ORIGINAL ARTICLE

Intisari Sains Medis 2024, Volume 15, Number 1: 86-91
P-ISSN: 2503-3638, E-ISSN: 2089-9084

Published by Intisari Sains Medis

Antihyperglycemic and pancreatoprotective effects of butterfly pea flower (*Clitoria ternatea*) aqueous extract in hyperglycemic mice

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Received: 2023-12-02
Accepted: 2024-01-16
Published: 2024-02-08

ABSTRACT

Background: Diabetes mellitus has become a global concern with cases that increase every year and contribute to various organ damage. Previous research shows that the butterfly pea flower (*Clitoria ternatea*) has the potential to lower blood glucose level and protect the pancreas through its antioxidant and antihyperglycemic effects.

Methods: An experimental analytic study was conducted using 15 mice which were divided into 5 groups, N (normal), HG (hyperglycemic without CTE), HG+MET (hyperglycemic + metformin), HG+CTE300 (hyperglycemic + CTE300mg/kgBB), and HG+CTE500 (hyperglycemic + CTE500mg/kgBB). CTE administration was carried out for 2 weeks in groups HG+CTE300 and HG+CTE500 which were then examined for blood glucose level and histological features of pancreatic β cells preparation with hematoxylin-eosin staining.

Results: The results of the data analysis showed that there was a significant difference in mean blood glucose level between the HG group and the treatment group with CTE administration (HG+CTE300 and HG+CTE500) ((MD: 53.67 95%; p=0.001) and (MD: 57.67 95%; p=0.001) respectively). In addition, a significant difference was found in the average number of pancreatic β cells per area between the HG group and the treatment group given CTE 500 mg/kg BW (MD: -8.83; p=0.012). There was no significant difference between the mean blood glucose and the number of pancreatic β cells per area between the groups with CTE compared to the metformin group.

Conclusion: Aqueous extract of butterfly pea flower (*Clitoria ternatea*) has potential effects on lowering blood glucose levels and improving pancreatic β cell features in hyperglycemic mice.

Keywords: antihyperglycemia, blood glucose, *Clitoria ternatea*, pancreatic β cells, pancreatoprotective effect.
Cite This Article: Sasmana, I.G.A.P., Surudarma, I.W., Wiryanthini, I.A.D., Sutadarma, I.W.G. 2024. Antihyperglycemic and pancreatoprotective effects of butterfly pea flower (*Clitoria ternatea*) aqueous extract in hyperglycemic mice. *Intisari Sains Medis* 15(1): 86-91. DOI: 10.15562/ism.v15i1.1906

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia or increased blood glucose which can cause damage to various body organs, including the heart, blood vessels, kidneys, nerves, and so on.[1] This disease has become a global concern with the incidence rate increasing every year and contributing to the development of various chronic diseases with high mortality and morbidity rates.[2–4] According to the International Diabetes Federation (IDF), around 537 million adults suffer from diabetes mellitus with a prevalence of around 9.8% of the world population.[5] Moreover, cases of diabetes mellitus were recorded to have increased quite significantly, up to more than 3 times compared to 1980. This is closely related to the increase in risk factors related to diabetes mellitus which is also increasing at this time, such as sedentary lifestyle, obesity, and consumption of foods high in sugar, which is also combined with other risk factors such as genetics, gestational diabetes, and so on.[6]

Hyperglycemia in diabetes mellitus occurs due to several pathological conditions. In type 1 diabetes mellitus, there is damage to cells in the pancreas which is generally caused by autoimmunity or certain chemicals which cause the inability of the pancreas to produce enough insulin to control blood sugar.[7] Meanwhile, type 2 diabetes mellitus is generally a chronic condition complicated by various risk factors, causing insulin resistance, and causing the body to fail to compensate for the increase in blood glucose.[8,9] This hyperglycemia condition causes various complications in the body's organs which contribute to the development of various diseases with high mortality and morbidity and contribute to 11.3% of deaths worldwide.[10] Diabetes mellitus patients have twice the risk of experiencing cardiovascular disease with an odds ratio for death of 4.56 (95% CI: 3.53 – 5.89).[11] In addition, patients with diabetes mellitus also have an 11.04 times higher risk (95% CI: 6.913 – 17.63) of developing chronic kidney disease compared to patients without diabetes mellitus.[12]

