea it became 245.13 mg/dL. Statistically, these results show a significant difference with a *p* value of 0.001¹².

Butterfly pea flowers contain active compounds that can act as antioxidants. Flavonoids are one of the compounds in butterfly pea flowers, including reducing compounds that function to inhibit oxidation reactions and capture free radicals¹⁸. Anthocyanins are active compounds and have antioxidant activity as indicated by their ability to contribute hydrogen to break chain radical reactions¹⁹. The content of flavones and flavanonols in butterfly pea flowers also plays a role in protecting the body from reactive oxygen species²⁰. The relationship in this study is that the flavonoid, anthocyanin, flavone, and flavanone compounds contained in butterfly pea flowers can act as antioxidants and protect the body from Reactive Oxygen Species (ROS) formed from a high-cholesterol diet which will then increase LDL levels which will form an oxidation reaction with ApoB100²¹.

CONCLUSION

Butterfly pea flower water extract has an effect on reducing total cholesterol levels in male white Wistar rats fed a high cholesterol diet.

Conflict-of-Interest Disclosure

All authors know of no conflict interest associated

with this publication, and there has been no significant financial support for this work that could have influenced its outcome. As corresponding author, Tania Octavia confirmed that the manuscript has been read and approved for submission by all the named authors.

Acknowledgements

This research is supported by Universitas Islamic Sultan Agung Semarang

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