

Results From An Obesity Model In White Male Wistar Rats After Administration Of Telang Flower (*Clitoria Ternatea*) Extract On Pancreatic Function And Histopathological Characteristics

Weni Yulisandra Rini¹, Fahrul Azmi Tanjung^{2*}, Wilvia³

^{1,2,3}Master of Biomedical Science Study Program Faculty of Medicine, Dentistry, and Health Sciences, Prima Indonesia University, Medan

* Corresponding Author:

Email: fahruzmitanjung@unprimdn.ac.id

Abstract.

Diabetes mellitus (DM) is a chronic disease affecting 95% of diabetics, characterized by high blood glucose levels due to decreased insulin production. Bioactive chemicals from plants like butterfly pea flower and Clitoria Ternatea can potentially treat DM, inhibit carbohydrate-digesting enzymes, and potentially regenerate pancreatic beta cells. This study tests butterfly pea flower extract on obesity-related pancreatic function in white rats. In obese rats given varied dosages of butterfly pea flower extract, the study evaluates pancreatic tissue histopathology and ethanol extract properties. The study examines male Wistar rats' acclimatization before treatment with butterfly pea flower extract, a high-fat diet, and butterfly pea flower extract, comparing the effects of the extract on rats. Pancreatic function is examined using enzymes amylase and lipase. Histopathological observation of pancreatic organs involves several steps, including fixation, washing, infiltration, embedding, cutting, attaching, deparaffinization, dealcoholization, staining, mounting, and scoring. A post-test-only control group design compared butterfly pea flower extract and pancreatic function in male white rats. Butterfly pea flower extract improved pancreatic histology in obese white rats at 600mg/KgWB, closer to the normal group. The extract contains secondary metabolites that repair cell tissue damaged by the rats' high-fat diet and obesity.

Keywords: *Butterfly pea Flower Extract, Obesity, and Diabetes Mellitus.*

I. INTRODUCTION

Persistent hyperglycemia due to insulin production, insulin action, or both characterizes diabetes mellitus (DM), a chronic disease of carbohydrate, protein, and lipid metabolism [1]. The pancreas secretes insulin into the circulation in response to elevated blood glucose levels. When insulin levels are adequate and its function is unimpaired, the body will store or use the surplus glucose in the blood for metabolism [2]. A high prevalence of diabetes is observed in Indonesia. By 2022, the number of people diagnosed with type 1 diabetes in Indonesia will reach 41.8 thousand [3]. Diabetes mellitus symptoms are not always severe. It takes a few years for the first signs of the illness to appear [4]. Although the symptoms are comparable to type 1 diabetes, they are not always easy to spot [5]. Consequently, the condition can be recognized even after problems have developed, which can be several years after it starts. We call it diabetes mellitus, affecting almost 95% of people with diabetes. Former names for diabetes mellitus include adult onset and non-insulin dependent [6]. This kind of diabetes was formerly exclusively found in adults, but it is now being observed more and more in youngsters as well [7]. The metabolic pathways that the body uses to generate energy are impacted by diabetes mellitus. Without timely treatment, the condition might prevent the body from effectively using insulin, perhaps resulting in elevated blood sugar levels [8]. Nerves and blood arteries are particularly vulnerable to the long-term effects of type 2 diabetes. Obesity, heredity, and insufficient physical activity are risk factors for developing type 2 diabetes [9].

An unhealthy amount of fat can build up to dangerous levels, a condition known as obesity [10]. Obesity begins with an imbalance between caloric intake and expenditure. Diabetes mellitus risk factors include obesity [11]. High blood glucose levels, resulting from decreased insulin production from pancreatic β -cells, are the hallmarks of diabetes mellitus (DM), a chronic metabolic illness [12]. The induction of toxic substances such as streptozotocin and alloxan, as well as viral infections and genetic abnormalities, can disrupt pancreatic β -cell function [13]. When administered to rats used in experiments, the chemical alloxan causes the rats to develop diabetes due to its harmful effects on pancreatic beta cells and other diabetogenic

features [14]. The destructive activity of alloxan on pancreatic beta cells occurs when the compound enters and is absorbed by these cells. The toxicity and diabetogenic effects of alloxan are determined by its capacity to be soaked by pancreatic beta cells. Multiple mechanisms, including the simultaneous oxidation of sulfhydryl groups and the generation of free radicals, harm pancreatic beta cells after substance absorption [15]. Alterations to the pancreatic histology are a common pathological finding in both human and animal models of diabetes mellitus. The pancreas is an integral part of diabetes management [16]. Diabetes is a metabolic condition that develops when the pancreas either does not produce enough insulin or does not respond appropriately to the insulin it makes. Alpha cell abnormalities in glucagon release are another feature of type 1 and type 2 diabetes [17].