Several therapeutic compounds were found to have less than optimal effects

and quite bad side effects, especially in long-term conditions. Metformin has been reported to cause lactic acidosis and thiazolidinediones have been reported to cause body fluid retention and worsen heart attacks.[13,14] In addition, SGLT-2 inhibitors have been reported to cause urinary tract infections due to increased glucose excretion.[15] Moreover, until now pharmacological therapy for the regeneration of pancreatic β cells has been minimally developed.[16]

Several studies reported the potential of the butterfly pea flower *(Clitoria ternatea)* to lower blood glucose but is still rarely discussed. The petal part of the butterfly pea flower has high levels of anthocyanins and flavonoids, namely 132 ppm and 23.99 mg/100g respectively.[17] Anthocyanins, tannins, and flavonoids are important compounds because they can inhibit increases in blood glucose through several mechanisms, such as protecting pancreatic β cells, reducing insulin resistance, as inhibitors of the enzymes α-amylase and α-glucosidase, and acting as antioxidant.[18] This study aims to further examine the antihyperglycemic and pancreatoprotective effects of butterfly pea flower extract *(Clitoria ternatea)* on hyperglycemic mice.

MATERIAL AND METHODS

Extract Preparation and Phytochemical Analysis

The butterfly pea flower (*Clitoria ternatea*) was obtained from Buleleng, Indonesia. The flowers are then dried and extracted according to the method of.[19] The dried blue butterfly pea flower petals were then crushed with a hammer mill crusher for 30 minutes. Then, the crushed sample (100 g) was extracted with 2 L of distilled water and boiled at 60°C for 20 minutes. The soluble extract was filtered through nylon. The filtrate was dried by lyophilization. The results of this extraction produced the aqueous extract from butterfly pea flowers which then was analyzed for phytochemical anthocyanin screening.

Animals Study Design and Feeding

The experimental animals used were male BALB/c mice, healthy, 8-12 weeks old with an average body weight of 28 grams. The experimental animals used were 15 mice divided into 5 groups, N (negative control), HG (positive control, hyperglycemic without CTE or metformin), HG+MET (hyperglycemic + 1.3 grams metformin), HG+CTE300 (hyperglycemic + CTE *(Clitoria ternatea* extract*)* 300 mg/kgBW), and HG+CTE500 (hyperglycemic + CTE *(Clitoria ternatea* extract*)* 500 mg/kgBW). The sample size was calculate based on Arifin and Zahiruddin, 2017 formula.[20]

Mice were placed in experimental cages (3 mice per cage), in a room with a temperature of 24 ± 1 °C, humidity of 55% ± 5%, and with a 12-hour light/dark cycle. Alloxan is injected intraperitoneally at a dose of 150 mg/kg BW. Diabetic condition will be achieved on the seventh day after injection. The intervention was given based on the dose for each group. Metformin is given according to the conversion calculation between the human dose and the mouse dose, namely 1.3 grams per day. Before administration, dilution will be carried out using distilled water until 0.4 ml of distilled water has the same content according to the concentration series given to each group. The intervention was provided for 2 weeks

Body Weight and Blood Glucose Measurement

Body weight was measured before the mice were induced by alloxan, after the mice were hyperglycemic, and after the treatment was given. Meanwhile, blood glucose measurements were carried out using a glucometer after the mice had hyperglycemia and after 2 weeks of administering the extract to test animals. The type of blood sugar checked is fasting blood sugar 8 hours after being given food.

Histology Evaluation

Pancreatic samples from mice were taken after the mice were euthanized and then histology preparations were made using Mayer's Hematoxyllin-Eosin. The preparations were then observed using a microscope with 40x and 400x magnification and documented using OptiLab ver. 2 each with 4 fields of view from each sample randomly. Calculation of the number of pancreatic beta cells per area of the islets of Langerhans was carried out using ImageJ software (NIH, Bethesda, Maryland, USA).

