The Effects of Ethanol Extract of Asian Pigeon Wings (*Clitoria ternatea* L.) Flower on Body Weight and Malondialdehyde Level in Diabetes Rat Model

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Abstract. Diabetes mellitus. is ai global metabolici disease with high morbidity and imortality rates. Many diabetes patients have difficulties lowering their blood glucose levels although they are taking anti-diabetes drugs. Flower of Asian pigeon wings (*Clitoria ternatea* L) contains flavonoid, anthocyanin, compounds and possess high antioxidant activity, which can lower postprandial glucose levels in Albino rats with diabetes. The purpose of this study was to analyze the "effect-of ethanol extract of Asian pigeon wings flower on body weight (BW) and malondialdehyde (MDA) level of diabetes rats. Male-wistar rats-aged 8–12 weeks old and weighed 150–200 g were used in this experimental study. Rats were induced with 45 mg/kg BW streptozotocin and 110 mg/kgBW nicotinamide to generate type 2 diabetes. Diabetes rats were randomly divided into two groups: -the T1 group was giveni1.8 mg/kg BW acarbose and the T2 group was given 150 mg/kgBW. Extract of Asian pigeon wings flower for 21 days. Collected data before, during and after treatment were analyzed using the repeated measure ANOVA. The mean rat BW at days 14^{th} and 21^{st} of T1 (189 ± 4.17 and 196 ± 3.65 g) was higher than the mean rat BW of T2 (184 ± 5.81 and 189 ± 6.15 g). Increased rat BW in both groups was significantly higher than that of day 1 (180 ± 3.84 and 179 ± 6.22 g respectively). The mean MDA levels of T1 significantly reduced from 9,45 ± 0,31 μmol/l at day 1 to 3.59 ± 0,22 μmol/l at day 14 and 3.00 ± 0.23 μmol/l at day 21 with p = 0.001. The T2 group also had significant reductions of mean MDA levels from 9,56 ± 0.18 μmol/l at day 1 to 5.45 ± 0.26 μmol/l a day 14 and 4.80 ± 0.25 μmol/l at day 21 with p = 0,001. In conclusion, administration of 150 mg/kg BW extract of Asian pigeonwings flower is able to increase BW and reduce MDA level of diabetes rats but its metabolic and anti-oxidant activities are lower than the acarbose drug.

Keywords: Clitoria ternatea L flower · Diabetes mellitus · Body weight MDA

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1 Introduction

Diabetes mellitus (DM) is a. global metabolic. Disease-with high morbidity and mortality rates. Diabetes mellitus is caused by abnormal insulin production and chronic hyperglycemia [1]. Hyperglycemia can lead the increasing production of free radicals or reactive-oxygen-species (ROS). The increasing production of, ROS in cell membranes will effect the formation of *malondialdehyde* (MDA) [2].

The high production of ROS will effect decreasing function of the insulin receptor and also the ability of pancreatic -cells to produce insulin. Thus, there will be a decreasing in glucose storage in the body and an increasing in the usage of muscle protein as a substrate in energy production so that weight loss can be occurred [3].

Management of DM can be done in various ways, starting from education, pharmacological therapy, nutritional therapy, and physical activity [4]. Pharmacological therapy is giving anti-hyperglycemic drugs both orally or injection [5]. Some *antihyperglycemic* drugs have side effects in DM patients. The Side effects from the use of Metformin and Glimepiride can cause nausea, while the consumption of glibenclamide can cause hypoglycemia [6].

One of the foodstuffs that contains high antioxidants is Asian pigeon wings flower (*Clitoria ternatea L*). Asian pigeon wingsflower contains chemical compounds such as tannins, saponins, triterpenoids, phenols, flavonoids, flavonol glycosides, proteins, alkaloids, anthocyanins, essential oils, and steroids [7, 8]. This study aimed to analyze the effect of ethanol extract of Asian pigeon wings flower on BW and MDA level of diabetes rats.

2 Materials and Methods

2.1 Animals

A total of 12 male Albino Wistar rats which aged 8–12 weeks and had 150–200 g, was obtained from the Center for Food and Nutrition Studies Gadjah Mada University Yogyakarta.