The endocrine pancreas secretes two primary hormones, glucagon and insulin, which are essential for regulating the body's energy metabolism [18]. One of the most common metabolic diseases in the world, type 2 diabetes mellitus is characterized by insulin resistance in target organs and impaired insulin production from pancreatic beta cells. Obesity is a risk factor for type 2 diabetes mellitus. When people eat too many lipids, they gain weight, and that excess fat ends up in other organs like the liver and pancreas, where it causes damage and poisoning [19]. A delicate balancing act of insulin secretion and action maintains normal regulation of glucose levels. Insulin, for instance, lowers fatty acid release from adipose tissue while increasing glucose absorption in skeletal muscle and the liver. Nevertheless, insulin signaling in target tissues is suppressed by an aberrant reduction in insulin production. When insulin cannot reach its target tissues, the body raises blood glucose and fatty acid levels [20]. Glucose and heavy acid levels in the blood continue to rise, which worsens insulin resistance and secretion [19]. Medications that lower blood sugar levels are called antidiabetic medications. Current antidiabetic medications, however, are prohibitively costly, ineffectual, and fraught with dangerous side effects. Patients with diabetes who simultaneously have renal, hepatic, or cardiac failure should not use the commonly prescribed oral antidiabetic medications biguanides, sulfonylureas, or thiazolidinediones. Treatment options for Diabetes Mellitus should thus include using bioactive chemicals produced from plants as they are effective, readily available, safe, and very inexpensive [1].

The healing properties of plants have been known for a long time to alleviate human illness; now, millions of people all over the globe depend on these plants for their basic needs, including health, money, and quality of life. Evidence suggests a strong demand for studies investigating plant-derived chemicals with the potential to develop novel anti-diabetic medications since 800 plants have been shown to have such action. The butterfly pea flower is one of several plants with potent antidyslipidemic and antidiabetic properties demonstrated in human clinical trials (*Clitoria Ternatea*). The butterfly pea flower is an example of an indigenous Indonesian plant with untapped commercial potential. Possibilities for study and development abound because of the extensive genetic variability of butterfly pea flowers based on physical traits. Another one of butterfly pea's numerous applications is as an attractive plant; it's also a natural dye, culinary colorant, and cancer preventive owing to its high antioxidant content. Using natural, nutrient-rich substances, such as anthocyanins, to boost endurance and prevent the spread of the COVID-19 pandemic is strongly suggested [11]. People utilize the butterfly pea flower to lower blood sugar levels; it is a plant native to China, India, the Philippines, and Madagascar (*Clitoria Ternatea*). Butterfly peas, often known as butterfly pea flowers, are natural plants that belong to the Fabaceae family [21]. Plant compounds discovered in butterfly pea flowers include proteins, alkaloids, anthraquinones, anthocyanins, 4-ena-3,6 Dion stigma, steroids, saponins, polysaccharides, and flobatins.

The composition of the fatty acids contains linoleic, palmitic, stearic, and linoleic oleic acids. Some antioxidants found in butterfly pea flowers have been studied for their ability to reduce blood sugar levels. These compounds include phenolics, flavonoids, anthocyanins, flavonol glycosides, kaempferol glycosides, quercetin glycosides, myricetin glycosides, terpenoids, tannins, and steroids [22]. In comparison to plants like *Centella Asiatica* (3,816 mg/mL), pearl grass (2,686 mg/mL), and hibiscus (*Hibiscus tiliaceus*), butterfly pea flower (*Clitoria Ternatea*) has a relatively high flavonoid concentration (1,425 mg/mL). The antioxidant values of butterfly pea flowers are $62.77 \mu\text{g/mL} \pm 0.21$, including 4.865 g QE/100 g extract of total flavonoids and 2.133 g GAE/100 g extract of total phenols [23]. Butterfly pea flowers (*Clitoria Ternatea*)

contain flavonoids that can protect pancreatic beta cells from injury. It has antioxidants that neutralize or absorb free radicals linked to phenolic OH groups. Consequently, disorders involving damaged tissue can be helped by flavonoid chemicals. *Clitoria Ternatea* has pharmacological qualities that have been documented in earlier research [24].

These include antioxidant, anti-platelet aggregation, vasodilation, and antidiabetic effects. A recent investigation indicated that vitro bay flower extract inhibits carbohydrate-digesting enzymes such as pancreatic α -amylase and intestine α -glucosidase [25]. The capacity to inhibit pancreatic α -amylase and antioxidant activity during simulated gastrointestinal digestion were both improved by microencapsulation of *Clitoria ternatea* flower extract, which improved bioaccessibility [26]. Previous studies have investigated the effects of an ethanol extract from *Clitoria Ternatea* on pancreatic regeneration. Injecting streptozotocin into diabetic rats allowed researchers to study the drug's antihyperlipidemic and antidiabetic properties and its correlation with antioxidant activity in living organisms and laboratory settings. Researchers found that pancreatic beta cells injured by butterfly pea flower extract might be regenerated [22]. In light of the preceding, scientists are keen to examine the effects of administering butterfly pea flower extract (*Clitoria Ternatea*) to an obese model of male Wistar strain white rats on pancreatic function and histological images of the pancreas. This study warrants more funding because of the widespread belief that plant-based treatments are safer and more cost-effective than pharmaceutical alternatives.

