The Effect Of Giving Butterfly Pea Flower Extract (*Clitoria Ternatea*) On Reducing Cholesterol Levels And Histopathological Features Of White Rat Testes (*Rattus Norvegicus*) Wistar Male Obesity Model

Risnaldo Jaya¹, Andre Budi^{2*}, Bungaran Sihombing³

^{1,2,3} Master of Biomedical Science Study Program Faculty of Medicine, Dentistry, and Health Sciences, Universitas Prima Indonesia, Medan, Indonesia *Corresponding Author: Email: <u>andrebudi@unprimdn.ac.id</u>

Abstract.

Obesity is a global, non-communicable condition that causes male infertility. Obesity impacts spermatogenesis and male fertility. Butterfly pea blossoms (Clitoria ternatea) contain antioxidants that fight free radicals and prevent obesity. This research looked at how butterfly pea flower extract (Clitoria ternatea) affected cholesterol levels, testicular function, and the histopathology of the testicles of obese male Wistar rats (Rattus norvegicus). This research is quantitative and uses a lab or actual experiment. The 24 test mice were randomly divided into four groups. The research data was analyzed using SPSS 25.0 for Windows. The normality test significance is 0.577. One-way ANOVA test results demonstrate 0.000 or greater than 0.05 significance. LSD post-hoc test values. The control and treatment groups 1-3 differed significantly (p = 0.000). No change was seen between treatment groups 2 and 3 (p =0.088). The research found butterfly pea flower extract therapy (Clitoria ternatea) at 400mg and 600 mg/kgBW lowers cholesterol. Flavonoids, saponins, tannins, and triterpenoids reduce cholesterol and improve testicular function in obese white rats.

Keywords: Butterfly Pea Flower, Obesity, Cholesterol and Infertility.

I. INTRODUCTION

Proper bodily function requires a nutrient-dense diet. Eating well makes individuals happy because it improves their social health, boosting their performance at work and overall well-being. Excessive caloric intake or insufficient caloric expenditure causes the body to accumulate excess energy. Kids and teens nowadays are big eaters of fast food. Nowadays, most food products, such as speedy food, have increased calories, fat, sugar, salt, and other unhealthy additives. At the same time, just 63.3% of Indonesians eat the required amount of fruits and vegetables. A lack of proper nourishment, brought on by these factors, might lead to weight gain [1]. When caloric intake exceeds energy expenditure for essential metabolic functions and physical activity, a state known as obesity develops [2]. The prevalence of obesity, a chronic condition, is on the rise around the world. A pandemic has grown due to the alarming increase in prevalence across all age groups [3]."Energy imbalance," defined as "energy input exceeding expenditure," is the primary factor that leads to obesity. One of the main causes of weight gain is consuming more calories than one burns off. Nutritional quality can impact energy balance Through various factors, including the intricate hormonal and neurological networks that regulate fullness [4]. Fast food is heavy in calories but low in fiber and sugar, and the general tendency toward inactivity and sedentary lifestyles are the root causes of this disease. Obesity has several potential causes, including environmental factors, smoking, sleep difficulties, and medication intake. Furthermore, it is believed that there is a hereditary component to obesity [5]. Mental health, cancer, cardiovascular disease, and musculoskeletal diseases are just a few of the many adverse health outcomes associated with obesity.

The disease impacts nearly every physiological function of the body. The above factors negatively affect people, the economy, living standards, and productivity in the workplace [3]. Depression, low self-esteem, and workplace and personal discrimination are some of the psychological impacts that obese people face [6]. Male infertility can be influenced by obesity as well [5].Male infertility is frequently linked to obesity, a non-communicable condition that affects a large percentage of the global population. Male

reproductive potential is impacted by obesity because it impacts spermatogenesis [5]. Worldwide, infertility affects about 10% of families, and in underdeveloped nations, that number might be much higher [7].Research into the impact of lifestyle and environmental variables on male reproductive capacity has been prompted by reports of an increasing frequency of male infertility along with decreasing semen quality [8]. Poor sperm quality is linked to a high body mass index. Compared to males of average weight, those who are overweight or obese are more likely to have deficient sperm concentrations [7].Changes in concentration, motility, and shape of semen are connected with aberrant gonadal hormone levels, which are abnormal in obese people. At a body mass index (BMI) of 32–35 kg/m2, infertility becomes more common, according to studies. As a result of estrogen's adverse effects on spermatogenesis through its feedback mechanisms, elevated estrogen levels in obese people may decrease spermatogenesis [9].

