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The Effect of Butterfly Pea (*Clitoria ternatea*) Extract on Reducing Total Cholesterol Levels in *Rattus norvegicus* with The Hypercholesterolemia Model

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Abstract

Hypercholesterolemia is a metabolic disorder disease characterized by increased blood cholesterol levels above normal limits. Many studies support the use of statins as cholesterol-lowering agents that work by inhibiting HMG-CoA reductase so that cholesterol secretion decreases. The use of statins is often prescribed in the long term which results in side effects such as myotoxicity, myopathy, myalgia, myositis, and rhabdomyolysis. A study is needed on butterfly pea flower or *Clitoria ternatea* which is known to contain secondary metabolites such as flavonoids, anthocyanin, triterpenoids, and phytosterols. This content can work as an antioxidant that helps lower cholesterol levels in the blood. The purpose of this study was to determine the effect of butterfly pea flower extract (*Clitoria ternatea*) on reducing total cholesterol levels in *Rattus norvegicus* with the hypercholesterolemia model. This research is based on laboratory experiments with pre and posttest control group design. The sample used in this study was male white rats (*Rattus norvegicus*), 2-3 months old, weighing ± 200 grams. It was found that the average difference in cholesterol levels in the control group (C) was 10.34 ± 0.06 which indicated that there was a not too significant increase. In the simvastatin group (S), treatment group 1 (G1), treatment 2 (G2), and treatment 3 (G3) had a mean difference in total cholesterol levels which showed a decrease with the results S = -62.17 ± 4.4 ; G1 = -52.33 ± 3.08 ; G2 = -55.17 ± 2.13 ; and G3 = -59.34 ± 0.49 . The One Way ANOVA test a significance value of $p < 0.05$ was obtained which indicated a significant difference. It can be concluded that, administration of ethanol extract of butterfly pea flower (*Clitoria ternatea*) can reduce total cholesterol levels in the blood.

Keywords: *Clitoria ternatea*, total cholesterol, hypercholesterolemia, rat, medicine, metabolic disease

1.1 Introduction

Hypercholesterolemia is a metabolic disorder characterized by increased levels of cholesterol in the blood above the normal limit (Yani, 2015). If cholesterol accumulates in the plasma for a long time, it will cause lipid peroxidation through macrophages to form atherosclerotic plaques and coronary heart disease (Wijaya, 1990). Cases of hypercholesterolemia are of great concern in Europe, America, Australia and Asia. According to WHO, in 2008, the global prevalence of increased total cholesterol in adults was 39% (37% for men and 40% for women) (WHO, 2009). As for in America, the prevalence of hypercholesterolemia reaches 16.2% in adulthood (Roger et al., 2011), while in Bangladesh and Nepal found 16% and 13% incidence of hypercholesterolemia (WHO, 2011). In Indonesia, cases of hypercholesterolemia are still relatively high. Based on the 2016 Non-Communicable Disease Profile, the percentage of cases of hypercholesterolemia recorded at Posbindu and Puskesmas was found to be 48% of men and 54.3% of women. For the percentage of someone with high cholesterol at the age of more than 60 years was found to be 58.7%. According to Posbindu and East Java FKTP data, out of 8,225 people examined, 2,967 or 36.1% were identified as having high cholesterol levels above 190 mg/dL. The latest data according to RISKESDAS 2018, found that 34,820 people over 15 years in Indonesia still have high cholesterol levels and cases will continue to increase over time.

Hypercholesterolemia can trigger various diseases, one of which is atherosclerosis and coronary heart disease¹. Treatment of high cholesterol levels involves lifestyle modification and pharmacotherapy. Many studies support the use of statins as pharmacological therapy to reduce the incidence of cardiovascular disease in adults and children (Pang et al., 2016). Statins work by inhibiting HMG-CoA reductase so that cholesterol secretion in the liver decreases. Statins have an elimination half-life of less than 5 hours and have first-pass effects in the liver, resulting in low systemic bioavailability (Bellosta, 2017).