2.2 Extraction of *Clitoria Ternatea L* Flower

C. ternatea flowers were obtained from a plantation in the Jogorogo, Ngawi Regency, East Java. The collected flowers were dried under the sun and then grounded using a milling machine to get powder. The simplicia of *C. ternatea*flowers was macerated using 96% ethanol with 1:10 ratio. *C. Ternatea* flowers powder soaked for 24 h, then filtered to separate the dregs. The filtrate was put into a rotary evaporator at speed of 100 rpm with temperature of 60 °C.

2.3 Research Design

This research was a laboratory experimental study with pre-posttests control group design. The sample size for this_study was determined using the Institutional Animal Care and Use Committee (IACUC) (2002), and got at least 6 rats/group. In this study, we used 2 rats groups, wich consisted of 6 rats by simple random sampling.

2.4 Research Protocol

This research protocol was approved by The Research Ethics Committeee of the Faculty of Medicine, Universitas Sebelas Maret with number 33/UN27.06.6.1/KEP/EC/2021. Selected male wistar rats were adapted for 7 days. Rats were kept in a room with_temperature 27–290 C, with 122 h of-bright light and 12 h of dark cycle (lights are turned on at 07.00 WIB), with 70–90% of humidity. The following day all rats were injected by using 45 mg/KgBW streptozotocin and 110 mg/KgBW nicotinamide intra peritoneally for 3 days. To verify wether or not rats with diabetes, fasting blood glucosa levelsswere measured in the 4th days after injection.

The treatment 1 (T1 group) received 1.8 mg/kgBW oral acarbose, and the T2 group received 150 mg/kgBW of Asian pigeon wings flower extract for 21 days. All rats were given standard Comfeed, given as much as 10% of the weight of rats for 2 times a day. The drinking water used is reverse osmosis was provided by adlibitum and inserted in the nipple which is placed on the cage.

2.5 Evaluation of BW and MDA Levels

The weighing all groups using a digital scale for every week in four times, were at the beginning before being given treatment, on the 7th day, 14th day of treatment, and on the 21st day of treatment.

All groups (T1 and T2) was checked for MDA with The Measurement of MDA levels can be done by using the TBARS (Thiobarbituric Acid Reactive Substance). Blood sample mixed with 15% of TCA and 0.37% of TBA in 0,25N of HCL. Then, the mixed were placed in a water bath for 60 min at 95 ^{0}C. The tube is placed on an ice bath and left for 15 min until cool. The cooled solution was centrifuged.for.15 min at 3000 rpm. The supernatant formed was transferred into a cuvette to measure the absorbance using a spectrophotometer at the wavelength of 532 rpm.

2.6 Data Analysis

All collected data were presented in mean ± standard deviation. The data were tested for normality and homogenity. Data were analyse using independent T test and repeated measure ANOVA with p-value < 0.05.

3 Results

3.1 Body Weight

The results of BW measurements in T2DM model rats on days 0, 14th, and 21st can be seen in Fig. 1. Based on Fig. 1, the results of the average body weight of T2DM rats in groups T1 and T2 had a significant difference ($p = < 0.05$). The average body weight at the beginning of the study was not much different, the T1 group of 180 ± 3.84 and the T2 group of 179 ± 6.22. On 14th day, the average weight of T1 rat was increased to 189 ± 4.17 and T2 to 184 ± 5.81. On 21st day, the weight of rats in group T1 was 196 ± 3.65 and T2 was 189 ± 6.15.

To see further the effect of Asian pigeon wingsflower extract on the bodyweight of DM rats could be seen in Table 1.

