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Administrations of Butterfly Pea Flower *(Clitoria Ternatea L)* Extract Reduce Oxidative Stress and Increase Body Weight of Male Wistar Rats with Diabetes

Pemberian Ekstrak Bunga Telang (Clitoria Ternatea L) Menurunkan Stres Oksidatif dan Meningkatkan Berat Badan pada Tikus Wistar Jantan Diabetes

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ABSTRACT

Background: Asian pigeonwings flower (Clitoria Ternatea L.) is a plant that contains high antioxidants. Numerous research studies have shown that CT flowers can reduce the blood glucose levels of diabetic rats. Lower blood glucose levels can reduce MDA in DM patients.
Objectives: This study aimed to analyze the effect of CT on serum malondialdehyde (MDA) levels and body weight of diabetic rats.
Methods: Male albino Wistar rats induced by streptozotocin 45 mg/kgBW and nicotinamide 110 mg/kgBW to generate type 2 diabetes. Diabetes rats were randomly divided into three groups: T1 was the control of diabetic rats, T2 was given 300 mg/kgBW extract of CT, and T3 was given 600 mg/kgBW extract of CT for 21 days. Data collected before, during, and after treatment were analyzed using One Way ANOVA and LSD posthoc.
Results: The mean of MDA in the T2 and T3 groups decreased on day 14 that was T2 4,67±0,17 µmol/l and T3 3,99±0,30 µmol/l, (p<0,001) and on day 21 also decreased that was T2 4,07±0,14 µmol/l and T3 3,34 ±0,23 µmol/l (p<0,001). While T1 did not experience a significant decrease. The mean of body weight in the T2 and T3 groups increased on day 14 that was T2 187,83±4,67 grams and T3 183,50±4,41 grams (p<0,001), and on day 21, also increased was T2 195,17±3,65 grams, 190,67±4,08 grams (<0,001). In contrast, T1 did not experience a significant increase.
Conclusion: Administration of CT flower extract 300mg/KgBW, and CT flower extract 600mg/KgBW reduces serum MDA levels of diabetic rats compared to the control of diabetic rats.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is the most prevalent chronic disease with an increasing prevalence worldwide[1]. T2DM causes metabolic alterations, hyperglycemia, and high levels of oxidative stress. It performs a critical function in reducing diabetes effects in each endothelial and cardiovascular system[2].

Free radicals produced by automobile oxidation of glycation products, glycosylation, tissue alteration, and the loss of antioxidant defense structures are a few of the mechanisms concerned with the oxidative stress of diabetes sufferers[3]. ROS increases the lipid peroxidation product of malondialdehyde (MDA), which leads to a lower degree of endogenous antioxidants. In recent years, numerous research studies have shown that antioxidants exert practical consequences in the organism and may help prevent and treat several metabolic diseases, including diabetes[4]. Several studies have shown that it consequently increases blood glucose levels because of delayed glucose absorption, then people with diabetes will experience weight loss because there is a problem with insulin as a glucose absorption receptor. If the body cannot obtain sufficient calories from glucose, the other substances, such as protein and fat, will be converted into energy, resulting in a weight decrease[5].

There are studies that provide foods that contain high antioxidants routinely and orally, either alone or in combination, proven to reduce oxidative stress and improve antioxidant fame in hyperglycemic rats[6]. Another study showed that giving Blue Congo extract as a natural antioxidant has proven to lower blood

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glucose, increase glucose tolerance, and reduce the quantity of glycated hemoglobin[7].

Clitoria Ternatea L., or Asian pigeonwings flower, is a plant that contains antioxidants and is often used by the community to be consumed as food and drink, as well as traditional medicine[8,9]. This plant is famed because it is proven to exhibit antioxidant, anti-inflammatory, and anti-diabetic effects[8,10]. Flavonoids, anthocyanins, saponins, phenols, and tannins are among the phytochemical substances found in CT flower[11,12]. According to Manivannan[13], CT flower extract has been proven to have anti-diabetic activity because it significantly reduces serum glucose levels in rats with diabetes mellitus[13]. In previous studies, the CT flower functions as an anti-diabetic. However, no more specific research has been conducted on MDA and body weight. Therefore, the cause of this observation is to investigate the effect of giving a dose of CT flower extract on MDA and BW of diabetic male Wistar rats.