The use of statins is often prescribed in the long term. This causes various adverse side effects such as myopathy (27.8%), myalgia (10%) (Abed et al., 2022), myositis (20%) (Selva-O'Callaghan et al., 2018), and rhabdomyolysis (1.5%) (Ezad et al., 2018). The number of other incidents were contradictory myopathic complaints. According to the US National Lipid Association Statin Safety Assessment Task Force, in a meta-analysis found that the myopathy that occurs due to the use of statins in 45 patients is damage to muscle protein and increased creatine kinase (Tomaszewski, 2011). Expert opinion states the need for a better alternative therapy to statins, especially assessing their dangerous side effects. It aims to create cholesterol-lowering therapeutic interventions with evidence-based, safe, and low cost for hypercholesterolemic patients.

The cause of hypercholesterolemia apart from cholesterol synthesis by the HMG-CoA reductase enzyme is an increase in oxidative stress due to lipid peroxidation by cells. Lipid peroxidation is considered to be the main molecular mechanism involved in oxidative damage to cell structures (Repetto et al., 2012). Oxidative stress is a condition caused by high levels of oxidants (free radicals) compared to levels of antioxidants in the body. Deficiencies in antioxidant levels can result in cascading damage that starts with cells up to higher level (Halliwell & Gutteridge, 2007). The human body also has the ability to produce its own antioxidants such as glutathione which can convert free radicals through a detoxification mechanism, so that they can overcome free radicals. The amount of antioxidants produced by the body is less likely to overcome oxidative stress, so an additional substance is needed that can provide sufficient levels of antioxidants.

Flavonoids are known as a group of phenolic compounds in plant tissues that can act as antioxidants (Redha, 2007). Indonesia, which is rich in various types of herbal plants, provides great potential as a source of antioxidants. Butterfly pea flower or *Clitoria ternatea* is one of the plants with the highest levels of flavonoids compared to other plants such as gotu kola or *Centella asiatica* (3,816 mg/mL), pearl grass or *Oldenlandia corymbosa* (2,686 mg/mL), and hibiscus or *Hibiscus tiliaceus* (1,425 mg/mL). Butterfly pea flowers are known to have antioxidant levels of $62.77 \mu\text{g/mL} \pm 0.21$ with a total flavonoid content of 4.865 g QE/100 g extract and a total phenol content of 2.133 g GAE/100 g extract (Nuriyah, 2021). Butterfly pea flowers are very easy to find and cultivate by local farmers in Indonesia. The morphological system that supports it can survive well in the dry season. In its use, people often use the butterfly pea flower as a traditional medicine for eye infections, ear disorders, skin disorders, throat disorders, and tumor prevention. Butterfly pea flowers also contain various other secondary metabolites such as anthocyanins, triterpenoids, and phytosterols (Aziza, 2021).

Research is needed regarding the effectiveness of the ethanol extract of butterfly pea flowers on reducing cholesterol levels. This research is experimental based using experimental animal subjects in the form of white rats (*Rattus norvegicus*) which will be given high cholesterol feed as an inducer of hypercholesterolemia. White rats were chosen because they have a body metabolism that is almost similar to humans, especially in lipid metabolism such as cholesterol (Heriansyah, 2013). If they have entered the dyslipidemia or hypercholesterolemia phase, the rats will be given ethanol extract of butterfly pea flowers with 3 different dose variations, such as 25 mg/Kg BW/day, 50 mg/Kg BW/day, and 100 mg/Kg BW/day. This research is expected to be a solution for alternative long-term use of statins. Especially when it is associated with cases of hypercholesterolemia which is triggered by increased levels of oxidants in the body.

Based on the explanation above, a study was conducted on "The Effect of Butterfly Pea Flower Extract (*Clitoria Ternatea*) on Reducing Total Cholesterol Levels in *Rattus norvegicus* with the Hypercholesterolemia Model".

1.2 Methods

This research is a laboratory experimental based research with pre and post test control group design. The experimental unit used in this study was male white rats (*Rattus norvegicus*), 2-3 months old, weighing ± 200 grams, and in good health condition which were reared and developed at the Pharmacology Laboratory, Faculty of Medicine, Airlangga University. Sampling was done by purposive random sampling. Randomization was carried out by labeling each experimental animal by applying paint to the tail of the rat. After that, draws were made that read the body parts of each mouse that were painted. A draw was conducted to determine which experimental animals would fall into groups C, S, G1, G2, and G3. This research will be carried out over a period of 5 months (May 2022 – September 2022).