II. METHODS

The research design utilized in this study is a post-test-only control group design, which observes the control and treatment groups after an activity has been administered. It is a true experimental study [27]. This study was conducted in the University of North Sumatra's Anatomical Pathology Laboratory and the Department of Pharmacology Pharmacy at the University of North Sumatra Hospital. The physiology and appearance of rats (*Rattus norvegicus*) make them a popular biomedical study model [28]. Labs are also ideal for white rats. Variables vary among the things being studied and have measurable and observable attributes [29]. Flower extract administration in Butterfly pea flower is done independently (*Clitoria Ternatea*). They were limiting factors in Histology and function of the pancreas in obese female Wistar rats (*Rattus norvegicus*) in white rats. The experiment necessitated a seven-day acclimatization period before treatment for the male Wistar rats. The butterfly pea flower extract was made by crushing and sieving the mangrove components found in the butterfly pea flower. To get a thick extract, it was filtered and then evaporated. Additionally, male Wistar strain white overweight rats had a phytochemical screening to find substances that affected the pancreas. Before receiving bay flower extract treatment, the rats were subjected to a 14-day high-fat, high-cholesterol diet. The Lee Index assessed obesity. For fourteen days, four groups of rats were subjected to varying treatments. The exocrine pancreatic enzymes amylase and lipase were utilized to measure pancreatic function. Amylase and lipase levels were used to evaluate impairment to pancreatic function.

Fixation, washing, cleaning, infiltration, embedding, cutting, attaching, deparaffinization, dealcoholization, staining, and mounting are all steps in the histopathological examination of pancreatic organs. The pancreas is fixed with a solution of 0.9% sodium chloride and 70% alcohol after dissecting and dislocating it. After an hour of xylol immersion, pure paraffin I, II, and III are injected into the pancreas. The ribboned pancreas is housed in a rectangular box. The paraffin should be deparaffinized and dealcoholized in a wooden container. The pancreas is mounted following staining with Hematoxylin and Eosin. A score of 0 indicates no necrosis, a score of 1 indicates half total, a score of 3 indicates three-quarters total and a score of 4 indicates complete cell necrosis; this grading system is used to quantify pancreatic damage. Keep doing this until the pancreas becomes bright enough to be seen under a 400x microscope. Data entry, cleansing, verification, coding, and storage are all parts of the data processing. Using SPSS 25.0, data is gathered, scored, tabulated, and analyzed from histopathological observations [30]. We utilize the Kolmogorov-Smirnov test to ensure that our data is usually distributed, and we use the One-Way ANOVA method to look for statistically significant differences between our test groups. For additional analysis, Post Hoc Tests using the LSD approach are also utilized.

III. RESULTS AND DISCUSSION

Research Result

Table 1. Characteristics of Test Animals

Component	Group			
	Control	P1	P2	P3
Rat Type	Wistar strain white rats			
Gender	Male			
General Conditions	White coat color, healthy and active			
Average Initial Body Weight	243gr	240gr	251gr	258gr
Average Final Weight	250gr	333gr	349gr	351gr

The study involved feeding rats a high-fat diet to induce obesity. The fat-restricted group was fasted for a week before testing. The high-fat diet, which contains quail egg yolk, artificially raises cholesterol levels in rats. Weights were measured before and after the diet, and after 14 days, the rats' Lee index values changed. The results suggest that rats fed a high-fat diet end up overweight. The test animals in the treatment group were already overweight before administering butterfly pea flower extract. The study suggests that a diet rich in fat can cause obesity.

Table 2. Mouse Body Weight

Parameters	Group	Average a high-fat diet	
		Before	After
Body Weight (gr)	Control	243gr	250gr
	P1	240gr	333gr
	P2	251gr	349gr
	P3	258gr	351gr
Naso-anal Length (mm)	Control	210mm	213mm
	P1	215mm	221mm
	P2	212mm	218mm
	P3	216mm	213mm
Index lee	Control	0.29	0.29
	P1	0.28	0.31
	P2	0.29	0.32
	P3	0.29	0.32

Lipase levels were monitored before and after a high-fat meal and butterfly pea flower extract administration. The control group's average lipase level was 22.38 U/L, which increased to 22.61 U/L after 14 days of treatment. Treatment groups 1 and 2 showed varying results in lipase levels. Treatment group 1 had 42.5 U/L after a high-fat meal but dropped to 31.98 U/L after butterfly pea flower extract administration. Treatment group 2 had 41.78 U/L after a high-fat meal, and treatment group 3 decreased from 42.31 U/L to 22.65 U/L after 600 mg/KgWB. The third treatment group, consisting of obese rats, had the most significant reduction in lipase levels.

Table 3. Lipase Level

No	Group	Repetition	Lipase levels after high-fat diet (U/L)	Lipase Level After Treatment (U/L)
1	Control	1	22.5	21.9
2		2	23.1	23.8
3		3	20.9	22.3
4		4	21.7	22.9
5		5	23.4	23.1
6		6	22.7	21.7
Average			22.38	22.61
7	Treatment I (200mg/KgWB)	1	41.2	31.5
8		2	44.8	33.2
9		3	43.2	32.3
10		4	40.8	30.9
11		5	41.8	31.1
12		6	43.2	32.9
Average			42.5	31.98
13	Treatment II (400mg/KgWB)	1	42.9	24.4
14		2	41.2	23.1

15		3	43.9	25.1
16		4	40.2	23.5
17		5	41.5	22.7
18		6	40.9	24.9
Average			41.78	23.95
19	Treatment III (600mg/KgWB)	1	42.8	24.4
20		2	43.1	22.9
21		3	41.9	22.4
22		4	40.7	21.7
23		5	41.8	23.2
24		6	43.5	21.3
Average			42.31	22.65

The study examined changes in amylase levels in rats following a high-fat diet and butterfly pea flower extract administration. Results showed that the treatment group demonstrated significant changes. The control group had an average amylase level of 60.3U/L before treatment and 59.48U/L after 14 days of distilled water. Treatment groups 1 and 2 showed varying amylase levels after a high-fat diet and butterfly pea flower extract. Treatment group 3 had the most significant decrease in serum amylase levels and was close to the control group. Treatment group 1 had the most minor decrease or improvement in lipase levels. The study highlights the importance of considering diet and butterfly pea flower extract in dietary management.