With a BMI of 30 kg/m² or higher, infertility has been identified as a crucial factor. Researchers have found a link between body mass index and the ability to conceive. Alterations to the overall number of sperm, concentration of sperm, shape of sperm, and motility all exhibit the same association [10]. An overabundance of energy substrates supplied to metabolic pathways in adipose and non-adipose cells can lead to increased reactive oxygen species (ROS) generation in obese persons due to high circulating glucose and lipids [11]. Reactive oxygen species (ROS) with at least one oxygen atom can steal electrons from other molecules to become electrically stable. These species are short-lived, unstable, and highly reactive [12]. Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) and antioxidants. The common consensus is that oxidative stress is the root cause of several ailments [13]. Infertility in men is one of these. Free radical oxidative stress is the root cause of most reproductive health issues, particularly those affecting testicular function. Arterial blockage and significant damage to cells in the reproductive system, including spermatogenesis, can result from a potentially fatal free radical attack [14]. Because of the same levels of unsaturated fatty acids in all tissues and the extraordinarily high cell division rate and mitochondrial oxygen consumption in testicular tissue, oxidative stress plays a significant role in the onset of male infertility. Also, the testicular arteries aren't very strong, so there's not a lot of oxygen pressure, which means that cells are fighting for what little oxygen there is. The male reproductive system, including the testicles, is highly vulnerable to oxidative stress due to these circumstances [14].

Overproduction of reactive oxygen species (ROS) beyond the antioxidant capacity of cells is a common cause of oxidative stress, which a variety of internal and external stimuli may induce. Both direct and indirect effects of oxidative stress on the hypothalamic-pituitary-gonadal (HPG) axis and disruption of interaction with other hormonal axes can lead to infertility in men [8]. Environmental elements, including food, contaminants, and toxins, can impact this capability. Because of this, antioxidant defenses are not enough to protect cells from oxidative stress and all free radicals. Antioxidants and their therapeutic development can halt the oxidative chain reaction, improving spermatogenesis by strengthening the immune system's ability to remove free radical-induced oxidative stress [14]. To protect testicular cells from this harm, antioxidants neutralize free radicals or stop them from forming in the first place [14]. Superoxide dismutase, catalase, and peroxidase are enzymatic antioxidants, whereas vitamins, steroids, and other nonenzymatic antioxidants work against ROS overproduction[8]. Oxidative stress (OS) is a condition that cells and the body go through when there is an imbalance between reactive oxygen species (ROS) and antioxidants. Therefore, spermatozoa and other testicular cells are susceptible to DNA, RNA, and protein dysfunction due to lipid peroxidation induced by high ROS [15]. [16], foods such as fruits, vegetables, drinks, cereals, and plants include exogenous antioxidants like ascorbic acid (Vitamin C), α -tocopherol (Vitamin E), carotenoids, and polyphenols. The butterfly pea flower is an example of a plant that contains antioxidants (*Clitoria ternatea*) [17]. The Indonesian name for *Clitoria ternatea* is *Bunga Telang*. This plant belongs to the family Fabaceae. Many Asian nations have employed the deep blue blossoms of *Clitoria* ternatea as an herbal remedy for various ailments for millennia [18].

Indonesia is a paradise for *Clitoria ternatea* growers. Local farmers in Indonesia can easily plant and cultivate butterfly pea flowers. The morphological mechanism that allows the butterfly pea bloom to survive the dry season is connected. Traditional Chinese medicine uses butterfly pea flowers to treat various ailments, including skin disorders, ear infections, throat difficulties, and tumor prevention. Butterfly pea

flowers also contain phytosterols, triterpenoids, and anthocyanins, among other secondary metabolites [19]. The phytochemicals included in *Clitoria ternatea* affect many different types of cells. The pharmacological significance of *Clitoria ternatea* has been well-documented, with several research demonstrating its anti-inflammatory, anticancer, and antioxidant properties [20]. Antioxidants found in abundance in butterfly pea flower (*Clitoria ternatea*) help fight off harmful free radicals [21]. Free radicals are detrimental to the organism because they react to biomolecules and their relationship with several disorders, including polycystic ovarian syndrome, endometriosis, intrauterine growth restriction, and preeclampsia. Protecting against oxidative damage and these reproductive illnesses may be possible with the help of the butterfly pea flower (*Clitoria ternatea*), which is abundant in bioactive compounds and natural antioxidants [22]. Consequently, the purpose of this research is to examine the effects of the butterfly pea flower (*Clitoria ternatea*) on the histopathology of the testes in rats that are overweight and to find out whether it has any antioxidant effects on lowering cholesterol levels (*Rattus norvegicus*).