The details of the division are as follows: Group C is the control where the hypercholesterolemia model rats are not given any therapy. Group (S) were hypercholesterolemic rats which were given simvastatin at a dose of 10 mg/time. Group I was the group of rats that were given the ethanol extract of butterfly pea flower dose I of 25 mg/200 gram BW/day for 14 days. Group II was a group of hypercholesterolemic rats which were given 50 mg/200 gam BW/day of ethanol extract of butterfly pea flower II for 14 days. Group III is a group of hypercholesterolemic rats that were given ethanol extract of sea cucumber flower dose III of 100 mg/200 gram BW/day for 14 days.

In this study, the research results obtained were analyzed using the One Way ANOVA. Sampling was done before and after treatment. The blood sample before treatment was taken through the aorta as much as 1 cc while after the treatment it was taken through the aorta as much as 2 cc. The sample is then centrifuged to get the serum. Cholesterol levels were measured using the CHOD-PAP method which was carried out at the Clinical Pathology Laboratory, RSUD dr. Soetomo, Surabaya. Examination of cholesterol levels was carried out twice, the first test was used when the experimental animals had received high-fat feed for 28 days to find out whether they had entered a state of hypercholesterolemia or not. As for the second examination, it was carried out after the rats received administration of ethanol extract of butterfly pea flowers and simvastatin for 14 days.

The data obtained was then analyzed using the IBM SPSS Statistics application version 23. The first tests were carried out, namely the Kolmogorov-Smirnov and Shapiro-Wilk to determine the distribution value of the data. Data are reported as percentages for categorical variables and as mean \pm SD in normally distributed numeric data or median (IQR) in non-normally distributed numeric data. If the results of the research data obtained have a normal and homogeneous distribution, then for inferential statistical analysis the One Way ANOVA. The parameter used is the result of a significance value <0.05 .

1.3 Results

A study was conducted on the effect of butterfly pea flower extract (*Clitoria Ternatea*) on reducing total cholesterol levels in *Rattus norvegicus* with a hypercholesterolemia model. The research was carried out at the Pharmacology Laboratory, Faculty of Medicine, Airlangga University from 3 June 2022 to 15 September 2022. This research used a sample of white rats (*Rattus norvegicus*). White rats were divided into 5 groups: control group (C), simvastatin group (S), treatment group 1 (G1), treatment group 2 (G2), and treatment group 3 (G3). Group K served as a control where hypercholesterolemic rats were not given any therapy. Group (S) were hypercholesterolemic rats which were given simvastatin at a dose of 10 mg/time (0.36 mg/day/head). Group I was the group of rats that were given the ethanol extract of

butterfly pea flower dose I of 25 mg/200 gram BW/day for 14 days. Group II was a group of hypercholesterolemic rats which were given 50 mg/200 gam BW/day of ethanol extract of butterfly pea flower II for 14 days. Group III is a group of hypercholesterolemic rats that were given ethanol extract of sea cucumber flower dose III of 100 mg/200 gram BW/day for 14 days. In this study, measurements of body weight and total cholesterol levels were carried out.

1.4 Weight White Ratt (*Rattus norvegicus*)

White rat (*Rattus norvegicus*) was acclimatized to the Pharmacology laboratory environment, Faculty of Medicine, Airlangga University for a week by being given standard food, namely A594K pellets. After adaptation, all rats were found to be in good condition.

Then the rats were randomly grouped into 5 predetermined groups and given high-fat feed with the composition of 80 grams/day of beef lard, 60 grams/day of duck egg yolk, and 60 grams of lard/day of lard. This high-fat feed was given for 28 days to the control group, simvastatin, treatment 1, treatment 2, and treatment 3 which were then weighed. The results of the body weight of the rats for each group can be seen in Tables 1, 2 and 3 below:

Table 1. Average (Mean) \pm SD (Standard Deviation) Body Weight of White Rats (Grams) After Acclimatization Period

No.	Group	N	Average Rat Body Weight \pm SD (Gram)
1.	Group 1	6	186,33 \pm 12,98
2.	Group 2	6	184 \pm 6,92
3.	Group 3	6	170,83 \pm 12,12
4.	Group 4	6	172,33 \pm 12,43
5.	Group 5	6	163 \pm 11,34

From the weight data above, a homogeneity test was carried out and a significance value of > 0.05 was obtained, which means that the variance of the white rat body weight data was homogeneous.