METHODS

Research Protocol

This study was a laboratory experiment using the Pretest and Posttest Control Group Design. The number of samples in each treatment group was determined using IACUC (2002): a minimum of 6 rats in one study group. The sampling method was carried out by simple random sampling. This research protocol was approved by The Research Ethics Committee of the Faculty of Medicine, Universitas Sebelas Maret, with number 33/UN27.06.6.1/KEP/EC/2021. The adaptation process for controlling the condition of the experimental animals was carried out for seven days. Rats were reared in a particular room and placed in clean polypropylene cages of 6 rats per large cage. Those cages were given a transparent barrier so that 1 rat inhabited 1 small cage. Then, the rats were reared in a room with temperature control (27-290^{0}C), 12 hours of vibrant mild, and 12 hours of dark cycle (lights turned on at 07.00 WIB). The humidity was 70-90%. The feed used in this study was standard Comfeed feed, and drinking water was given using ad libitum sampling.

The establishment of DM model rats induction by using streptozotocin 45mg/kgBW/day and nicotinamide 110 mg/kgBW/day intraperitoneally was conducted for three days, and then blood was drawn for testing blood glucose and MDA levels. The T1 group was given standard feed without intervention, and the T2 group received 300 mg/KgBW/day of CT flower extract; the T3 group received 600mg/KgBW/day of CT flower extract for 21 days.

Determination of Malondialdehyde

MDA levels were measured using the TBARS method, which measures the concentration of Thiobarbituric Acid Reactive Substance. Blood samples were mixed with 15% TCA and 0,37% TBA in 0.25N of HCL. Then, it was heated in a water bath for 60 minutes at 95^{0}C. Next, the tube was placed in an ice bath and left for 15 minutes to be cooled. The cooling solution was centrifuged for 15 minutes at 3000 rpm. The supernatant formed was transferred into a cuvette to measure its absorbance using a spectrophotometer at 532 nm.

Determinant of Body Weight

The weighing for all groups used a digital scale four times a week, and it occurred at the beginning before treatment was given, on the 7th day of treatment, 14th day of treatment, and 21st day of treatment.

Statistical Analysis

Univariate analysis to determine descriptive characteristics of the variables expressed by the mean and standard deviation. Normality facts of using the Shapiro-Wilk test were normally distributed when the p-value was >0.05. Bivariate analysis used One Way Anova with p-value <0.05.

RESULTS AND DISCUSSION

The Effect of Dosage of CT Extract on MDA

The results of the serum MDA examination in the three treatment groups of DMT2 rats are shown in Table 1. According to Table 1, the average results of giving CT flower extract to MDA of DMT2 rats in T1, T2, and T3 groups, which was previously given treatment, were T1 9.54 ± 0,42 µmol/l, T2 9.58 ± 0.22 µmol/l, T3 9.52 ± 0.28 µmol/l with p-value=0.698, it was not significantly different because the mean MDA values of T1, T2, and T3 of rats were nearly the same. The mean results of giving CT flower extract to MDA of DMT2 rats in T1, T2, and T3 groups on day 14 were T1 8.64 ± 2.54 µmol/l, T2 4.67 ± 0.17 µmol/l, T3 3.99 ± 0.30 and it had a significant difference (p<0.001). Then, the mean results of giving CT flower extract to the MDA of DMT2 rats in T1, T2, and T3 groups on day 21 were T1 8.68 ± 2.81 µmol/l, T2 4.07 ± 0.14 µmol/l T3 3.34 ±0.23 µmol/l and it also had a significant difference (p<0.001).

Table 1. Table 1. Analysis of differences in doses of ct flower extract in MDA serum levels of male wistar rats with diabetes

Duration	Treatment Group			p-value
	T1 (µmol/l)	T2 (µmol/l)	T3 (µmol/l)	
0	9.54 ± 0.42	9.58 ± 2.54	9.52 ± 2.81	0.698
14	8.68 ± 0.22	4.67 ± 0.17	3.99 ± 0.14	<0.001*
21	8.68 ± 0.28	4.07 ± 0.14	3 ± 0.23	<0.001*

One Way ANOVA test, *) Significant if p-value <0.05, the T1 group was given standard feed without intervention, the T2 group received 300 mg/KgBW of CT flower extract, the T3 group received 600 mg/KgBW.