II. METHODS

True experiments or experimental designs in a controlled environment are the basis of this study's practical quantitative methodology. To conduct a genuine experiment, researchers must exclude outside influences that may skew the results. In this work, rats (*Rattus norvegicus*) male Wistar strains that were obese were examined using a pre-test-post-test control group design to find out how butterfly pea flower extract (*Clitoria ternatea*) affected cholesterol levels and the histological image of the testes [15]. This study utilized male Wistar rats (*Rattus norvegicus*) who were 2-3 months old, weighed 160-200 gr, and participated in the experiment [23]. Because their anatomy and physiology are very similar to humans and their status is a popular choice among biomedical researchers, Wistar male rats were selected as study test subjects. Six animals per group are the bare minimum for testing purposes. Each batch of experimental rats consisted of 24 Wistar rats.

The animals used in the experiments were divided into four groups at random. A variable is any observable quality that differs among the individuals or groups under study [24]. Administering butterfly pea flower extract to obese Wistar male rats reduces cholesterol levels and testicular histopathological pictures; this is the variable under investigation in this study (*Rattus norvegicus*). The administration of butterfly pea flower extract (*Clitoria ternatea*) is the independent variable, with the subsequent reduction in cholesterol levels and description of testicular histopathology as the dependent variable. Obesity is induced by a high-cholesterol diet, which also serves as the precondition variable. The study used various tools and equipment to analyze samples, including rat cages, evaporators, and test tubes. Different substances were also used. Statistical analysis was performed using SPSS 25.0, with Kolmogorov-Smirnov and One Way ANOVA used for significance analysis [25].

III. RESULTS AND DISCUSSION Research Result

This study tested 200-300gr white rats (*Rattus norvegicus*) male Wistar strain. The control group got simply regular food and drink. In contrast, the experimental group received either a high-fat diet or butterfly pea flower extract (*Clitoria ternatea*) at 200mg, 400mg, or 600mg/KgBW. This study employed 24 rats since the Ferderer formula was used to calculate the sample size for four groups with a maximum of six heads per group. According to animal characteristics, the rats in this study seemed healthy before and after therapy. The experiment continued with 24 test animals. Rats were fed a high-fat, cholesterol diet daily. Duck egg yolk was fed. This dish raises cholesterol. The butterfly pea flower (*Clitoria ternatea*) extract therapy began after 14 days of high-fat, high-cholesterol eating. Weight-wise, the mice were obese according to the Lee index.

			lillians	
Component	Group K	Group P1	Group P2	Group P3
Rat Type	White Rattus norvegicus wistar strain			
Gender		Μ	Iale	

Table 1. Characteristics of	of Test	Animals
-----------------------------	---------	---------

General Conditions	White coat color, healthy and active			
Average Initial Body Weight	238gr	235gr	244gr	242gr
Average Final Weight	240gr	325gr	334gr	333gr

Rat body weight and nasoanal length were measured before and after a high-fat diet induction. The Lee index value was calculated to determine obesity. The rats in the treatment group were obese before the diet, and the Lee index value increased after 14 days of duck egg yolk consumption. The administration of butterfly pea flower extract improved testicular function.

Demonstern	Comm	Average			
ParameterGroupBody Weight (gr)P1P2P3ControlP1P3ControlP1P2P3ControlP1P2P3Control	Group	Before a high-fat diet	After a high-fat diet		
	Control	238gr	240gr		
Body Weight (gr)	P1	235gr	325gr		
	P2	244gr	334gr		
	P3	242gr	333gr		
Naso-anal Length (mm)	Control	214mm	215mm		
	P1	216mm	221mm		
	P2	217mm	220mm		
	P3	216mm	217mm		
To be the	Control	0.28	0.28		
	P1	0.28	0.31		
muex lee	P2	0.28	0.31		
	P3	0.28	0.32		

 Table 2. Mouse Body Weight

The study examined the effects of a high-fat, high-cholesterol diet on white Wistar rats. The rats were given butterfly pea flower extract therapy for 14 days, which showed a decrease in cholesterol levels. The control group had an average cholesterol level of 39.18mg/dl, while treatment group 1 declined from 70.1mg/dl to 58.91mg/dl. The treatment group 2 also decreased from 70.68mg/dl to 46.56mg/dl, and the treatment group 3 experienced the most significant decrease from 70.48mg/dl to 40.83mg/dl. The results showed that the rats no longer experienced high cholesterol levels due to the butterfly pea flower extract.