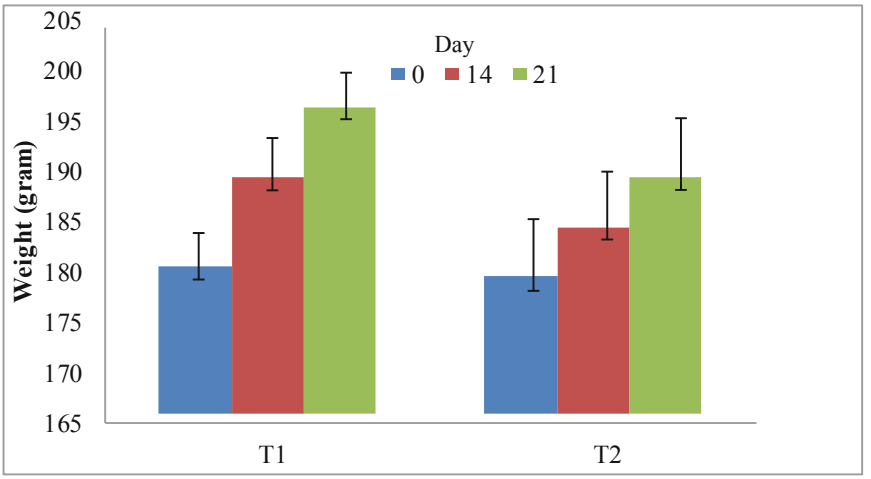

Fig. 1. The mean of body weight of rats before and after giving asian pigeon wings flowers extract. Note: T1 = T2DM rats were given acarbose 1.8 mg/KgBW; T2 = T2DM rats were given 150 mg/200 g BW of Asian pigeon wingsflower extract. T1 and T2 weight statistical tests = $P < 0.05$.

Table 1. The significant.differences on body weight In T2DM rats

Group	Δ (μmol/l)	P-value
Acarbose 1.8 mg /kgBW		
0 day		
14 days	-9.333*	<0.001
21 days	-16.167*	<0.001
14 days		
0 day	9.333*	<0.001
21 days	-6.833*	<0.001
21 days		
0 day	16.167*	<0.001
14 days	6.833*	<0.001
Asian pigeon wings flower extract 150 mg /kgBW		
0 day		
14 days	-5.667*	<0.001
21 days	-10.333*	<0.001
14 days		
0 day	5.667*	<0.001

(*continued*)

Table 1. (*continued*)

Group	Δ (μmol/l)	P-value
21 days	-4.667*	<0.001
21 days		
0 day	10.333*	<0.001
14 days	4.667*	<0.001

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

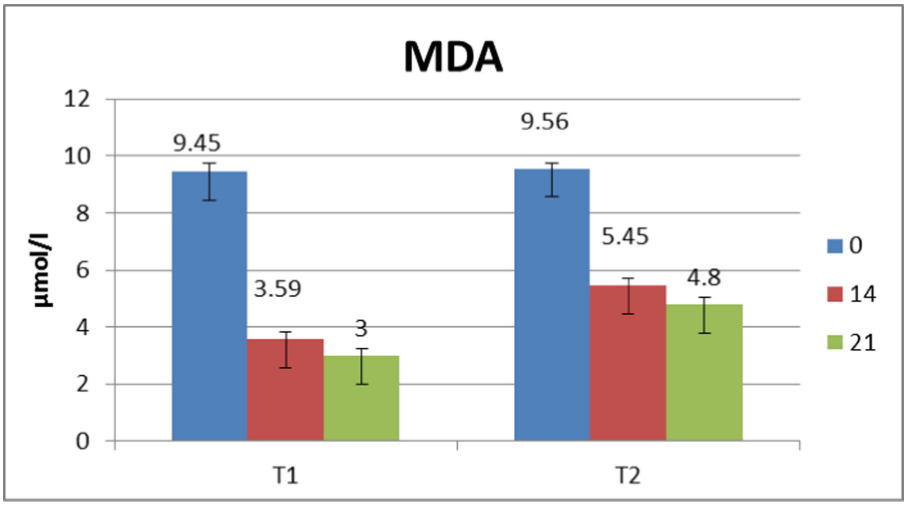

Fig. 2. The mean of MDA of rats before and after giving asian pigeon wings flowers extract. Note: T1 = T2DM rats were given acarbose 1.8 mg/KgBW; T2 = T2DM rats were given 150 mg/200g BW of Asian pigeon wingsflower extract. T1 and T2 weight statistical tests = P < 0.05

3.2 MDA

Based on Fig. 2, the average. MDA results of T2DM rats in groups T1 and T2 had a significant difference (p = < 0.05). The average MDA of the T1 and T2 groups at the beginning of the study was almost the same, namely 9.45 ± 0.31 and 9.56 ± 0.18, while on day 14th, it was decreased to T1 by 3.59 ± 0.22 and T2 to 5.45 ± 0.26. On day 21st, the average MDA of the T1 group was 3.00 ± 0.23 and T2 was 4.80 ± 0.25. To see further the effect of Asian pigeon wings flower extract on decreasing MDA can be seen in Table 2.

The MDA decrease can be shown in Table 2, group T1 could reduce MDA levels in DM rats up to 6,455 mol/l for 21 days. The T2 group was able to reduce the MDA levels of DM rats by 4,678 mol/l for 21 days. There was a significant difference in the MDA of groups T1 and T2, both on 14th day and 21st day (p < 0.05).