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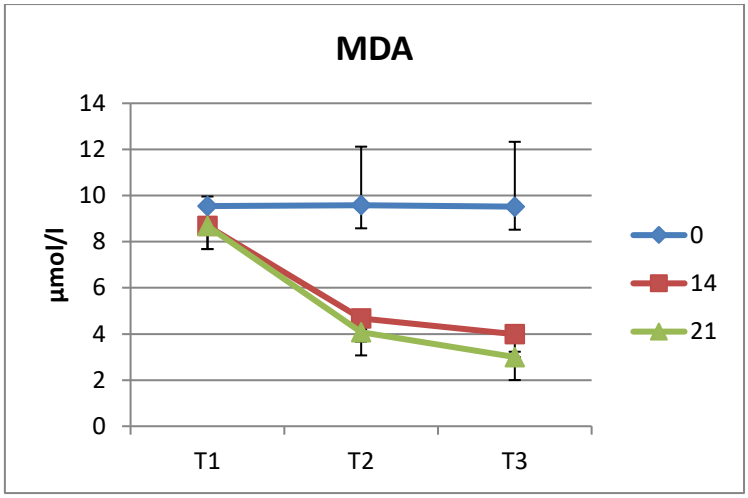

The T1 group was given standard feed without intervention, and the T2 group received 300mg/KgBW of CT flower extract. The T3 group received 600mg/KgBW.

Figure 1. The effect of dosage of CT flower extract on serum MDA in Diabetes Mellitus rats

Other information regarding the effect of giving a dose of CT flower extract on the decrease in MDA can be found in Table 2. Following Table 1, on day 14 of intervention, there was a significant effect (<0.001) on the lower MDA in the control group compared to the DM rat group, which was given CT flower extract 300 mg/KgBW, and the DM rat group given was CT flower extract 600 mg/kgBW. In addition, the DM rat group was given CT flower extract 300 mg/kgBW compared to the DM rat group given that CT flower extracts 600 mg/kgBW, which also has a significant difference (0.002). While on day 21 after the intervention, all groups experienced a significant difference (<0.001).

Table 2. The least significant difference doses of ct flower extract of post hoc test results in MDA serum levels of male wistar rats with diabetes

Group	Δ (µmol/l) day 14	p-value	Δ (µmol/l) day 21	p-value
MDA				
Control with				
CT flower extract 300 mg/kg BW	4.92**	<0.001	5.68**	<0.001
CT flower extract 600 mg/kg BW	5.60**	<0.001	6.41**	<0.001
CT flower extracts 300 mg/kg BW.				
Control	-4.92**	<0.001	-5.68**	<0.001
CT flower extract 600 mg/kg BW	0.68**	0.002	0.72**	<0.001
CT flower extract 600 mg/kg BW				
Control	-5.60**	<0.001	-6.41**	<0.001
CT flower extract 600 mg/kg BW	-0.68**	0.002	-0.72**	<0.001

LSD posthoc, **) The mean distinction is sizable at 0.05

The high antioxidant content found in CT flowers can reduce MDA levels in DM rats. CT flower contained high antioxidants such as flavonoids and anthocyanins[11,14]. According to Jaafar's research[14], the extractable phenolic, flavonoid, and anthocyanin components showed that *C. Ternatea* is a good source of natural antioxidants. Flavonoids exhibit antioxidant activity in vitro as they reduce the formation of free radicals and bind free radicals to reduce the rate of formation of ROS[15,16]. ROS is considered a crucial factor in the pathogenesis of type 2 diabetes mellitus[17]. This procedure contributes to improving plentiful illnesses and complications of the disease. ROS will affect a variety of biological components, causing cells to become destroyed. In this process, antioxidants are needed to protect organisms from ROS[18].

Reactive oxygen species (ROS) production is linked to increased MDA levels. MDA is a marker of lipid peroxides in the body produced by free radicals on polyunsaturated fatty acids in biological membranes. MDA levels were found to be considerably greater in diabetic patients with and without problems, indicating that free radicals are the cause of oxidative lipid damage[2,19]. The Aluwong[20] study found that when an increase followed hyperglycemia in lipid peroxidation, MDA concentrations in the serum, brain, and kidneys of untreated diabetic rats increased significantly. Increased serum MDA levels in untreated diabetic rats may be linked to oxidative stress-induced damage of erythrocyte membranes and tissues[20]. According to Kavitha's[21] study, Wistar rats given a dosage of 400 mg/KgBW/day of C. Ethanol leaf extract for 28 days showed a significant reduction in blood glucose, glycosylated hemoglobin,

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creatinine, and serum levels than diabetes controls[21]. According to another study, a limited clinical study involving 15 healthy males found that giving them 1 or 2 grams of CT flower extract mixed with 50 grams of sugar as a drink resulted in lower plasma glucose and insulin levels[22].