Table 3. Total Cholesterol Level of Rats

No	Group	Repetition	Whole cholesterol level after high-fat diet (mg/dl)	Whole cholesterol level after administration of butterfly pea flower extract (mg/dl)
1		1	40.1	40.2
2		2	39.2	39.6
3	Control	3	40.2	40.8
4	Control	4	38.4	38.7
5		5	39.1	39.9
6		6	38.1	38.4
		Average	39.18	39.6
7		1	70.1	59.9
8		2	68.9	59.5
9	Treatment I	3	71.2	60.1
10		4	70.5	57.2
11		5	69.7	58.2
12		6	70.2	58.6
		Average	70.1	58.91
13		1	70.6	46.5
14		2	69.1	44.6
15	Traatmont II	3	71.5	47.4
16	Treatment II	4	71.9	47.1
17		5	70.2	45.7
18		6	70.8	48.1
		Average	70.68	46.56
19		1	70.2	40.4
20	Trastmant III	2	71.2	41.4
21	r reatment III	3	69.6	42.7
22		4	72.2	41.9

23	5	69.3	39.5
24	6	70.4	39.1
	Average	70.48	40.83

To improve testicular function in obese white rats (*Rattus norvegicus*) Wistar strain, researchers also performed a phytochemical test on butterfly pea flower extract (*Clitoria ternatea*) to determine the concentration of secondary metabolite chemicals in the extract.Phytochemical testing determines how many secondary metabolite chemicals are in butterfly pea flower extract (*Clitoria ternatea*) [18]. The following assays are available: steroid/triterpenoid, tannin, alkaloid, flavonoid, and saponin. The production of red or yellow color indicates flavonoid tests, whereas the formation of foam and froth indicates saponin testing [26]. Catechol tannin and blue-black liquid result from a tannin test, whereas white or yellow sediments result from an alkaloid test. Color creation can be observed in steroid and steroid/triterpenoid assays as red or yellow, respectively.These analyses help determine what chemicals make up the secondary metabolites in the butterfly pea flower extract. According to the data, are flavonoids, saponins, tannins, alkaloids, and triterpenoids all substances found in the extract?

 Table 4. Phytochemical Test

Secondary metabolite	Testing	Color	Results
Flavonoid	Wilstater	Red	+
Saponin	Forth	Blue and effervescent	+
Tannin	FeC13	Blackish green	+
Alkaloid	Wagner	Red	-
Triterpenoid	Lieberman – Burchard	Red	+

The phytochemical analysis of butterfly pea flower extract (*Clitoria ternatea*) revealed the presence of secondary metabolites, including tannins, triterpenoids, flavonoids, and saponins [26]. Researchers discovered tannin, saponin, and flavonoid components in butterfly pea flower extract. The testicular tissue specimens from the control and treatment groups were examined histopathologically. The treatment group received daily dosages of 200mg/KgBW, 400mg/KgBW, and 600mg/KgBW of butterfly pea flower extract. Routine testicular histopathology, a score of 10, was seen in the control group that consumed distilled water and conventional pellets. In contrast to the control group, which destroyed a low-fat diet and got butterfly pea flower extract, this served as a benchmark for the experimental group. This study examined the testicular structure with a high-fat diet and butterfly pea flower extract. The organization of spermatogenic cells was looser in the first group, scoring 3 out of 5. The second group's testicular structure improved, but their spermatozoa cell count remained below 10, falling into the 8th scoring category. Histological examination of the testicles in the third group was indistinguishable from that in the control group.

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

Table 5. Histopathologic Features of Testicular Tissue



Data Analysis Results

We need the data normality test to prove that data with a normal distribution is typical of the population. A p-value greater than 0.05 indicates regularly distributed data, whereas a smaller p-value indicates otherwise. We found periodically a significance level of 0.200 after confirming normality with the Kolmogorov-Smirnov test. A normal distribution is shown by a p-value over 0.05. This suggests a normal distribution of the data. Next, we may check for homogeneity by using the Levene test to examine each study population subgroup, assuming a normal distribution.