Statistical Analyses

Data analysis was carried out using SPSS version 26 software (SPSS Inc., Chicago, IL, USA). Data were analyzed using the One-Way ANOVA test and continued with the LSD Post Hoc Test to find out which groups had significant differences with a significance value of $p < 0.05$.

RESULTS

Body Weight and Blood Glucose

Analysis of changes in body weight over time showed that there was an increase in the body weight of the mice before the alloxan injection compared to 7 days after the alloxan injection (a state of hyperglycemia was reached). There was also a significant decrease in body weight

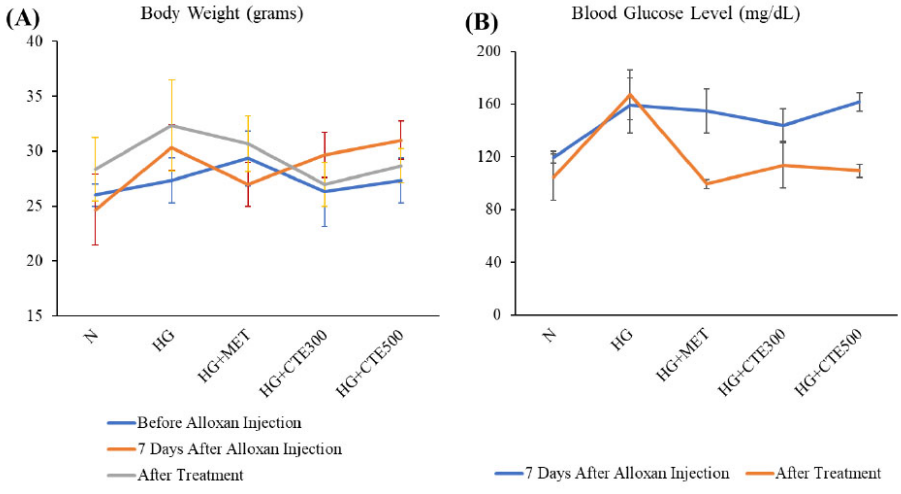

Figure 1. Line graphs show changes in body weight (A) and blood glucose level (B) in mice over several time series (before and after treatment).

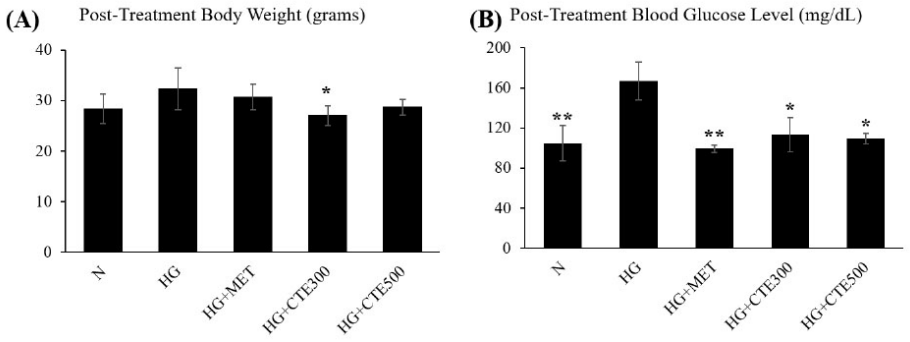

Figure 2. Comparison between intervention groups from post-hoc analysis of post-treatment body weight (A) and post-treatment blood glucose levels (B) in mice. (* $p < 0.05$, ** $p < 0.05$) vs positive control group (HG group).