Table 4. Average Amylase Level (U/L)

No	Group	Repetition	Amylase levels after high-fat diet (U/L)	Amylase Level after Treatment (U/L)
1	Control	1	60.1	58.6
2		2	59.8	58.3
3		3	59.2	59.8
4		4	60.6	59.1
5		5	61.5	60.4
6		6	60.6	59.3
Average			60.3	59.25
7	Treatment I	1	68.9	66.7
8		2	67.8	68.2
9		3	68.8	67.1
10		4	69.4	68.8
11		5	69.6	66.1
12		6	68.1	67.4
Average			68.76	67.38
13	Treatment II	1	68.3	60.1
14		2	69.7	61.2
15		3	66.2	60.6
16		4	67.6	59.1
17		5	69.3	62.1
18		6	66.3	59.7
Average			67.9	60.46
19	Treatment III	1	69.2	60.3
20		2	68.7	57.7
21		3	69.1	59.9
22		4	67.3	58.7
23		5	67.4	60.7
24		6	69.5	59.6
Average			68.53	59.48

Researchers also conducted a phytochemical test on butterfly pea flower extract (*Clitoria Ternatea*) to see the content of secondary metabolite compounds in the extract, which can be used to improve pancreatic function in obese white rats (*Rattus norvegicus*) Wistar strain. The phytochemical analysis of butterfly pea flower extract (*Clitoria Ternatea*) reveals the presence of secondary metabolites such as tannins, triterpenoids, flavonoids, and saponins. The flavonoid test produces a crimson extract, while the saponin test produces a foam that does not dissolve when HCl 2 N is applied. The tannin test results show a blue-green or blue-black color, while the alkaloid test results in a white or yellow precipitate. The steroid test

results in a reddish or yellowish tint, and the steroid/triterpenoids test results in a red color. The phytochemical analysis of butterfly pea flower extract confirms previous findings [31], indicating that the extract contains tannin, saponin, and flavonoid components. The study's findings confirm the presence of these secondary metabolites [31]. The findings of the phytochemical analysis of butterfly pea flower extract confirm the presence of tannin, saponin, and flavonoid components.

Table 5. Phytochemical Test

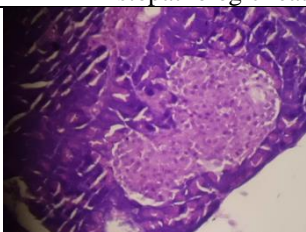
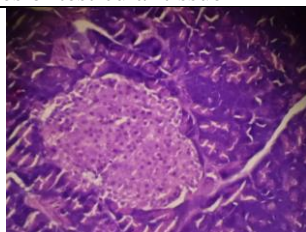
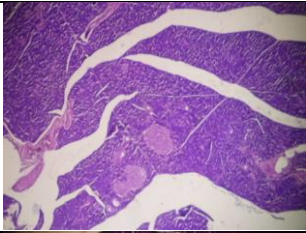
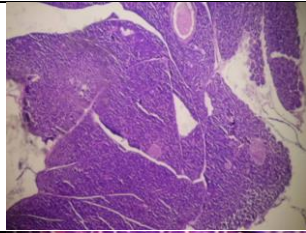
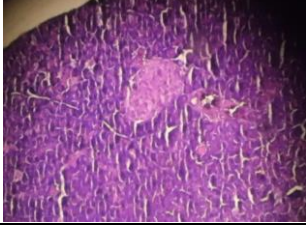
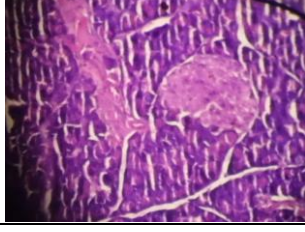
Secondary Metabolites	Testing	Color	Results
Flavonoid	Wilstater	Red	+
Saponin	Forth	Blue and effervescent	+
Tannin	FeCl ₃	Blackish green	+
Alkaloid	Wagner	Red	-
Triterpenoid	Lieberman – Burchard	Red	+

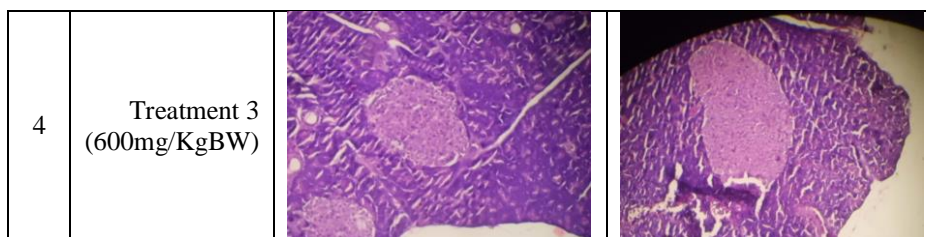
(+) = Contains the tested compound class