Crown		Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Oloup		df	Sig.	Statistic	df	Sig.
	Control	.172	6	.200*	.957	6	.798
Results	P1	.199	6	.200*	.935	6	.621
	P2	.164	6	$.200^{*}$.972	6	.906
	P3	.161	6	$.200^{*}$.955	6	.779

Table 6. Normality Test Results

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

a. Lilliefors Significance Correction

Table 7. Homogeneity Test Result	S
---	---

		Levene Statistic	df1	df2	Sig.
	Based on Mean	.676	3	20	.577
Results	Based on Median	.620	3	20	.610
	Based on the Median and with adjusted df	.620	3	18.274	.611
	Based on trimmed mean	.675	3	20	.577

We checked for group homogeneity using the Levene test at a 5% significance level. According to the rule of thumb for decision-making, data is considered homogenous if the significance value is more significant than 0.05 and non-homogeneous if the value is less than 0.05. The table above displays the results of the Levene test for homogeneity. There is a significance column value of 0.577 for the likelihood. The calculated significance probability value is more significant than 0.05, indicating that the control and treatment groups are drawn from similar and homogenous populations with the same variance.

	5				
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1403.385	3	467.795	330.889	.000
Within Groups	28.275	20	1.414		
Total	1431.660	23			

Table 8. One-Way ANOVA Test Results

The researchers do a one-way ANOVA test to examine if the trial groups' efficacy differs statistically after confirming that the data is normal and homogeneous and that the outcomes follow a normal distribution with homogenous variances. The table shows that the One-Way ANOVA test yielded 0.000, less than 0.05. These data suggest that the treatment group varies considerably from the control group. Researchers utilized post hoc LSD to compare groups' average total cholesterol levels.

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	P1	-19.31667*	.68648	.000	-20.7486	-17.8847
	P2	-6.96667*	.68648	.000	-8.3986	-5.5347
	P3	-1.23333	.68648	.088	-2.6653	.1986
P1	Control	19.31667*	.68648	.000	17.8847	20.7486
	P2	12.35000*	.68648	.000	10.9180	13.7820
	P3	18.08333*	.68648	.000	16.6514	19.5153
P2	Control	6.96667*	.68648	.000	5.5347	8.3986
	P1	-12.35000*	.68648	.000	-13.7820	-10.9180
	P3	5.73333*	.68648	.000	4.3014	7.1653
Р3	Control	1.23333	.68648	.088	1986	2.6653
	P1	-18.08333*	.68648	.000	-19.5153	-16.6514
	P2	-5.73333*	.68648	.000	-7.1653	-4.3014

 Table 9. LSD Post-hoc Test Results

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

Groups were compared for significant differences in wound healing using the LSD Post Hoc test. The analysis revealed a substantial difference between the control group and treatment groups 1, 2, and 3 (p = 0.000) and between the control group and group 4 (p = 0.000). There was no statistically significant difference between treatment groups 2 and 3 (p = 0.088).

Research Discussion

The study tested the effectiveness of butterfly pea flower extract in reducing cholesterol levels and improving testicular function in obese male Wistar rats. The rats were divided into control, treatment, and control. After a 14-day high-fat diet, the rats showed a 0.31 Lee index value in treatment groups 1, 2, and 3. When caloric intake exceeds energy expenditure for essential metabolic functions and physical activity, a condition known as obesity develops [2]. "Energy imbalance," defined as "energy input exceeding expenditure," is the primary factor that leads to obesity. One of the main causes of weight gain is when caloric intake is higher than energy expenditure [4].Significant health effects of obesity include mental health issues, cancer, cardiovascular illnesses, and musculoskeletal abnormalities, among almost all physiological processes of the body. The above negatively affects productivity, quality of life, the economy, and individuals [3]. An overabundance of energy substrates supplied to metabolic pathways in adipose and nonadipose cells can lead to increased reactive oxygen species (ROS) generation in obese persons due to high circulating glucose and lipids [11]. Oxidative stress results from an unbalanced ratio of reactive oxygen species (ROS) to antioxidants. It is widely believed that oxidative stress is the initial stage of several illnesses [13]. One of these conditions is infertility in men [21]. To protect testicular cells from this harm, antioxidants neutralize free radicals or stop them from forming in the first place [14]. Superoxide dismutase, catalase, and peroxidase are enzymatic antioxidants, whereas vitamins, steroids, and other non-enzymatic antioxidants work against ROS overproduction [27].