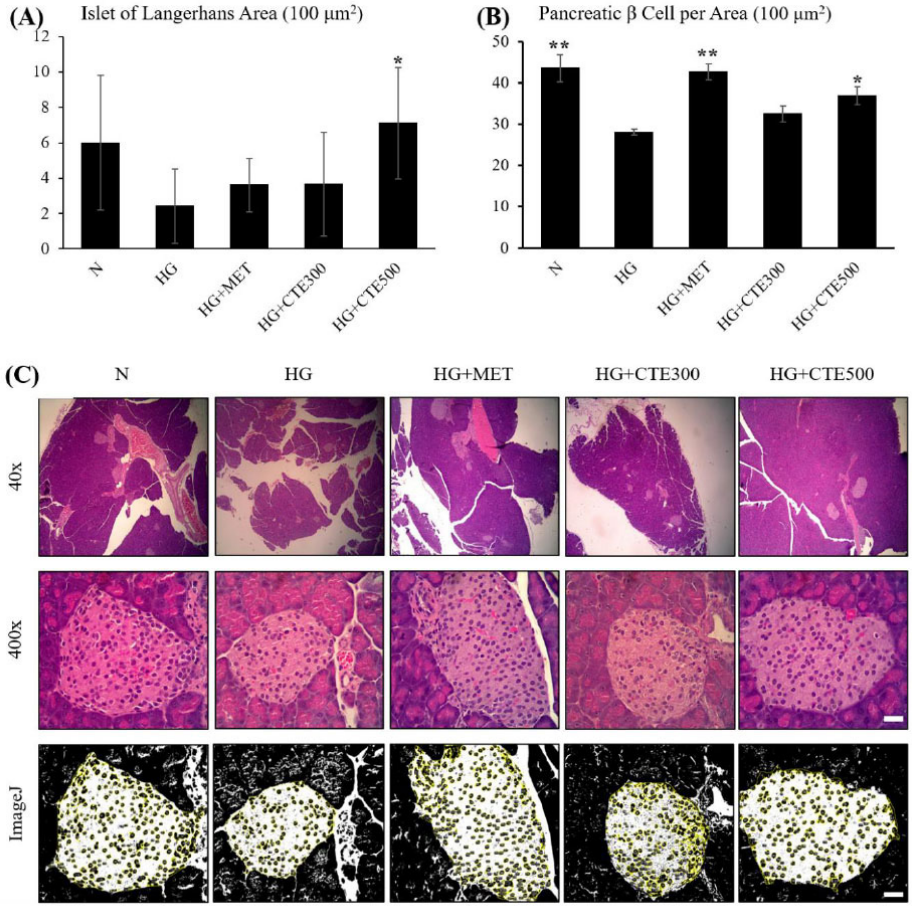

Figure 3. Comparison between intervention groups from a post-hoc analysis of Islet of Langerhans Area (A) and Pancreatic β Cell per Area (100um) in mice. The histological finding shows the number of pancreatic β cells and the islet of Langerhans qualitatively (C). (* $p < 0.05$, ** $p < 0.05$) vs positive control group (HG group).

7 days after alloxan induction compared to after treatment in the HG+CTE300 and HG+CTE500 groups. Meanwhile, there was an increase in body weight in the HG group over time (Figure 1A). Meanwhile, there was a significant reduction in blood glucose levels in the HG+MET, HG+CTE300, and HG+CTE 500 groups before and after treatment. Meanwhile, there were no significant changes in the N and HG groups (Figure 1B).

Figure 2A shows that there was a significantly lower value of post-therapy body weight in the HG group compared to the HG+CTE300 group (Mean Difference: 95%CI: p = 0.04). Meanwhile, there was a significant difference in post-treatment blood glucose levels in the HG group compared to the HG+MET (MD: 67.67; 95% CI: 41.81 – 93.53; p <0.001), HG+CTE300 groups (MD: 53.67 KI 95% 27.81- 79.53; p = 0.001), and HT+ CTE500 (MD: 57.67 KI 95% 31.81 - 83.53; p = 0.001) (Figure 2B). Meanwhile, there is no significant difference between the effectiveness of metformin and butterfly pea flower extract. This shows the potential of butterfly pea flower extract (*Clitoria ternatea*) in reducing body weight and blood glucose in hyperglycemic mice.

Pancreatic β Cell

The area of Langerhans Island shows very varied results. From these variations, it was found that there was a significant mean difference between the HG group and the HG+CTE500 group (Figure 3A). Meanwhile, there was a significant increase in the number of pancreatic β cells per islet area of Langerhans in the HG+MET (MD: 14.57 95%CI: 7.99-21.15; p<0.001) and HG+CTE500 groups (MD: 8.83 KI 95%: 2.25 to 15.41; p = 0.012) compared with the HG group. Meanwhile, there was no significant difference between the HG+MET and HG+CTE500 groups (Figure 3B). The results of the histology examination (Figure 3C) show that the qualitative description of the area of the islets of Langerhans and the number of pancreatic β cells per region are higher in the group with butterfly pea flower extract (CTE300 and CTE500) compared to the HG group.