(-) = Does not contain the tested compound

The phytochemical investigation on butterfly pea flower extract (*Clitoria Ternatea*) aims to identify secondary metabolites such as flavonoids, tannins, saponins, flavonoids, and steroids/triterpenoids. The flavonoid test involves adding one gram of butterfly pea flower extract to a water bath and cooking in a water bath for fifteen minutes. A reddish-yellow hue indicates the presence of flavonoids. The saponin test involves adding 1 gram of butterfly pea flower extract to boiling water and stirring vigorously for 10 seconds. A foam is created at a height of 1-10 cm for a minimum of 10 minutes and does not dissolve when one drop of HCl 2 N is administered. The tannin test involves mixing 1 gram of butterfly pea flower extract with hot water and adding FeCl₃. A blue-green or blue-black hue shows a positive result for catechol tannin, while a blue-black color indicates a positive outcome for tannin. The alkaloid test produces a white or yellow residue, suggesting the presence of alkaloids. The steroid test results show a reddish or yellowish tinge, indicating the presence of terpenoids. The extract from butterfly pea flowers included tannin, saponin, and flavonoids [31].

Table 6. Histopathologic Features Of Pancreatic Tissue

No	Group	Histopathologic features of testicular tissue	
1	Control		
2	Treatment 1 (200mg/KgBW)		
3	Treatment 2 (400mg/KgBW)		



Data Analysis Results

Results of Serum Lipase Level Observation

Table 7. Normality Test Results

Group		Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Results	Control	.155	6	.200*	.956	6	.786
	P1	.193	6	.200*	.916	6	.478
	P2	.175	6	.200*	.921	6	.514
	P3	.144	6	.200*	.972	6	.908

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

The normalcy test is used to determine if data follows a normal distribution, with the Kolmogorov-Smirnov test being employed in this research. A normal distribution represents the population, and a p-value greater than 0.05 indicates a normal distribution. A significance level of 0.200 was found for normalcy, indicating a regularly distributed data set. The Levene test is then used to assess homogeneity, the degree to which different population subsets are similar. The Levene test was used to check for group homogeneity using a 5% significance threshold. A value less than 0.05 indicates non-homogeneous data, while a value over 0.05 indicates homogeneous data. The table shows a probability value of 0.827, meaning that all three groups are likely from similar or homogenous populations.

Table 8. Homogeneity Test Results

		Levene Statistic	df1	df2	Sig.
Results	Based on Mean	.297	3	20	.827
	Based on Median	.294	3	20	.829
	Based on the Median and with adjusted df	.294	3	14.925	.829
	Based on trimmed mean	.297	3	20	.827

We utilize the One-way ANOVA test to see if the trial groups' efficacy differs statistically after ensuring that the data is normal and homogeneous and that the outcomes follow a normal distribution with homogenous variances. In the table above, the One-Way ANOVA test had a significance value of 0.000, below 0.05. These data suggest that the treatment group varies considerably from the control group. A post hoc LSD test examined group lipase variation. The two groups differ statistically if the significance value is less than 0.05.

Table 9. One-Way ANOVA Test Results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	364.273	3	121.424	128.446	.000
Within Groups	18.907	20	.945		
Total	383.180	23			

The LSD Post Hoc test was used to determine whether groups had significant differences from other groups. The analysis showed an essential difference between the control and treatment groups 1 (p = 0.000) and 2 (p = 0.028). The control and treatment groups 3 had no significant difference (p = 0.953).

Table 10. LSD Post-hoc Test Results

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	P1	-9.36667*	.56135	.000	-10.5376	-8.1957
	P2	-1.33333*	.56135	.028	-2.5043	-.1624
	P3	-.03333	.56135	.953	-1.2043	1.1376
P1	Control	9.36667*	.56135	.000	8.1957	10.5376
	P2	8.03333*	.56135	.000	6.8624	9.2043

	P3	9.33333*	.56135	.000	8.1624	10.5043
P2	Control	1.33333*	.56135	.028	.1624	2.5043
	P1	-8.03333*	.56135	.000	-9.2043	-6.8624
	P3	1.30000*	.56135	.031	.1290	2.4710
P3	Control	.03333	.56135	.953	-1.1376	1.2043
	P1	-9.33333*	.56135	.000	-10.5043	-8.1624
	P2	-1.30000*	.56135	.031	-2.4710	-.1290

*. The mean difference is significant at the 0.05 level.

Serum Amylase Level Observation Results

The research used the Kolmogorov-Smirnov test to check for normal distribution, ensuring the data is representative of the population. A p-value more significant than 0.05 indicates normal distribution, while a p-value less than 0.05 indicates non-normal distribution. The significance level was 0.200, indicating normal distribution. The Levene test was used to check for homogeneity.

