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Effect of Clitoria ternatea Tea on Triglyceride levels in High Fat High Fructose Diet Fed Rats

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High triglyceride levels are one of the criteria for metabolic syndrome, which has a high prevalence. Butterfly pea flowers (*Clitoria ternatea L.*) contain flavonoids and polyphenols that have the potential to lower triglyceride levels. However, tea preparations have not yet been proven to lower triglycerides. This study aimed to determine the effect of butterfly pea flower tea on triglyceride levels in male Wistar rats (*Rattus norvegicus*) induced by a high-fat, high-fructose diet. This is an experimental study with a post-test only control group design. The study was conducted at the Center for Food and Nutrition Studies, Gadjah Mada University. For 22 days, 30 male Wistar rats were randomly divided into 5 groups. Group K0 was given standard feed, group K(–) was given a high-fat diet (2 ml/200 gBW/day) and fructose (1 ml/200 gBW/day), group P1 was given a high-fat high-fructose diet and butterfly pea flower extract at a dose of 80 mg/200 gBW, group P2 was given a high-fat high-fructose diet and butterfly pea flower tea at a dose of 36 mg/200 gBW, and group P3 was given a high-fat high-fructose diet and butterfly pea flower tea at a dose of 72 mg/200 gBW. The data obtained were analyzed using the One-Way ANOVA test and continued with the Post Hoc LSD test. The average triglyceride levels showed that the groups were K(-) 136.73 mg/dL, P2 111.23 mg/dL, P3 88.77 mg/dL, P1 85.96 mg/dL, and K0 70.99 mg/dL. One-Way ANOVA test showed significant differences between groups ($p<0.05$), the results of Post Hoc LSD K(-) and P3 were significantly different. Administration of butterfly pea flower tea (*Clitoria ternatea L.*) has an effect on triglyceride levels in high fat high fructose diet model mice.
Keywords: butterfly pea flower tea, high fat high fructose diet, triglyceride levels

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1. Introduction

Metabolic syndrome is a cluster of metabolic disorders characterized by hypertriglyceridemia, insulin resistance, hypertension, coagulation disorders, and visceral obesity. This condition is a global concern because it is closely linked to an increased risk of type 2 diabetes mellitus. According to the American Diabetes Association (ADA), high triglyceride levels are a key parameter in diagnosing metabolic syndrome, so controlling triglyceride levels plays a crucial role in preventing further complications [1].

Globally, metabolic syndrome is estimated to affect about a quarter of the adult population, with prevalence continuing to rise in line with high obesity rates. In Indonesia, 2018 RISKESDAS data showed a relatively high prevalence of metabolic syndrome in various provinces, such as Jakarta, West Java, South Kalimantan, and North Sulawesi. This high incidence is a serious concern because metabolic syndrome is a major risk factor for cardiovascular disease, which contributes significantly to morbidity and mortality [2], [3].

Various efforts have been made to control metabolic syndrome, one of which is through the use of herbal plants as supportive therapy. Herbal plants are considered to have great potential because they contain bioactive compounds that are natural, relatively safe, and have minimal side effects. One herbal plant that is beginning to be widely studied is the butterfly pea flower (*Clitoria ternatea* L.), which is known to contain various active compounds such as flavonoids, anthocyanins, tannins, alkaloids, phenolic acids, and

terpenoids. These compounds have antioxidant activity that plays a role in improving lipid profiles, including lowering blood triglyceride levels [4].

Butterfly pea flowers can be processed into herbal tea, a beverage made from plant parts other than Camellia sinensis leaves. The tea preparation was chosen because it maintains the flavonoid and anthocyanin content without degradation due to excessive heating. Furthermore, butterfly pea flower tea has a characteristic bluish-purple color, characteristic of anthocyanins, a mild aroma, and a neutral taste, thus maintaining sensory quality and being readily accepted by the public [5]. The anthocyanin and tannin content in butterfly pea flowers is known to play a role in reducing triglyceride levels through various mechanisms, such as inhibiting the lipase enzyme, reducing lipogenesis, inhibiting HMG-CoA reductase, and increasing lipid metabolism and energy expenditure.

Several previous studies have shown that butterfly pea flower has antihyperlipidemic potential and can significantly lower triglyceride levels. However, research specifically examining the effect of butterfly pea flower tea on triglyceride levels in metabolic syndrome is still limited. Therefore, experimental studies using animal models, such as male Wistar rats fed a high-fat, high-fructose diet, are needed to determine the effectiveness of butterfly pea flower tea in lowering triglyceride levels, one of the main indicators of metabolic syndrome.