Table 2. Average (Mean) \pm SD (Standard Deviation) Body Weight of White Rats (Grams) After Feeding Cholesterol

No.	Group	N	Average Body Weight Rats \pm SD (Gram)
1.	Control (C)	6	243,67 \pm 13,64
2.	Simvastatin (S)	6	225,33 \pm 7,96
3.	Group 1 (G1)	6	226,67 \pm 12,5
4.	Group 2 (G2)	6	232,67 \pm 12,89
5.	Group 3 (G3)	6	218,17 \pm 11,95

Description:

- C: Control group
- S: The group that received simvastatin
- G1: The treatment group that received the extract 25 mg/Kg BW
- G2: The treatment group that received the extract 50 mg/Kg BW
- G3: The treatment group that received the extract 100 mg/Kg BW

From the above body weight data a homogeneity test was carried out and a significance value of > 0.05 was obtained, which means that the variance of the white rats' body weight data was homogeneous.

Table 3. Average (Mean) \pm SD (Standard Deviation) of White Rats' Body Weight (Grams) After Giving Treatment

No.	Group	N	Average Body Weight Rats \pm SD (Gram)
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No.	Group	N	Average Body Weight Rats \pm SD (Gram)
1.	Control (C)	6	260,0 \pm 26,75
2.	Simvastatin (S)	6	283,3 \pm 27,69
3.	Group 1 (G1)	6	281,3 \pm 24,09
4.	Group 2 (G2)	6	286,3 \pm 14,17
5.	Group 3 (G3)	6	325,0 \pm 37,89

Description:

- a. C: Control group
- b. S: The group that received simvastatin
- c. G1: The treatment group that received the extract 25 mg/Kg BW
- d. G2: The treatment group that received the extract 50 mg/Kg BW
- e. G3: The treatment group that received the extract 100 mg/Kg BW

From the weight data above, a homogeneity test was carried out and a significance value of > 0.05 was obtained, which means that the variance of the white rats' body weight data was homogeneous.

1.5 Total Cholesterol Levels White Ratt (*Rattus norvegicus*)

Measurement of total cholesterol levels was carried out by taking the blood of rats anesthetized using ether. Blood was collected using a 2 cc syringe from the rat heart (aorta), put into an EDTA tube to prevent clotting, and then taken to the Clinical Pathology Laboratory (PK) RSUD dr. Sutomo Surabaya to be tested with the CHOD-PAP method. The results of the analysis of total cholesterol levels from the rats are as follows:

Table 4. Average (Mean) \pm Standard Deviation (SD) Total Cholesterol Levels in White Mice (mg/dL)

GROUP	Average Total Cholesterol Levels \pm SD (mg/dL)					
	N	Pretest	N	Postest	N	Delta
Control (C)	6	115,83 \pm 3,71	6	126,17 \pm 3,65	6	10,34 \pm 0,06
Simvastatin (S)	6	124,5 \pm 1,87	6	62,33 \pm 6,31	6	-62,17 \pm 4,4
Group 1 (G1)	6	108,33 \pm 3,01	6	56 \pm 6,09	6	-52,33 \pm 3,08
Group 2 (G2)	6	109,17 \pm 6,73	6	54 \pm 4,6	6	-55,17 \pm 2,13
Group 3 (G3)	6	110,17 \pm 4,75	6	50,83 \pm 4,26	6	-59,34 \pm 0,49

Description:

- a. C: Control group
- b. S: The group that received simvastatin
- c. G1: The treatment group that received the extract 25 mg/Kg BW
- d. G2: The treatment group that received the extract 50 mg/Kg BW
- e. G3: The treatment group that received the extract 100 mg/Kg BW

The average results, standard deviation, and delta were found in the control group (C) there was an increase in the average LDL-C level in the blood of white rats (*Rattus norvegicus*). The simvastatin (S) group showed the greatest decrease among the five groups. Treatment group 1 (G1), treatment 2 (G2), and treatment 3 (G3) all experienced a decrease. Treatment group 3 (G3) experienced the most decrease when compared to treatment group 1 (G1) and treatment 2 (G2). Treatment group 1 (G1) was the group with the least decrease compared to the simvastatin group, treatment 2 (G2), and treatment 3 (G3).