Table 11. Normality Test Results

Group		Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Results	Control	.141	6	.200*	.977	6	.935
	P1	.160	6	.200*	.979	6	.948
	P2	.133	6	.200*	.986	6	.977
	P3	.209	6	.200*	.943	6	.687

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

The Levene test was used with a significance level of 5% to ensure that the groups were similar. Conventional wisdom is that a significance value of less than 0.05 indicates that the data is not homogeneous, while more than 0.05 suggests that the data is homogeneous. You may view the results of the Levene test for homogeneity in the table shown above. A probability of 0.814 is displayed in the significance column. Because the calculated significant probability value is more than 0.05, we may deduce that the control group and treatment groups 1, 2, and 3 are from distinct populations.

Table 12. Homogeneity Test Results

		Levene Statistic	df1	df2	Sig.
Results	Based on Mean	.315	3	20	.814
	Based on Median	.229	3	20	.875
	Based on the Median and with adjusted df	.229	3	17.989	.875
	Based on trimmed mean	.295	3	20	.828

Tests for normality and homogeneity show that the study data follows a normal distribution with consistent variances. A post hoc LSD test examined average amylase levels, and a One-way ANOVA test verified that the treatment and control groups were significantly different.

Table 13. One-Way ANOVA Test Results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	268.355	3	89.452	90.424	.000
Within Groups	19.785	20	.989		
Total	288.140	23			

To find out if there were any significant variations in amylase between the groups, the Post Hoc LSD test was utilized. Analysis revealed a statistically significant difference (p = 0.000) between the control and treatment groups. The p-value for the control group and the second treatment group is 0.047. A statistically significant difference (p = 0.689) exists between the control and treatment groups 3.

Table 14. LSD Post-hoc Test Results

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	P1	-8.13333*	.57424	.000	-9.3312	-6.9355
	P2	-1.21667*	.57424	.047	-2.4145	-.0188
	P3	-.23333	.57424	.689	-1.4312	.9645
P1	Control	8.13333*	.57424	.000	6.9355	9.3312
	P2	6.91667*	.57424	.000	5.7188	8.1145
	P3	7.90000*	.57424	.000	6.7022	9.0978

P2	Control	1.21667*	.57424	.047	.0188	2.4145
	P1	-6.91667*	.57424	.000	-8.1145	-5.7188
	P3	.98333	.57424	.102	-.2145	2.1812
P3	Control	.23333	.57424	.689	-.9645	1.4312
	P1	-7.90000*	.57424	.000	-9.0978	-6.7022
	P2	-.98333	.57424	.102	-2.1812	.2145

*. The mean difference is significant at the 0.05 level.

Research Discussion

A study on male Wistar rats used butterfly pea flower extract to investigate its effects on pancreatic function. The rats were divided into four groups: a control group, an experimental group with a high-fat diet, and a control group with varying butterfly pea flower extract dosages. The study aims to determine if butterfly pea flower extract can improve pancreatic function in overweight rats. The study involved animals fed a high-fat diet, primarily quail egg yolk, for 14 days. The mice's body weight and nasal length were recalculated to determine their obesity. After the high-fat meal, rats in treatment groups 1 and 2 had Lee index values of 0.31 and 0.32, respectively, indicating they may be classified as obese. After the diet, rats were treated with butterfly pea flower extract at different dosages. Data analysis was conducted using tests for normality, homogeneity, and significance. The data passed the normalcy test, and the results showed a normal distribution of blood lipase and amylase levels in all groups. This data can be used to conclude the population as a whole, as it follows a normal distribution. The Levene test was used to determine if serum lipase and amylase levels were from similar or homogeneous populations. The results showed a significance value of 0.827 for lipase levels and 0.814 for amylase levels, indicating that the data from the control, treatment 1, and therapy three groups represent the same population. The One-Way ANOVA test was then used to assess efficacy and significance in the homogenous and normally distributed data. The study found significant differences in serum lipase and amylase levels between the control group and treatment groups 1, 2, and 3, necessitating additional post hoc LSD testing.

The control group had significantly higher lipase levels than the treatment groups, but no significant difference was found with treatment group 3. The study concluded that the lipase levels of treatment group 3, which received 600mg/KgWB of butterfly pea flower extract, were similar to those of the control group. However, there was a difference between the control group and groups 1 and 2, which were treated differently. There was also a significant difference in blood amylase levels between the control and treatment groups 1, with p-values of 0.047 and 0.689, respectively. No significant difference was found between treatment group 3's and control group's serum amylase levels. Histopathological images of pancreatic tissue supplemented microscopical examinations. Those in the control group had standard pancreatic histology images. The outcomes of the pancreatic histopathology observation in the control group serve as a benchmark against which the other groups are described and evaluated. Treatment group 1 got a high-fat diet in addition to 200mg/KgWB of butterfly pea flower extract (*Clitoria Ternatea*), which caused histological abnormalities in the pancreas organ. This led to form differences. Complete necrosis of the pancreatic cells was indicated by the maximum achievable score of 4 for all study subjects. Patients in the second treatment group who took 400 mg/kg of butterfly pea flower extract saw an improvement in pancreatic histological structure (*Clitoria Ternatea*). One patient had a score of 0, four subjects had a score of 4, and one subject had a score of 2 among this group. Group 3's histological pancreatic structure was identical to the control group's, with four subjects scoring 0 and two others scoring 1.