DISCUSSION

Aqueous extract of butterfly pea flowers *(Clitoria ternatea)* is a therapeutic modality that comes from nature and contains various pharmacological compounds that can decrease body weight and blood glucose levels. Metabolic diseases (diabetes mellitus, dyslipidemia, hypertension, etc.) are closely related to increased body weight. In diabetes mellitus, excess blood glucose will be stored in the form of white adipose tissue which can cause an increase in inflammatory mediators and a

worse prognosis.[21–23] *Clitoria ternatea* can reduce the concentration of white adipose tissue.[24] This extract has the potential to inhibit adipogenesis thereby preventing fat deposition.[25] Previous research shows that anthocyanins have the potential to reduce the expression of the Sterol Response Element Binding Proteins-1 (SREBP-1) and Fatty Acid Synthase (FAS) genes which are involved in the conversion of glucose into fatty acids and the deposition of white adipose tissue.[26] Moreover, the effectiveness of butterfly pea flowers (*Clitoria ternatea*) in reducing blood glucose is in line with previous research by Bhosale, et al (2013) which evaluated the potential of *Clitoria ternatea* ethanol extract. The ethanol extract of butterfly pea flowers was reported to reduce fasting blood glucose at a dose of 400 mg/kgBW in streptozotocin-induced diabetic rats $p<0.001$ within 2 weeks.[27]

The potential for antihyperglycemia can occur due to the pharmacological effects of the various components that make up the butterfly pea flower (*Clitoria ternatea*), including anthocyanins, tannins, and so on. Liquid extract of butterfly pea flowers (*Clitoria ternatea*) also contains flavonoids reaching 1771.39 mg/100g.[28] These anthocyanins and flavonoids have the potential to increase insulin sensitivity and secretion in the body. This occurs through increased translocation of GLUT4. GLUT4 is a transporter in peripheral organs that is used to deliver insulin to these organs so that insulin can provide an adequate response.[29] These transporters are found abundantly in energy storage organs such as adipose tissue, striated muscle cells, heart muscle cells, liver, and several other organs. In conditions of oxidative stress, this transporter also experiences disruption, thereby minimizing insulin uptake. Increasing insulin uptake by anthocyanins will increase the absorption and conversion of glucose in peripheral organs which will be converted into glycogen, fat components, or other energy reserve components. In addition, the increase in glucose uptake in peripheral tissues is also mediated by peroxisome proliferator-activated receptor (PPAR), whose activation can also be increased by anthocyanins. Increasing activation of these receptors will increase insulin sensitivity.[30]

Evaluation of the potential of butterfly pea flower extract (*Clitoria ternatea*) as an anti-diabetic agent was also evaluated by Widowati (2023). This research shows the potential of butterfly pea flower extract to reduce blood glucose through antioxidant, anti-inflammatory effects, reducing hepatic GSK-3b, and reducing pancreatic glycogen in mice with diabetes mellitus and dyslipidemia. Butterfly flower extract at a dose of 800mg/kgBW was found to reduce blood glucose and increase insulin levels significantly ($p < 0.05$).[31] This can be mediated by the potential of anthocyanins in butterfly pea flower extract (*Clitoria ternatea*) in modulating enzymes related to glucose metabolism. Anthocyanins have the potential to inhibit the enzymes α-amylase and α-glucosidase, enzymes involved in breaking down carbohydrate molecules into monosaccharides so that inhibition of these enzymes causes less glucose to be absorbed in the intestine and reduces the increase in blood glucose, especially post-prandial glucose.[32] In addition, purified anthocyanin extract from *Berberis integerrima* bunge fruit was found to inhibit the α-glucosidase enzyme with an IC50 of 0.71 ± 0.085 mg/ml (acarbose: 8.8 ± 0.14 mg/ml, $p < 0.001$) and inhibits the α-amylase enzyme with an IC50 of 1.14 ± 0.003 mg/ml (acarbose: 1 ± 0.085 mg/ml, $p < 0.05$).[33]