Butterfly pea flower (*Clitoria ternatea*) is one plant that has antioxidants [28], [29].Protect yourself from harmful free radicals with the help of the many natural antioxidants found in the butterfly pea flower (*Clitoria ternatea*). Antioxidants found in abundance in the butterfly pea flower (*Clitoria ternatea*) can neutralize harmful free radicals [21]. The reactivity of free radicals to biomolecules and their link to several disorders, such as polycystic ovarian syndrome, endometriosis, preeclampsia, and intrauterine growth restriction, make them detrimental to the organism. The butterfly pea flower, or *Clitoria ternatea*, can protect against oxidative damage and reproductive illnesses since it is rich in numerous bioactive compounds and natural antioxidants [22]. This finding motivated the current investigation into the potential of butterfly pea flower extract (*Clitoria ternatea*) as an all-natural remedy for obesity-related issues, namely lowering total cholesterol and enhancing testicular function[30]. Data analysis was required to examine and test the data generated by this 14-day observational study approach. The next step is to run a normal test on the processed data. The data used to conduct the normality test were acquired with the assistance of SPSS and the Kolmogorov-Smirnov test. With a significance level of 0.200 across the board, the data from each group followed a normal distribution. This data may be used to conclude the population since it follows a normal

distribution. The Levene test confirmed the homogeneity of the normally distributed data, indicating that the control group and treatment groups 1, 2, and 3 are all representatives of the same population, and the efficacy and significance were assessed using the One-Way ANOVA test. The ANOVA test results indicate a significant difference between the control, treatment 1, and treatment three groups, necessitating more post hoc LSD testing.

The analysis showed a significant difference between the control and treatment groups 1, 2, and 3 but no significant difference between treatment groups 2 and 3. Obese male white rats (*Rattus norvegicus*) in this study showed a reduction in total cholesterol levels across all groups. By comparing the various average numbers, we can observe that the decrease in total cholesterol levels varies. A drop from 70.1mg/dl to 58.91mg/dl was seen after treatment group 1 received 200mg/KgBW of butterfly pea flower extract (Clitoria *ternatea*). Although there has been a reduction, the cholesterol levels in the first therapy group are still considered excessive. In group 2, the butterfly pea flower extract (Clitoria ternatea) was administered at a dose of 400 mg/kg of body weight, which resulted in a decline from 70.68 mg/dl to 46.56 mg/dl. Group 3, which received a dose of 600 mg/dl, had the most significant decrease, going from 70.48 mg/dl to 40.83 mg/dl. Because their cholesterol levels dropped below 54 mg/dl, the group that received 400 mg/KgBW or 600 mg/KgBW of butterfly pea flower extract (Clitoria ternatea) no longer had elevated cholesterol, according to the data. Histopathological examinations revealed various cell appearances. The testicular histology of the control group, which was given standard pellets and distilled water, was expected, and they scored a 10, which means they had normal tubule epithelium, complete spermatogenesis, and at least ten spermatozoa cells. The average testicular histopathology in the control group served as a benchmark for describing the other groups and a comparison to the treatment group that received a high-fat diet in addition to butterfly pea flower extract. The control group did not undergo any dietary changes (*Clitoria ternatea*).

Because the organs had been exposed to the high-fat diet and obesity, there were changes in the form of the testicular structure in treatment group 1, which was administered a high-fat diet and butterfly pea flower extract (Clitoria ternatea) at a level of 200mg/KgBW. Group 1, which received 200 mg/kg of butterfly pea flower extract (Clitoria ternatea), had a score of 3 on the histology image because its spermatogenic cell arrangement was less organized than the control group's (Spermatogenic cells consist only of spermatogonium cells). The histological structure of the testes improved in treatment group 2, which received 400mg / KgBW of butterfly pea flower extract (*Clitoria ternatea*). However, the group still had spermatozoa cells below 10, placing them in scoring category 8. The third treatment group, which received a high-fat diet and 600 mg/kg of butterfly pea flower extract (Clitoria ternatea), had a test histological structure similar to the control group, earning them a score of 10. The content of butterfly pea flower extract is inseparable from its improvement of testicular function in obese rats.Phytochemical studies have shown that butterfly pea flower extract has triterpenoids, tannins, saponins, and flavonoids, among other phytochemical components [26]. Research testing revealed that butterfly pea flower extract included tannin, saponin, and flavonoids. The results showed that compared to the group that received merely distilled water, the one given butterfly pea flower extract (Clitoria ternatea) had a better testicular function and reduced cholesterol levels. [28], who also discovered that the butterfly pea flower extract (Clitoria ternatea) might lower cholesterol levels in the Wistar strain of white rats (Rattus norvegicus).