This group was given a high-fat diet in addition to 600mg/KgWB of butterfly pea flower extract (*Clitoria Ternatea*). The study found that obese male Wistar rats (*Rattus norvegicus*) showed improved pancreatic histological structure after receiving 600 mg/kg of butterfly pea flower extract. The first treatment group altered the pancreatic structure, which obtained a high-fat meal and 200mg/KgWB of butterfly pea flower extract. The second treatment group, which received 400 mg/kg of butterfly pea flower extract, showed pancreatic histology similar to that of the control group. The compound content of butterfly pea flower extract was detachable from improving pancreatic organ structure. The phytochemical studies have shown that the butterfly pea flower extract (*Clitoria Ternatea*) includes secondary metabolites such as tannins, triterpenoids, flavonoids, and saponins [31]. Research testing revealed that butterfly pea flower

extract contained tannin, saponin, and flavonoids. This study's results align with previous research conducted by [22]. The study examined the effect of butterfly pea flower ethanol extract on pancreatic regeneration and found that butterfly pea flower extract can help the regeneration process of damaged pancreatic beta cells.

IV. CONCLUSION

The study found that butterfly pea flower extract (*Clitoria Ternatea*) at 600mg/KgWB can improve pancreatic function in obese Wistar white rats. The treatment group 3 showed the most significant improvement, approaching the control group. The extract contains secondary metabolites like saponins, tannins, flavonoids, and triterpenoids that help repair pancreatic cells damaged by obesity. The results suggest that further research is needed to understand the effectiveness and toxicity of butterfly pea flower extract in obese conditions and to conduct other tests on its application to humans.

V. ACKNOWLEDGMENTS

The Department of Pharmacology Laboratory and the Anatomical Pathology Laboratory at the University of North Sumatra played a significant role in making this initiative a reality, and the authors are highly grateful for their help. Also, we have a favorable impression of the manager and the personnel. Because of their informative comments, we would like to express our most sincere gratitude to our study collaborators.

REFERENCES

- [1] Y. M. Belayneh, Z. Birhanu, E. M. Birru, and G. Getenet, "Evaluation of in vivo antidiabetic, antidyslipidemic, and in vitro antioxidant activities of hydromethanolic root extract of datura stramonium L. (Solanaceae)," *J. Exp. Pharmacol.*, vol. 11, pp. 29–38, 2019, doi: 10.2147/JEP.S192264.
- [2] H. Kolb, K. Kempf, M. Röhlhng, and S. Martin, "Insulin: Too much of a good thing is bad," *BMC Med.*, vol. 18, no. 1, pp. 1–12, Aug. 2020, doi: 10.1186/S12916-020-01688-6/TABLES/1.
- [3] D. J. Magliano, E. J. Boyko, and I. D. F. D. Atlas, "What is diabetes?," in *IDF DIABETES ATLAS [Internet]. 10th edition*, International Diabetes Federation, 2021.
- [4] H. N. Wilkinson and M. J. Hardman, "Wound healing: cellular mechanisms and pathological outcomes," *Open Biol.*, vol. 10, no. 9, Sep. 2020, doi: 10.1098/rsob.200223.
- [5] S. A. Paschou, N. Papadopoulou-Marketou, G. P. Chrousos, and C. Kanaka-Gantenbein, "On type 1 diabetes mellitus pathogenesis," *Endocr. Connect.*, vol. 7, no. 1, pp. R38–R46, Jan. 2018, doi: 10.1530/EC-17-0347.
- [6] A. P. Kengne, F. Turnbull, and S. MacMahon, "The Framingham Study, Diabetes Mellitus and Cardiovascular Disease: Turning Back the Clock," *Prog. Cardiovasc. Dis.*, vol. 53, no. 1, pp. 45–51, Jul. 2010.
- [7] G. Roglic, "WHO Global report on diabetes: A summary," *Int. J. Noncommunicable Dis.*, vol. 1, no. 1, p. 3, 2016, doi: 10.4103/2468-8827.184853.
- [8] D. K. Patel, R. Kumar, D. Laloo, and S. Hemalatha, "Diabetes mellitus: An overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity," *Asian Pac. J. Trop. Biomed.*, vol. 2, no. 5, pp. 411–420, 2012, doi: 10.1016/S2221-1691(12)60067-7.
- [9] WHO, "Obesity and overweight." Accessed: Jan. 13, 2024. [Online]. Available: <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight>
- [10] D. Kim, "Predictors of obesity," in *Obesity*, Elsevier, 2020, pp. 191–194. doi: 10.1016/B978-0-12-818839-2.00015-6.
- [11] M. Zakir *et al.*, "Cardiovascular Complications of Diabetes: From Microvascular to Macrovascular Pathways," *Cureus*, vol. 15, no. 9, 2023, doi: 10.7759/cureus.45835.
- [12] K. C. Agu, "Diabetes mellitus: A review of some of the prognostic markers of response to treatment and management," *J. Insul. Resist.*, vol. 3, no. 1, pp. 1–10, 2018.
- [13] A. Roosdiana, Sutrisno, C. Mahdi, and A. Safitri, "The Influence of Ethanolic Root Extracts of *Ruellia tuberosa* L. on Pancreatic Protease Activity and MDA Level of Rats (*Rattus norvegicus*) Induced by MLD-STZ," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 217, no. 1, p. 012041, Dec. 2018, doi: 10.1088/1755-1315/217/1/012041.
- [14] O. Lekan *et al.*, "A New model for Alloxan-induced diabetes mellitus in rats," *Artic. J. Bangladesh Soc. Physiol.*
- [15] O. M. Prameswari and S. B. Widjanarko, "Uji Efek Ekstrak Air Daun Pandan Wangi Terhadap Penurunan Kadar Glukosa Darah Dan Histopatologi Tikus Diabetes Mellitus [IN PRESS 2014]," *J. Pangan dan Agroindustri*, vol. 2, no. 2, pp. 16–27, 2014, Accessed: Jan. 24, 2024. [Online].