Table 2. The significant differences on MDA in T2DM rats

Group	Δ (μmol/l)	P-value.
Acarbose 1.8 mg/kgBW		
0 day		
14 days	-9.333*	<0.001
21 days	-16.167*	<0.001
14 days		
0 day	9.333*	<0.001
21 days	-6.833*	<0.001
21 days		
0 day	16.167*	<0.001
14 days	6.833*	<0.001
Asian pigeon wings flower extract 150 mg/kgBW		
0 day		
14 days	4.115*	<0.001
21 days	4.678*	<0.001
14 days		
0 day	-4.115*	<0.001
21 days	.563*	<0.001
21 days		
0 day	-4.678*	<0.001
14 days	-.563*	<0.001

*: the mean difference is significant at the 0.05 level

MDA in the beginning of the treatment in the T1 and T2 groups was not significantly different ($p > 0.05$), this is because MDA of the rats is almost the same. But MDA in the 14th days were had significant between T1 and T2 groups ($p < 0.05$). In 21st days, MDA of T1 and T2 groups had significant ($p < 0,05$).

4 Discussion

MDA levels of rats in the study group were very high. This was because STZ and NA induction increased insulin resistance, affected glucose oxidation and decreased insulin biosynthesis and secretion. STZ entered pancreatic -cells via the glucose transporter GLUT2 causing decreased expression of GLUT2 which causes hyperglycemia and reduces glycogen content in muscle [9, 10].

Hyperglycemia precipitated and increase inside the manufacturing of reactive oxygen species (ROS) [6, 9]. High ROS may want to increase the amendment of lipids DNA, and proteins in diverse tissues. Molecular adjustments in those diverse tissues effected

an imbalance among protecting antioxidants (antioxidant defense) and improved manufacturing of free radicals. This became the beginning of oxidative harm referred to as oxidative stress [11]. The negative impact on the cell membrane will occur in a chain reaction called lipid peroxidation [12]. The final result of this chain reaction was the breaking of the fatty acid chain into various compounds that are toxic to cells, including malondialdehyde (MDA), ethane, and pentane [9, 11].

The results of the study above showed that giving the Asian pigeon wingsflower as much as 150 mg/KgBW affected the reduction of MDA levels in T2DM rats. However, the reduction was not as much as the administration of *acarbose* as an oral anti-diabetic drug.

Antioxidant therapy may want to reduce the risk of growing diabetes and its headaches in type 2 diabetic patients. Various plant life containing antioxidant marketers together with flavonoids had a good healing effect inside the treatment of T2DM [13]. According to Tsounapi [14] administration of antioxidants could reduce the concentration of MDA and reduce oxidative DNA damage in the bladder.

Clitoria terntea L contained antioxidants such as flavonoids, *saponins, anthocyanins,* tannins, triterpenoids, polyphenols, and essential oils [7]. The polyphenol content contained in *Clitoria ternatea* L may have antioxidant activity in vitro that can increase antioxidant capacity and reduce lipid peroxide [15].

The content of flavonoids within the Asian pigeon wings flower stimulated glucose uptake in peripheral tissues and regulated the pastime or expression of enzymes concerned in carbohydrate metabolism which includes hexokinase [16].

The effect of Asian pigeon wingsflower extract as a natural antioxidant could prevent weight loss in T2DM rats. This was in line with several studies which stated that antioxidants contained in food can prevent weight loss in T2DM rats [17, 18].

Flavonoids as antioxidants could repair hyperglycemia, body weight and tissue, oxidative stress, skeletal-muscle glycogen, content, insulin resistance, cell-function index, Glut-4 gene expression, and pancreatic apoptosis that has been altered-by STZ and NA induced T2DM rats [10].

The administration of 150 mg/kgBW of Asian pigeon wingsflower extract for 21 days can prevent weight loss in T2DM rats and reduce MDA levels, but its activity is still less than the *acarbose* drug.