Tannin can also act as an inhibitor of α-amylase and α-glucosidase. Previous research by Kato, et al (2017) showed the potential of condensed and hydrolyzed tannins in inhibiting α-amylase with concentrations to inhibit 50% of the substrate (IC50) being 47 and 285.4 μM for salivary α-amylase and 141.1 and 248.1 μM for α-amylase pancreas. This shows the potential of tannin to inhibit the breakdown of carbohydrates, thereby inhibiting glucose absorption, which has an impact on inhibiting the increase in body glucose.[34]

The potential effect of *Clitoria ternatea* to reduce pancreatic damage as represented by a higher number of pancreatic β cells can be mediated by several mechanisms related to oxidative stress caused by alloxan as an inducer of hyperglycemia.[35,36] The components in water extract of butterfly pea flowers (*Clitoria ternatea*) have antioxidant functions so they can reduce oxidative stress and protect pancreatic β cells or are pancreatoprotective. Butterfly pea flowers (*Clitoria ternatea*) are reported to have several pharmacological compounds that can play this role, including anthocyanins, tannins, glycosides, flavonoids, steroids, saponins, and phenols.[37]

The potential of antioxidant-rich extracts that can reduce damage to the pancreas was also evaluated in research by Abdel-Rahman, et al (2019) which evaluated *Scorpio maurus palmatus* extract in alloxan-induced diabetic mice. This research showed that the group that received the treatment had a significantly greater number of pancreatic β cells/area compared to the positive control group ($p < 0.05$), one of which was mediated by the antioxidant mechanism of the extract.[38] In this concept, butterfly pea flower extract can also play a similar potential. As an extract containing high anthocyanins, previous research by Fu, et al (2021) showed the potential of butterfly pea flower extract *(Clitoria ternatea)* to inhibit free radicals. This research used a method for measuring the inhibition of the intracellular antioxidant 1,1-diphenyl-2-picrylhydrazyl (DPPH). The results of the research show that butterfly pea flower extract can inhibit up to 85% of free radicals at pH 1 and 80% at pH 5. This antioxidant potential can reduce free radicals which are one of the main causes of damage to pancreatic β cells in diabetes mellitus.[39] The antioxidant ability of anthocyanins can also prevent the worsening of conditions caused by oxidative stress, such as chronic kidney disease, cardiovascular disease, susceptibility to infection, cancer, and even death.[40–42]

Concerning genetic modulation, research by Minelko, et al (2020) shows the potential of butterfly pea flowers (*Clitoria ternatea*) in increasing the regulation of genes related to pancreatic regeneration, including PPARγ gene expression.[43] Apart from that, research by Kang, et al (2020) also shows the potential of anthocyanins to increase the expression of genes involved in the formation of pancreatic β cells, including glucose transporter 2 (Glut2), silent mating type information regulation 2 homolog 1 (Sirt1), mitochondrial

transcription factor A (Tfam), pancreatic/duodenal homeobox protein 1 (Pdx-1) and insulin 1 (Ins1). These genes were found to increase significantly at an anthocyanin concentration of 250 μg/mL.[44] The increase in these genes plays a role in restoring the function of pancreatic β cells through inhibiting oxidative stress in the endoplasmic reticulum, protecting the structure of pancreatic euchromatin, and regulating signaling pathways related to regeneration and maturation of pancreatic β cells that have been damaged.[38,45] Further research is needed to improve this research, especially in evaluating the long-term impact of administering *Clitoria ternatea* extract and its benefits on diabetes mellitus complications.

CONCLUSION

This study suggests that butterfly pea flower extract can reduce blood glucose and repair damage to pancreatic β cells by increasing cell viability and reducing body weight. The efficacy of *Clitoria ternatea* aqueous extract was similar to metformin related to blood glucose level, pancreatic protection, and body weight reduction in mice with hyperglycemia.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of the results of this research.

FUNDING

In preparing and conducting the research, the researcher used independent funds

ETHICS

This study was declared ethically feasible before the research was carried out by the Ethics Commission of the Faculty of Medicine, Udayana University with ethical number: 212/UN14.2.2.VII/LT/2023.

AUTHORS CONTRIBUTION

In the process of conducting and preparing the article, all authors were actively involved.

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