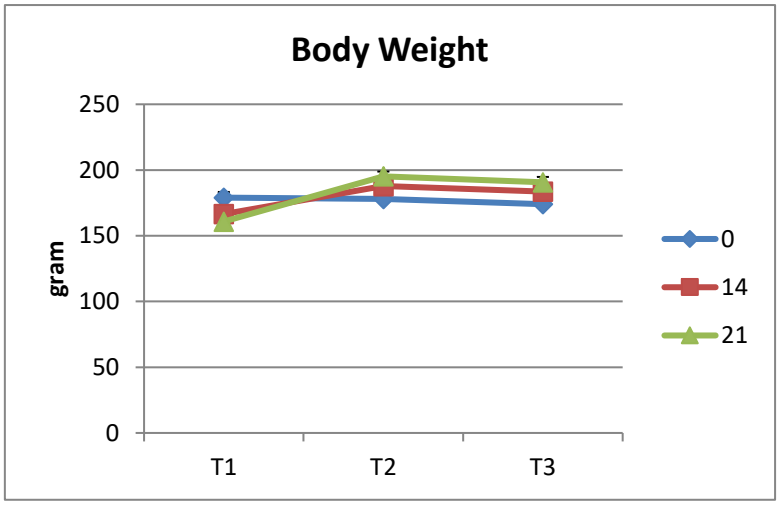
The Effect of Dosage of CT Extract on Body Weight

The results of the dose of CT extract on body weight are shown in Table 3. The body weight of DM rats in the T1, T2, and T3 groups at the beginning of the study was not significantly different due to the three treatment groups that had not received treatment. On day 14 of intervention, there were already visible alterations. The T1 group tended to decrease on day 14, while the T2 and T3 groups experienced increased body weight (p<0.001). On day 21, T1, T2, and T3 experienced a significant difference with p<0.001.

Table 3. Analysis of differences in doses of ct flower extract in body weight of male wistar rats with diabetes

Duration	Group			p-value
	T1 (g)	T2 (g)	T3 (g)	
0	179 ± 4.05	178 ± 4.05	174 ± 4.60	0.131
14	166.5 ± 3.728	187.83 ± 4.67	183.50 ± 4.41	<0 001*
21	160.83 ± 4.02	195.17 ± 3.65	190.67 ± 4.08	<0.001*

One Way ANOVA test, *) Significant if p-value <0.05, the T1 group was given standard feed without intervention; the T2 group received 300mg/KgBW of CT flower extract, the T3 group received 600 mg/KgBW.



The T1 group was given standard feed without intervention; the T2 group received 300mg/KgBW of CT flower extract, and the T3 group received 600mg/KgBW.

Figure 2. The effect of dosage of CT flower extract on body weight in DM rats

Further information about the differences in each group is attached with the LSD posthoc results in Table 4. Under Table 4, on day 14 and day 21 of intervention, there was a prominent effect (<0.001) on changes in body weight within the control group compared to the DM rat group that used CT flower extract 300 mg/kgBW and the DM rat group that used the flower extract. The DM rat group given CT flower extract 300 mg/kgBW compared to the DM rat group given 600 mg/kgBW CT flower extract did not experience a significant difference (0.088). On day 14 and day 21 also did not differ significantly (0.066).

Table 4. The least significant difference doses of ct flower extract of post hoc test results in body weight of male wistar rats with diabetes

Group	Δ (gram) 14 days	p-value	Δ (gram) 21 days	p-value
Body Weight				
Control with				
CT flower extract 300 mg/kg BW	-21.33**	<0.001	-34.33**	<0.001
CT flower extract 600 mg/kg BW	-17.00**	<0.001	-29.83**	<0.001
CT flower extract 300 mg/kg BW				
Control	21.33**	<0.001	34.33**	<0.001
CT flower extract 600 mg/kg BW	4.33	0.088	4.50	0.066
CT flower extract 600 mg/kg BW				
Control	17.00**	<0.001	29.83**	<0.001
CT flower extract 600 mg/kg BW	-4.33	0.088	-4.50	0.066

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LSD posthoc, **) The mean distinction is sizable at 0.05

According to Ramadhani's[23] research, allocating pomegranate peel extract as a natural antioxidant in DM rats increased BW[23]. The same thing was also found in Obasi's study, in which the allocation of phenolic aqueous leaf extract could increase body weight in DMT2 rats[24]. The high antioxidant contents in the CT flower could reduce free radicals in the body. Yakubu says high antioxidants can reduce ROS production and increase SOD activity[25]. The decrease in ROS can increase insulin secretion in the body so that there is an increase in glucose absorption in all tissues[24,26].

CONCLUSIONS

Applying CT flower extract at 300 mg/KgBW and 600 mg/KgBW as natural antioxidants for 21 days can reduce MDA levels and increase body weight in DM rats compared to DM control rats. However, the administration of CT flower extract at doses of 300 mg/KgBW and 600 mg/KgBW was not significantly different in weight gain.

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Conflict of Interest and Funding Disclosure

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