- [16] D. K. Andersen *et al.*, “Diabetes, Pancreatogenic Diabetes, and Pancreatic Cancer,” *Diabetes*, vol. 66, no. 5, pp. 1103–1110, May 2017, doi: 10.2337/DB16-1477.
- [17] P. Gilon, “The Role of α -Cells in Islet Function and Glucose Homeostasis in Health and Type 2 Diabetes,” *J. Mol. Biol.*, vol. 432, no. 5, pp. 1367–1394, Mar. 2020, doi: 10.1016/j.jmb.2020.01.004.
- [18] M. A. Atkinson, M. Campbell-Thompson, I. Kusmartseva, and K. H. Kaestner, “Organisation of the human pancreas in health and diabetes,” *Diabetologia*, vol. 63, no. 10, pp. 1966–1973, Oct. 2020, doi: 10.1007/S00125-020-05203-7/FIGURES/2.
- [19] H. Li *et al.*, “Phenolic-enriched blueberry-leaf extract attenuates glucose homeostasis, pancreatic β -cell function, and insulin sensitivity in high-fat diet-induced diabetic mice,” *Nutr. Res.*, vol. 73, pp. 83–96, Jan. 2020, doi: 10.1016/J.NUTRES.2019.09.005.
- [20] M. C. Petersen and G. I. Shulman, “Mechanisms of insulin action and insulin resistance,” *Physiol. Rev.*, vol. 98, no. 4, pp. 2133–2223, Oct. 2018.
- [21] G. K. Oguis, E. K. Gilding, M. A. Jackson, and D. J. Craik, “Butterfly pea (*Clitoria ternatea*), a cyclotide-bearing plant with applications in agriculture and medicine,” *Front. Plant Sci.*, vol. 10, p. 448370, May 2019.
- [22] V. Valentine, A. Agung, G. Budhiarta, and A. N. Susraini, “Administration of Butterfly Pea (*Clitoria ternatea*) Flower Etanol Extract Oral Increased the Number of Pancreatic Beta Cells and Decrease the Level of Glycated Hemoglobin (HbA1C) of Diabetic Male Wistar Rats (*Rattus norvegicus*),” *Int. J. Sci. Res.*, pp. 2319–7064, 2019, doi: 10.21275/SR21126085109.
- [23] S. Nuriyah Azizah, “Penetapan Kadar Flavonoid Total dan Fenol Total serta Aktivitas Antioksidan dari Ekstrak Bunga Telang (*Clitoria ternatea* L.),” 2021.
- [24] H. Hawari, B. Pujiasmanto, and E. Triharyanto, “Morfologi dan kandungan flavonoid total bunga telang di berbagai ketinggian tempat tumbuh berbeda,” *Kultivasi*, vol. 21, no. 1, pp. 88–96, Apr. 2022.
- [25] U. F. Shaik Mohamed Sayed *et al.*, “Natural products as novel anti-obesity agents: insights into mechanisms of action and potential for therapeutic management,” *Front. Pharmacol.*, vol. 14, Jun. 2023.
- [26] C. Chusak, C. J. Henry, P. Chantarasinlapin, V. Techasukthavorn, and S. Adisakwattana, “Influence of *Clitoria ternatea* Flower Extract on the In Vitro Enzymatic Digestibility of Starch and Its Application in Bread,” *Foods* 2018, Vol. 7, Page 102, vol. 7, no. 7, p. 102, Jul. 2018, doi: 10.3390/FOODS7070102.
- [27] S. Notoatmodjo, *Metodologi Penelitian Kesehatan*, 3rd ed. Jakarta: Rineka Cipta, 2022.
- [28] R. H. Weichbrod, G. A. (Heidbrink) Thompson, and J. N. Norton, *Management of Animal Care and Use Programs in Research, Education, and Testing*, 2nd ed. Boca Raton: CRC Press Taylor & Francis Group, 2018.
- [29] B. Suwarno and A. Nugroho, *Kumpulan Variabel-Variabel Penelitian Manajemen Pemasaran (Definisi & Artikel Publikasi)*, 1st ed. Bogor: Halaman Moeka Publishing, 2023.
- [30] I. Ghozali, *Aplikasi Analisis Multivariate dengan Program IBM SPSS 25*. Semarang, 2018.
- [31] L. Fikayuniar *et al.*, “Skrinning Fitokimia Serta Uji Karakteristik Simplisia dan Ekstrak Bunga Telang (*Clitoria ternatea* L.) Dengan Berbagai Metode,” *J. Ilm. Wahana Pendidik.*, vol. 9, no. 15, pp. 308–320, Aug. 2023, doi: 10.5281/ZENODO.8208374.