The normality test aims to determine the distribution of data. In this study, the Shapiro-Wilk to determine whether the research data were normally distributed or not. The parameter used is the significance value. If the significance value is $>$

0.05, it can be concluded that the data is normally distributed.

Table 5. Normality Test Results for Total Cholesterol Levels of White Rats

Kelompok	N	Sig.	Keterangan
Control (C)	6	0,804	Normal Distribution
Simvastatin (S)	6	0,981	Normal Distribution
Group 1 (G1)	6	0,410	Normal Distribution
Group 2 (G2)	6	0,610	Normal Distribution
Group 3 (G3)	6	0,643	Normal Distribution

Description:

- C: Control group
- S: The group that received simvastatin
- G1: The treatment group that received the extract 25 mg/Kg BW
- G2: The treatment group that received the extract 50 mg/Kg BW
- G3: The treatment group that received the extract 100 mg/Kg BW

The homogeneity test aims to determine the variance of the data. In this study, the Levene-Test to determine whether the research data was homogeneous or not. The parameter used is the significance value. If the significance value (Based on Mean) is obtained > 0.05 , it is concluded that the data in this study are homogeneous.

Table 6. Homogeneity Test Results Data on Total Cholesterol Levels of White Rats

Variable	Sig.	Description
Difference in total cholesterol levels	0,13	Homogeneous

The results of the homogeneity test on total cholesterol levels in rat blood obtained a significance value (Based on Mean) > 0.05 which can be concluded that the data is homogeneous.

The results of the research data obtained have a normal and homogeneous distribution, so for inferential statistical analysis the One Way ANOVA. The parameters used are the results of the significance value. If the significance value < 0.05 , it can be said that there is a significant difference in LDL-C levels between groups.

Table 7. Results of One Way ANOVA Test and LSD Test Data on Total Cholesterol Levels of White Rats

GROUP	N	Average difference in total cholesterol \pm	P ANOVA
		SD levels (mg/dL)	
Control (C)	6	10,34 \pm 0,06 ^a	0.000
Simvastatin (S)	6	-62,17 \pm 4,4 ^b	
Group 1 (G1)	6	-52,33 \pm 3,08 ^b	
Group 2 (G2)	6	-55,17 \pm 2,13 ^b	
Group 3 (G3)	6	-59,34 \pm 0,49 ^b	

Description:

- Superscripts indicate a significant difference
- C: Control group
- S: The group that received simvastatin
- G1: The treatment group that received the extract 25 mg/Kg BW
- G2: The treatment group that received the extract 50 mg/Kg BW
- G3: The treatment group that received the extract 100 mg/Kg BW

Test results One Way ANOVA above get a significance value of < 0.05 which indicates a significant difference.

Because the results of the ANOVA showed a significant difference, it was followed by using the LSD test to find out the differences in each group. LSD test results showed a significant difference between the control group (C) and the simvastatin group (S), treatment 1 (G1), treatment 2 (G2), and treatment 3 (G3), but no significant difference was found between the simvastatin group (S) and treatment groups 1 (G1), 2 (G2), and 3 (G3).

Based on the results above, it can be interpreted that the simvastatin (S) group with treatment groups 1 (G1), 2 (G2), and 3 (G3), when viewed from the difference in the mean value between the pre and post tests results were obtained negative indicating a decrease in total cholesterol levels. The reduction in cholesterol levels was highest in the simvastatin group. This is normal because simvastatin is a standard drug for treating hypercholesterolemia. But the provision of butterfly pea extract in the treatment groups 1, 2, and 3 was able to reduce total cholesterol levels, which had a significant difference in value compared to the group that was not given any treatment. Even though the mean total cholesterol levels in treatment groups 1, 2, and 3 were still below simvastatin, after being tested statistically there was no significant difference. This shows that the ethanol extract of the butterfly pea flower has the potential to reduce total cholesterol levels. Be seen that in the treatment of increasing doses, trend that was not significantly different. This is likely to have a different result if the dose is increased again (> 100 mg/Kg BW/day).