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References

1. International Diabetes Federation. IDF Diabetes Atlas 2017. Eighth 2017.

2. Pitocco D., Tesauro M., Alessandro R., Ghirlanda G., Cardillo C. Oxidative stress in diabetes: implications for vascular and other complications. International Journal of Molecular Sciences. 2013; 14(11):21525–21550. doi: https://doi.org/10.3390/ijms141121525
3. Rahul, C., Thangaraj P., and Saravanan S. 2016. Antidiabetic activity of Syzygium Calophyllifolium in Streptozotocin-Nicotinamide induced Type-2 diabetic rats. Biomedicine and Pharmacotherapy journal.
4. PERKENI. 2019. Pedoman dan Pencegahan Diabetes Mellitus Tipe 2 di Indonesia. Jakarta: Pengurus Besar Perkumpulan Endokrinologi Indonesia.
5. Unuofin JO, Lebelo SL. Antioxidant Effects and Mechanisms of Medicinal Plants and Their Bioactive Compounds for the Prevention and Treatment of Type 2 Diabetes: An Updated Review. *Oxid Med Cell Longev*. 2020; 2020:1356893
6. Choi SW, Ho CK. Antioxidant properties of drugs used in Type 2 diabetes management: could they contribute to, confound or conceal effects of antioxidant therapy?. *Redox Rep*. 2018; 23(1):1–24. doi: https://doi.org/10.1080/13510002.2017.1324381
7. Al-Snafi, AE. 2016. Pharmacological importance of Clitoriaternatea – A review. (www.iosrphr.org*) IOSR Journal of Pharmacy 6(3)*March: 68–83
8. Chaiyasut, C, dkk. 2016. Anthocyanin profile and its antioxidant activity of widely used fruits, vegetables, and flowers in Thailand. *Asian Journal of Pharmaceutical and Clinical Research, 9(6),* 218–224.
9. Ghasemi A., Khalifi S., dan Jedi S. 2014. Streptozotocin-Nicotinamide-Induced Rat Model Of Type 2 Diabetes. Journal of Diabetes and Metabolic Disorders, vol.12.
10. Ahangarpour A, Oroojan AA, Khorsandi L, Kouchak M, Badavi M. Solid Lipid Nanoparticles of Myricitrin Have Antioxidant and Antidiabetic Effects on Streptozotocin-Nicotinamide-Induced Diabetic Model and Myotube Cell of Male Mouse. *Oxid Med Cell Longev*. 2018; 2018:7496936. doi: https://doi.org/10.1155/2018/7496936
11. Wang CH, Chang RW, Ko YH, et al. Prevention of arterial stiffening by using low-dose atorvastatin in diabetes is associated with decreased malondialdehyde. *PLoS One*. 2014; 9(3): e90471.
12. Zimmet P. Z., Magliano D. J., Herman W. H., Shaw J. E. Diabetes: a 21st century challenge. *The Lancet Diabetes and Endocrinology*. 2014;2(1):56–64.
13. Zatalia S. R., Sanusi H. The role of antioxidants in the pathophysiology, complications, and management of diabetes mellitus. *Acta Medica Indonesiana*. 2013;45(2):141–147
14. Tsounapi P, Honda M, Hikita K, Sofikitis N, Takenaka A. Oxidative Stress Alterations in the Bladder of a Short-period Type 2 Diabetes Rat Model: Antioxidant Treatment Can Be Beneficial for the Bladder. *In Vivo*. 2019;33(6):1819-1826. doi: https://doi.org/10.21873/invivo.11674
15. Chusak C, Thilavech T, Henry CJ, Adisakwattana, S. Acute effect of Clitoriaternatea flower beverage on glycemic response and antioxidant capacity in healthy subjects: a randomized crossover trial. *BMC Complement Altern Med.* 2018. 18(1):6.
16. Gupta R., Mathur M., Bajaj V. K., et al. Evaluation of antidiabetic and antioxidant activity of *Moringaoleifera* in experimental diabetes. *Journal of Diabetes*. 2012;4(2):164171.doi: https://doi.org/10.1111/j.1753-0407.2011.00173.x
17. Ramadhani, D., Amradani, R., Ulfia, M., Utami, S., Indarto, D., Wasita, B. The Comparative Effect of Pomegranate Peel Extract and Dapagliflozin on Body Weight of Male Albino Wistar Rats with Type 2 Diabetes Mellitus. IOP Conference Series: Materials Science and Engineering. 2019. VL - 546. doi:https://doi.org/10.1088/1757-899X/546/6/062023
18. Novia, D., Sugiarto., Dewi, YLR., The Effect of Tamarind Leaves (tamarin dusindica linn) and Acarbose on Body Weight in Rats Type 2 Diabetes Mellitus Model. Atlantis Press. 2021.43–45. https://doi.org/10.2991/ahsr.k.210127