IV. CONCLUSION

Results from studies on obese Wistar white rats show that treatment with butterfly pea flower extract (*Clitoria ternatea*) at 400 mg/kgBW and 600 mg/kgBW successfully brings total cholesterol levels down to normal. The cholesterol levels in both groups are below 54 mg/dl; thus, it's clear. Treatment group 3, which included butterfly pea flower extract (*Clitoria ternatea*) at a dose of 600mg/KgBW, showed the most significant improvement and was close to the control group according to histological examinations of testicular tissue. The phytochemical testing of butterfly pea flower extract (*Clitoria ternatea*) revealed that it includes triterpenoids, tannins, saponins, and flavonoids, among other phytochemical components. The Wistar strain of obese white rats (*Rattus norvegicus*) shows improvements in testicular function and total cholesterol levels when given these chemicals.

V. ACKNOWLEDGMENTS

University of North Sumatra's Department of Pharmacology Laboratory and Anatomical Pathology Laboratory were instrumental in making this endeavor a reality, and the authors sincerely appreciate their support. In addition, we like the manager and the crew. Our deepest gratitude goes to our research collaborators for their insightful comments.

REFERENCES

- [1] T. Mahmudiono *et al.*, "The Effect of Online Order Development on Fast Food, Vegetable, and Fruit Consumption Behavior on Students in Surabaya," *Open Access Maced. J. Med. Sci.*, vol. 10, no. E, pp. 630– 637, Mar. 2022.
- [2] K. D. Hall and J. Guo, "Obesity Energetics: Body Weight Regulation and the Effects of Diet Composition," *Gastroenterology*, vol. 152, no. 7, pp. 1718-1727.e3, May 2017, doi: 10.1053/j.gastro.2017.01.052.
- [3] R. Mehrzad, "Etiology of obesity," in Obesity, Elsevier, 2020, pp. 43-54.
- [4] I. Romieu *et al.*, "Energy balance and obesity: what are the main drivers?" *Cancer Causes Control*, vol. 28, no. 3, pp. 247–258, Mar. 2017, doi: 10.1007/s10552-017-0869-z.
- [5] M. A. A. El Salam, "Obesity, An Enemy of Male Fertility: A Mini Review," *Oman Med. J.*, vol. 33, no. 1, pp. 3–6, Jan. 2018, doi: 10.5001/omj.2018.02.
- [6] F. Rubino *et al.*, "Joint international consensus statement for ending stigma of obesity," *Nat. Med.*, vol. 26, no. 4, pp. 485–497, Apr. 2020, doi: 10.1038/s41591-020-0803-x.
- [7] E.-Y. Wang, Y. Huang, Q.-Y. Du, G.-D. Yao, and Y.-P. Sun, "Body mass index effects sperm quality: a retrospective study in Northern China," *Asian J. Androl.*, vol. 19, no. 2, p. 234, 2017.
- [8] M. Darbandi *et al.*, "Reactive oxygen species and male reproductive hormones," *Reprod. Biol. Endocrinol.*, vol. 16, no. 1, p. 87, Dec. 2018, doi: 10.1186/s12958-018-0406-2.
- P. Sengupta, S. Dutta, and S. Muhamad, "Male reproductive hormones and semen quality," *Artic. Asian Pacific J. Reprod.*, 2019, doi: 10.4103/2305-0500.268132.
- [10] G. R. Chaudhuri *et al.*, "Obesity and male infertility: multifaceted reproductive disruption," *Middle East Fertil. Soc. J.*, vol. 27, no. 1, p. 8, Apr. 2022, doi: 10.1186/s43043-022-00099-2.
- [11] F. McMurray, D. A. Patten, and M. Harper, "Reactive Oxygen Species and Oxidative Stress in Obesity—Recent Findings and Empirical Approaches," *Obesity*, vol. 24, no. 11, pp. 2301–2310, Nov. 2016.
- [12] S. Bisht, M. Faiq, M. Tolahunase, and R. Dada, "Oxidative stress and male infertility," *Nat. Rev. Urol.*, vol. 14, no. 8, pp. 470–485, Aug. 2017, doi: 10.1038/nrurol.2017.69.
- [13] L. Giampietro, M. Gallorini, B. De Filippis, R. Amoroso, A. Cataldi, and V. di Giacomo, "PPAR-γ agonist GL516 reduces oxidative stress and apoptosis occurrence in a rat astrocyte cell line," *Neurochem. Int.*, vol. 126, pp. 239–245, Jun. 2019, doi: 10.1016/j.neuint.2019.03.021.
- [14] N. Asadi, "The Impact of Oxidative Stress on Testicular Function and the Role of Antioxidants in Improving it: A Review," J. Clin. DIAGNOSTIC Res., 2017, doi: 10.7860/JCDR/2017/23927.9886.
- [15] K. R. Feingold, "Lipid and Lipoprotein Metabolism," *Endocrinol. Metab. Clin. North Am.*, vol. 51, no. 3, pp. 437–458, Sep. 2022, doi: 10.1016/j.ecl.2022.02.008.
- [16] S. C. Lourenço, M. Moldão-Martins, and V. D. Alves, "Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications," *Molecules*, vol. 24, no. 22, p. 4132, Nov. 2019, doi: 10.3390/molecules24224132.
- [17] W. Suarna, I. Made, and S. Wijaya, "Butterfly Pea (*Clitoria ternatea* L.: Fabaceae) and Its Morphological Variations in Bali," 2021, doi: 10.22146/jtbb.63013.
- [18] A. Arsianti, S. Pasha Mahindra, N. Nur Azizah, A. Megawati Fajrin, and L. Dameria Nadapdap, "Phytochemical Analysis, Antioxidant and Anticancer Effects of Clitoria ternatae Extract on Breast T47D Cancer Cells," *Indones. J. Med. Chem. Bioinforma.*, vol. 1, no. 1, Aug. 2022, doi: 10.7454/ijmcb.v1i1.1003.
- [19] V. Aziza *et al.*, "Keragaman Fenotipik Bunga Buterfly pea Double Petal Asal Indonesia dan Thailand Berdasarkan Morfologi Bunga," *Al-Kauniyah J. Biol.*, vol. 14, no. 1, pp. 78–89, 2021.
- [20] A. E. Al-Snafi, "Pharmacological importance of *Clitoria ternatea* A review," *IOSR J. Pharm. www.iosrphr.org*, vol. 6, no. 3, pp. 68–83, 2016, Accessed: Jan. 15, 2024. [Online]. Available: www.iosrphr.org
- [21] W. Phrueksanan, S. Yibchok-anun, and S. Adisakwattana, "Protection of *Clitoria ternatea* flower petal extract against free radical-induced hemolysis and oxidative damage in canine erythrocytes," *Res. Vet. Sci.*, vol. 97, no. 2, pp. 357–363, Oct. 2014, doi: 10.1016/j.rvsc.2014.08.010.
- [22] S. E. GOH, P. J. KWONG, C. L. NG, W. J. NG, and K. Y. EE, "Antioxidant-rich *Clitoria ternatea* L. flower and its benefits in improving murine reproductive performance," *Food Sci. Technol.*, vol. 42, 2022.