2. Literature Review and Problem Statement

High-fat high-fructose (HFHF) diets serve as a well validated rodent model for inducing hypertriglyceridemia and metabolic syndrome by promoting hepatic de novo lipogenesis, VLDL overproduction, and insulin resistance [6]–[8]. Butterfly pea flower (*Clitoria ternatea* L.) has emerged as a promising phytotherapeutic agent due to its rich content of flavonoids, anthocyanins, tannins, and phenolic acids, which exert antioxidant, anti-inflammatory, and antihyperlipidemic effects. These compounds act through multiple mechanisms, including inhibition of lipoprotein lipase and HMG-CoA reductase, suppression of lipogenesis, and activation of PPAR-α and AMPK pathway to enhance fatty acid β-oxidation [4], [9], [10]. Preclinical studies using ethanolic or aqueous extracts of *C. ternatea* have consistently demonstrated significant reductions in serum triglycerides, total cholesterol, and LDL in high-fat diet induced models [11]–[13]. However most existing research has relied on concentrated laboratory extracts, wich may not translate directly to consumer-accessible preparations such as herbal tea. Some studies also report variability in efficacy depending on processing methods, with heat exposure during tea brewing potentially affecting the stability and bioavailability of heat-sensitive anthocyanins and polyphenols [12], [14].

Despite the established antihyperlipidemic potential of *Clitoria ternatea* extracts, a critical research gap remains regarding the efficacy of its tea preparation a more practical, everyday consumption form on triglyceride levels in HFHF induced metabolic syndrome models. Previous extract-based studies do not adequately represent real world use, where brewing processes may alter bioactive compound concentrations, and direct dose dependent comparisons between tea and extract within the same experimental protocol are lacking. This limits the translational value of existing evidence for public health applications. The present study therefore addresses the following problem: Does administration of butterfly pea flower tea (*Clitoria ternatea* L.) at doses of 36 mg/200 gBW/day and 72 mg/200 gBW/day significantly reduce triglyceride levels in male Wistar rats fed a high-fat high fructose diet compared to extract and control groups? It is hypothesized that butterfly pea flower tea will produce a significant, dose dependent reduction in triglyceride levels, although the magnitude may be slightly lower than that of the concentrated extract due to potential partial degradation of active compounds during tea processing.

3. Method

This study was a purely experimental study with a post-test only control group design. The subjects were 25 male Wistar rats (*Rattus norvegicus*) divided into five groups using simple random sampling. The study groups consisted of a normal group (K0) given only standard feed and distilled water, a negative control group (K-) induced by a high-fat and high-fructose diet, and three treatment groups: P1, P2, and P3.

Group P1 was given a high-fat and high-fructose diet and butterfly pea flower extract at a dose of 80 mg/200 gBW/day, group P2 was given a high-fat and high-fructose diet and butterfly pea flower tea at a dose of 36 mg/200 gBW/day, and group P3 was given a high-fat and high-fructose diet and butterfly pea flower tea at a dose of 72 mg/200 gBW/day.

The sample size was determined using the Federer formula to obtain a minimum of five rats in each group. Subjects met the inclusion criteria: 8–12-week-old male Wistar rats, weighing 180–200 grams, healthy, active, and free of anatomical abnormalities. Rats that became ill or died during the study were excluded and categorized as dropouts.

The study began with a seven-day acclimatization period, followed by 14 days of treatment. A high-fat diet was administered in the form of quail egg yolk at a dose of 2 ml/200 gBW/day, while a high-fructose diet was administered in the form of a pure fructose solution at a dose of 1 ml/200 gBW/day orally using a tube. Butterfly pea flower tea was made through a process of washing, withering, drying, grinding, and sieving until a powder was obtained, then brewed and administered orally according to the treatment dose. Butterfly pea flower extract was made through a process of drying, brewing, homogenizing, filtering, and thickening using a rotary evaporator.

Triglyceride levels were examined after the mice were fasted for 8 hours. Blood samples were taken from the retroorbital vein after the mice were anesthetized with ketamine. The blood was centrifuged to obtain serum, and triglyceride levels were then measured using the colorimetric enzymatic glycerol-3-phosphate-oxidase (GPO) method using a spectrophotometer, with results expressed in mg/dL.

The data obtained were analyzed using a one-way ANOVA test and followed by a post hoc test if significant differences were found. Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) software with a significance level of $p < 0.05$.