1.6 Discussion

In this study the results showed that there was a statistically significant decrease after administration of ethanol extract of butterfly pea flowers ($p < 0.05$). Based on descriptive analysis, reductions in total cholesterol levels were found in almost all groups, namely the simvastatin group (S), treatment 1 (G1), treatment 2 (G2), and treatment 3 (G3). The group with the highest decrease was found in the simvastatin (S) group, while the group with the dose of butterfly pea flower extract was found in treatment group 3 (G3), with a difference value of -59.34 ± 0.49 and a dose of 100 grams BW/day.

The decrease in cholesterol levels by butterfly pea flower extract (*Clitoria ternatea*) is thought to be due to the presence of substances in it, namely flavonoids, alkaloids, saponins, and tannins. The content of flavonoids, alkaloids, and tannins contained in butterfly pea flower extract acts as an antioxidant to reduce cholesterol levels in the blood of rats (Mulyani, 2012).

Flavonoids are exogenous antioxidants that have been shown to be useful in preventing cell damage due to oxidative stress through two mechanisms, namely direct and indirect (Sumardika & Jawi, 2012). The process directly occurs through the contribution of hydrogen ions so that it can neutralize the toxic effects of free radicals, whereas if it occurs indirectly, namely by increasing the expression of endogenous antioxidant genes.

In addition to acting as antioxidants, the results of in vitro studies show that flavonoids function by inhibiting the HMG-CoA reductase enzyme so that they can reduce cholesterol synthesis (Ekananda, 2015). When cholesterol is transported from the periphery of the intestine to the liver, the HMG-CoA reductase enzyme which is responsible for converting Acetyl-CoA to mevalonate in the process of cholesterol synthesis will be inhibited because the liver reduces the amount of its production (Wahyudi, 2009).

Alkaloids act as antioxidants by donating hydrogen ions like flavonoids. This compound can also inhibit the activity of the pancreatic lipase enzyme thereby increasing the excretion of fat through the feces. Absorption of fat by the liver becomes inhibited and cannot be synthesized into cholesterol (Lajuck, 2012). A decrease in the activity of the pancreatic lipase enzyme can reduce the deposition of triglycerides that enter the small intestine due to the change of triglycerides into two monoglycerides and two free fatty acids, so they do not enter the blood circulation (Wahyudi, 2009). Based on this, it is

said that butterfly pea flower extract can increase fat excretion through feces.

Saponins work by lowering blood cholesterol by binding to bile acids in the intestine thereby inhibiting the reabsorption of bile acids by the liver. Thus, bile acids will be excreted again with cholesterol. And so on until the cholesterol in the blood decreases. The tannins themselves are divided into two groups, namely hydrolyzed tannins and condensed tannins. Tannins can inhibit the absorption of fat in the intestine by reacting with proteins in the mucosa and intestinal epithelial cells (Ekananda, 2015). Tannins in the body can precipitate a protein layer on the surface of the small intestine so that it can reduce the efficiency of absorption of cholesterol and fat (Wahyudi, 2009) Proteins and amino acids present in food are likely to be precipitated by the tannins contained in butterfly pea flower extract (*Clitoria ternatea*), so that the absorption of fat in food will be disturbed. This process will cause the amount of cholesterol transported by chylomicrons to the liver to be unbalanced with the concentration of cholesterol from the food consumed.

1.7 Conclusions and Recommendations

This study found the effect of butterfly pea flower extract (*Clitoria Ternatea*) on reducing total cholesterol levels in *Rattus norvegicus* with a hypercholesterolemia model. In this study there were limitations, namely the nature of the experimental research with a pre and post test control group design so that it was not compared to the negative control group in the form of healthy rats without high-fat diet and any therapy. Researchers suggest that future studies use a negative control group by considering the effects of using butterfly pea (*Clitoria ternatea*) extract to find out the differences in each group. In addition, it is necessary to look for the relationship of other factors such as the influence of the optimum dose of butterfly pea extract by adding even higher dose variations (> 100 mg/Kg BW/day).

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1.8 Acknowledgements

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