- [23] S. Wolfensohn and M. Lloyd, *Handbook of laboratory animal management and welfare*. John Wiley & Sons, 2013.
- [24] B. Suwarno and A. Nugroho, *Kumpulan Variabel-Variabel Penelitian Manajemen Pemasaran (Definisi & Artikel Publikasi)*, 1st ed. Bogor: Halaman Moeka Publishing, 2023.
- [25] I. Ghozali, Aplikasi Analisis Multivariate dengan Program IBM SPSS 25. Semarang, 2018.
- [26] U. M. S. Purwanto, K. Aprilia, and Sulistiyani, "Antioxidant Activity of Butterfly pea (*Clitoria ternatea* L.) Extract in Inhibiting Lipid Peroxidation," *Curr. Biochem.*, vol. 9, no. 1, pp. 26–37, Jun. 2022.
- [27] T. Huff, B. Boyd, and I. Jialal, *Physiology, Cholesterol.* 2023.
- [28] A. M. Marpaung, "Tinjauan manfaat bunga buterfly pea (*Clitoria ternatea* l.) bagi kesehatan manusia," J. Funct. Food Nutraceutical, vol. 1, no. 2, pp. 63–85, Feb. 2020, doi: 10.33555/jffn.v1i2.30.
- [29] P. Chayaratanasin *et al.*, "Clitoria ternatea Flower Petal Extract Inhibits Adipogenesis and Lipid Accumulation in 3T3-L1 Preadipocytes by Downregulating Adipogenic Gene Expression," Molecules, vol. 24, no. 10, p. 1894, May 2019.
- [30] H. Hawari, B. Pujiasmanto, and E. Triharyanto, "Morfologi dan kandungan flavonoid total bunga buterfly pea (*Clitoria ternatea* L.) di berbagai ketinggian," *Kultivasi*, vol. 21, no. 1, Apr. 2022.