4. Results And Discussion

Table 1. Mean Triglyceride Levels, Normality Test, Homogeneity Test, and One-Way ANOVA Test

	Group					*p-value*
	K-0	K(-)	P1	P2	P3	
Mean ± SD	70,99± 1,81	136,37 ± 2,73	85,96 ± 2,77	111,23 ±3,70	88,77 ± 1,45	
Shapiro Wilk test*	0,953	0,760	0,944	0,991	0,759	
Levene test						0.377
One Way Anova						0,000

The results of triglyceride level measurements after treatment showed that the highest average triglyceride level was in the negative control group K (-) at 136.37 ± 2.73 mg/dL, while the lowest level was in the normal group K0 at 70.99 ± 1.81 mg/dL. Treatment groups P1 and P3 had relatively similar triglyceride

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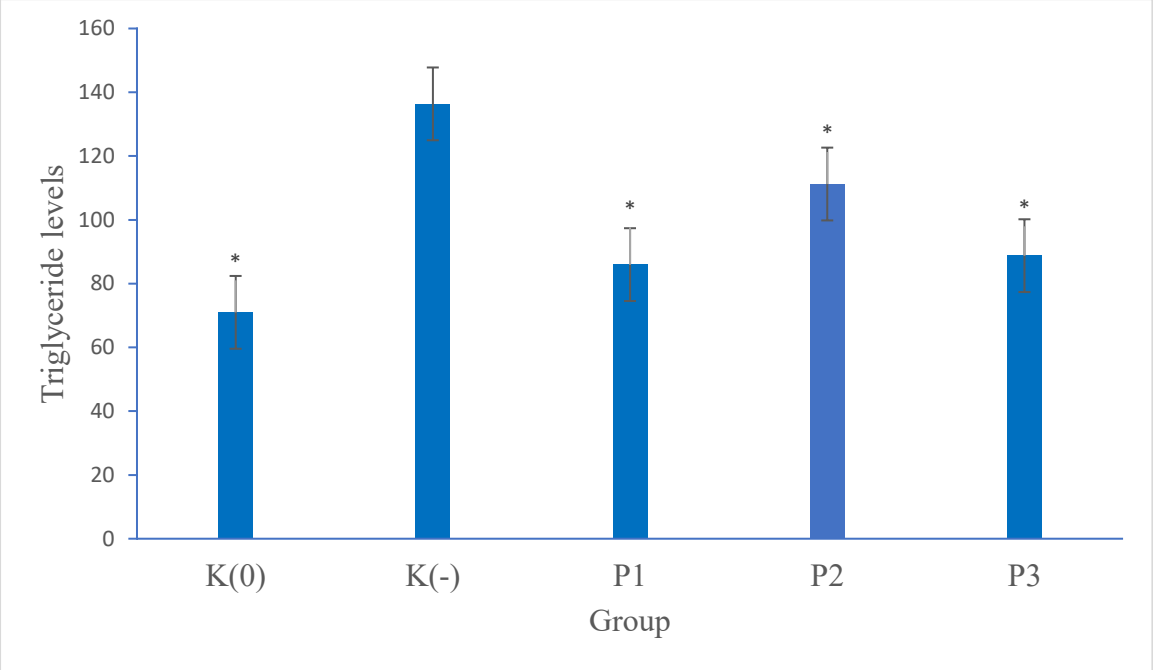
levels, respectively at 85.96 ± 2.77 mg/dL and 88.77 ± 1.45 mg/dL, while group P2 showed higher triglyceride levels at 111.23 ± 3.70 mg/dL.



Note: (*) has a significant difference with respect to group K(-) with a P value <0.001

Figure 1. Diagram of Average Triglyceride Levels after Treatment

The Shapiro–Wilk normality test showed that all data were normally distributed ($p > 0.05$) and the Levene homogeneity test showed homogeneous variance between groups ($p = 0.377$). Analysis using the one-way ANOVA test showed a significant difference in triglyceride levels between treatment groups ($p < 0.001$), which was also illustrated in the comparison diagram of the average triglyceride levels with the highest value in the K(-) group.

Table 2. LSD Post-Hoc Test Results Table

Group		*p value post hoc LSD*
K0	K(-)	0.000
	P1	0.000
	P2	0.000
	P3	0.000
K(-)	P1	0.000
	P2	0.000
	P3	0.000
P1	P2	0.000
	P3	0.077
P2	P3	0.000

Further post hoc LSD tests showed that almost all comparisons between groups were significantly different ($p < 0.05$), except between groups P1 and P3 which did not show a significant difference ($p = 0.077$), so it can be concluded that administration of butterfly pea flower tea at a dose of 36 mg/200 gBW/day and 72 mg/200 gBW/day provided a relatively similar effect on reducing triglyceride levels.

Discussion

This study shows that induction of a high-fat, high-fructose (HFHF) diet can cause significant metabolic changes in male Wistar rats, characterized by increased body weight, fasting blood sugar levels, and triglyceride levels. The highest average body weight was found in the negative control group K(-) given the HFHF diet without butterfly pea flower intervention, compared to the normal control group. This condition indicates that excessive energy intake from a combination of fat and fructose plays a major role in the accumulation of adipose tissue. A high-fat and high-fructose diet is known to disrupt the regulation of energy balance through the mechanisms of leptin resistance and insulin resistance, which causes increased appetite accompanied by decreased energy expenditure, thus triggering obesity and metabolic disorders [6].

The increase in mean fasting blood sugar levels in the K(-) group compared to the K0 group also confirms that the HFHF diet successfully triggered glucose metabolism disorders. The resulting insulin resistance leads to reduced glucose uptake by peripheral tissues and increased adipose tissue lipolysis, thereby increasing the release of free fatty acids (FFA) into the circulation. This increase in FFA further exacerbates insulin resistance and contributes to increased fasting blood glucose [15]. These results align with previous research showing that a high-fat and high-fructose diet can significantly increase blood glucose levels and trigger metabolic syndrome in animal models [16].

The main results of this study indicate that the administration of butterfly pea flowers (*Clitoria ternatea L.*), either in the form of extract or tea preparations, has a significant effect on reducing triglyceride levels. The highest mean triglyceride levels were found in the K(-) group, while all treatment groups showed lower triglyceride levels. Almost all comparisons between groups showed statistically significant differences ($p < 0.05$), which supports the hypothesis that the bioactive compounds in butterfly pea flowers play a role in improving lipid profiles. Flavonoids contained in butterfly pea flowers are known to inhibit the enzyme α-glucosidase, thereby reducing glucose absorption and reducing substrates for lipogenesis. In addition, anthocyanins and tannins play a role in increasing insulin sensitivity through activation of the PPAR-α and AMPK pathways, which in turn increase fatty acid β-oxidation and reduce triglyceride levels [9].

Comparison between the K0 and K(-) groups showed a very clear difference in triglyceride levels, indicating that the induction of a high-fat and fructose diet successfully increased blood triglycerides. The HFHF diet increased lipogenesis activity in the liver, increased FFA release, and stimulated the formation of very-low-density lipoprotein (VLDL), which directly contributed to increased plasma triglyceride levels [17]. This finding is in line with research [18] which states that the combination of a high-fat and high-fructose diet is an effective model for causing hypertriglyceridemia and insulin resistance.

A comparison between the K(-) group and the P1, P2, and P3 treatment groups showed that the administration of butterfly pea flowers significantly reduced triglyceride levels. This reduction is related to the antioxidant and anti-inflammatory properties of the active compounds in butterfly pea flowers, particularly phenolic acids, anthocyanins, and tannins. Phenolic acids are known to suppress the production of pro-inflammatory cytokines such as TNF-α and IL-6, which play a role in activating the JNK and IKKβ pathways that cause insulin resistance. Meanwhile, anthocyanins and tannins play a role in reducing oxidative stress, increasing insulin sensitivity, and suppressing the release of FFA, thus impacting triglyceride levels [3]. Several other studies also reported that butterfly pea flower extract was consistently able to reduce triglyceride levels in animal models [11].

Comparisons between treatment groups showed that the form of the butterfly pea flower preparation affected the effectiveness of triglyceride reduction. Group P1, which received butterfly pea flower extract, showed lower triglyceride levels compared to groups P2 and P3, which received tea preparations. This is

likely due to the more stable concentration of active compounds such as anthocyanins, flavonoids, and phenolics in the extract. The brewing process for tea preparations involves heating, which can cause degradation or variations in the levels of active compounds, resulting in a lower hypolipidemic effect compared to the extract [12], [14].

A comparison between groups P2 and P3 showed an effect of the dose of butterfly pea flower tea on triglyceride levels. Group P3 with a dose of 72 mg/200 gBW had lower triglyceride levels compared to P2 with a dose of 36 mg/200 gBW. This indicates that increasing the dose of butterfly pea flower tea increases the polyphenol content, including phenolic acids, anthocyanins, and tannins, which work more effectively in reducing pro-inflammatory cytokines, increasing PPAR-α activation, and reducing FFA release, thereby increasing fatty acid oxidation [19]. This finding is in line with research [20] which states that the decrease in triglycerides is proportional to the increase in the dose of butterfly pea flowers.

Groups P2 and P3 showed significant differences, with triglyceride levels lower in P3 than in P2. This indicates that in this study's dosing scheme, 72 mg/200 gBW was more effective than 36 mg/200 gBW in lowering triglyceride levels. These results align with previous research showing that higher doses of butterfly pea flower extract provide a more optimal hypolipidemic effect [21].

In the P1 group, the reduction in triglyceride levels approached that of the K0 group, and in some comparisons, no significant differences were observed. This suggests a possible optimal dose range that provides maximum effect without the need for further dose escalation. Furthermore, the 22-day treatment duration may not have been sufficient to fully restore lipid profiles to baseline. Previous research has shown that longer-duration interventions tend to provide a more comprehensive picture of the hypolipidemic effect [12].

Limitations of this study include the lack of quantitative measurements of flavonoid and antioxidant compounds in butterfly pea flower extracts or tea preparations, thus preventing a more specific dose-response relationship. Furthermore, this study did not measure visceral fat thickness as an additional parameter to assess obesity and the safety of long-term use. Therefore, further research is recommended to quantitatively evaluate the active compounds and include other metabolic parameters to obtain a more comprehensive picture of the effects of butterfly pea flower on metabolic syndrome.

The administration of butterfly pea flower tea (Clitoria ternatea L.) has been shown to affect triglyceride levels in male white Wistar rats induced by a high-fat, high-fructose diet ($p < 0.05$). Rats fed standard feed and distilled water had lower average triglyceride levels compared to the group induced by a high-fat, high-fructose diet. Administration of butterfly pea flower, either in the form of extract or tea, was able to reduce triglyceride levels compared to the negative control group. Butterfly pea flower extract at a dose of 80 mg/200 gBW/day showed lower average triglyceride levels compared to tea preparations at doses of 36 mg/200 g BW/day and 72 mg/200 gBW/day. In addition, administration of butterfly pea flower tea at a dose of 72 mg/200 gBW/day resulted in a greater reduction in triglyceride levels compared to a dose of 36 mg/200 gBW/day. Overall, there were significant differences between groups ($p<0.05$), especially in the Post Hoc LSD K(-) and P3 results which were significantly different.

5. Conclusion

The present study demonstrates that administration of butterfly pea flower (*Clitoria ternatea* L.), whether as extract or tea preparation, significantly reduces serum triglyceride levels in male Wistar rats induced with a high-fat high-fructose diet ($p<0.05$). The HFHF diet successfully induced hypertriglyceridemia, as evidenced by markedly elevated levels in the negative control group (136.37 ± 2.73 mg/dL) compared to the normal control (70.99 ± 1.81 mg/dL). Both the extract (80 mg/200 gBW/day) and higher dose tea (72

mg/ 200 gBW/day) produced substantial reductions, approaching levels observed in the normal group, while the lower tea dose (36 mg/200 gBW/day) showed a more moderate but still significant effect. Post hoc analyses confirmed significant differences between the negative control and treatment groups, supporting the dose dependent hypolipidemic activity of butterfly pea flower preparations.

These findings align with and extend previous research on the antihyperlipidemic properties of *Clitoria ternatea* by demonstrating that the more accessible tea form retains meaningful bioactivity, albeit potentially slightly less potent than concentrated extracts due to processing effects. The results reinforce the role of flavonoids, anthocyanins, and tannins in modulating lipid metabolism through antioxidant, anti-inflammatory, and PPAR-α or AMPK mediated pathways. Practically, this supports the potential use of butterfly pea flower tea as supports the potential use of butterfly pea flower tea as a supportive dietary intervention for managing hypertriglyceridemia and metabolic syndrome. Limitations include the relatively short intervention period, absence of direct quantification of bioactive compounds, and lack of additional metabolic parameters such as visceral fat. Future studies should incorporate longer durations, phytochemical profiling, and optimize dosing for translational applications. Overall, butterfly pea flower tea offers a promising, natural, and consumer friendly approach to improving lipid profiles in metabolic disorders.

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