

# ANTIDIABETIC POTENTIAL OF MATOA BARK EXTRACT (*Pometia pinnata*) IN ALLOXAN-INDUCED DIABETIC MALE RAT STRAIN WISTAR (*Rattus norvegicus*)

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## ABSTRACT

Diabetes mellitus is characterized by a hyperglycemia, characterized by increased blood sugar level and glycosylated hemoglobin (HbA1c). Hyperglycemia also produced excessive amount of Reactive Oxygen Species (ROS) which caused increased level of serum Malondialdehyde (MDA) and progressive changes in the mean amount of the pancreatic islets. Matoa bark extract is used as an antidiabetic agent containing polyphenols and flavonoids which acts as antioxidants against Reactive Oxygen Species (ROS) and also as an  $\alpha$ -glucosidase enzyme inhibitors. To determine the antidiabetic effect of matoa stem bark extract on alloxan-induced Wistar rats. Experimental research design with post-test only control group design. The control group was positive, negative, and 4 treatment groups consisted of 5 experimental animals in each group for 38 days. Blood sugar and MDA are measured using a spectrophotometer. HbA1c was measured using the HPLC method. Langerhans Island was dyed HE checked using a microscope. Data were analyzed using ANOVA, Post Hoc, and Regression tests. ANOVA test results have a significant effect ( $p = 0,000$ ). Post Hoc test results obtained a significant difference ( $p < 0.05$ ) in all treatment groups. MDA levels At a new dose of 300mg / 200gBW, there was a significant difference ( $p < 0.005$ ). Matoa bark 89% to decrease blood sugar, 96.5% to decrease in HbA1c, 72% to decrease MDA, 82% to the average picture of Langerhans Island. Doses that have a significant effect have begun to appear at a dose of 300 mg / 200 gBW to the highest dose of 400 mg / 200 gBW. Matoa stem bark extract has been shown to have antidiabetic effects that affect blood sugar levels, HbA1c, MDA, and the mean of Langerhans Island alloxan-induced male Wistar rats (*Rattus norvegicus*).

**Keywords:** Matoa Bark (*Pometia pinnata*, Sp.), Alloxan, Blood Sugar, HbA1c, Langerhans Island Average, MDA

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## INTRODUCTION

Diabetes mellitus is a serious disease that happens because the pancreas does not produce enough insulin or the compilation cannot be used effectively to produce insulin and it is one of the most common diseases in the world presently (Saeedi et al., 2019). In 2014, around 422 million young adults were affected by diabetes mellitus (WHO, 2016). Indonesia itself ranked sixth in 2017 as a country with a population of 10.3 million people with diabetes mellitus at the age of 20-79 years (Saeedi et al., 2019).

Diabetes mellitus is a metabolic disease characterized by hyperglycemia that happen due to abnormal insulin secretion and progressive changes in the structure of the island of Langerhans (Purnamasari et al., 2013). The state of chronic hyperglycemia in diabetes mellitus can cause the binding of glucose aldehyde groups by hemoglobin beta chain proteins to form glycosylic hemoglobin. Measurement of blood glycosylate hemoglobin (HbA1c) levels are often used as a reference in the examination of diabetes mellitus patients as an indicator of the state of chronic

hyperglycemia (Florkowski, 2013). In addition, hyperglycemia increases free radicals in the body, making the production of Reactive Oxygen Species (ROS) excess and cause cell death  $\beta$  pankreas (. Excessive ROS production led to ROS binds to unsaturated fatty acids and this is a sign of oxidative stress (Newsholme et al., 2016). Oxidative stress can be known through increased serum Malondialdehyde (MDA) levels.

The uncontrolled increased of blood sugar levels in the body can cause diabetes mellitus and if it is not managed properly it can lead to complications such as cerebrovascular disease, coronary heart disease, limb vascular disease, disorders of the eye, kidney and nerve (Soelistijo et al., 2015). As many as 43% of deaths due to high blood sugar levels happen before the age of 70 years (WHO, 2016).

All this time the treatment of diabetes performed by administering oral anti diabetic drug (OAD) or insulin injections which if it is keep continue happen, it will spendt huge costs and a heavy burden for the sufferer. These socio-economic factors that often cause suffering failure in controlling the blood sugar levels so important to consider alternative treatments that are effective, inexpensive, and easy to obtain. The occurrence of side effects also often occurs at the beginning of the use of antidiabetic oral drugs that cause sufferers to stop using the drug, so that blood glucose control fails (Riwu et al., 2015).

As a preference of back to nature, the use of traditional herbs is again looked at to be explored and examined its efficacy in helping to cure various diseases. One of the well-known traditional Indonesian medicinal plants and their efficacy that is believed to cure diabetes is the matoa plant or *Pometia pinnata* (Mataputun and Pontoh, 2013). This plant belongs to the genus *Pometia* and family Sapindaceae. In ethnobotany, matoa plants have been used in the treatment of several diseases or health problems. Matoa bark has antidiabetic activity which can control postprandial hyperglycemia in the treatment of diabetes mellitus by inhibiting the enzymes  $\alpha$ -glucosidase and  $\alpha$ -amylase better than acarbose (Elya et al., 2015).

Matoa bark is a material that is easily found around that has antioxidant content including polyphenols, flavonoids, tannins, saponins, quinones (Rachmawati et al, 2016) alkaloids and glycosides (Elya et al, (2015), Elya et al., (2015), Rachmawati et al., (2016).On the bark of the matoa (*Pometia pinnata*) has antioxidant activity with a polyphenol value of  $28.93 \pm 2.64$  mg GAE / g (Kawamura et al., 2010). Several previous studies have shown that antioxidant polyphenols can reduce oxidative stress and inhibit endocrine cell damage on the island of Langerhans by inhibiting the formation of chain reactions to convert superoxide into hydrogen superoxide by

donating hydrogen atoms to bind to free radicals and remove them through excretion systems (Prameswari and Widjanarko, 2014). Flavonoids have a role in controlling diabetes mellitus through various mechanisms among others by increasing insulin secretion, decreasing apoptosis and increasing proliferation of pancreatic beta cells, decreasing insulin resistance, inflammation and oxidative stress in cells and increasing GLUT4 activity (Vinayagam et al., 2015).

Seeing this potential, researchers are interested in providing more data related to the potential of this matoa bark extract as an antihyperglycemic agent for alternative therapies for diabetes mellitus. The author's interest is also supported by the lack of similar research and years of research that are long enough and need to be renewed.

## METHODS

### Design

This type of research is an experimental research (True Experiment Research) with Post Test Only Control Group Design method.

### Sample

The population and sample in this study were male white rats (*Rattus norvegicus* wistar strain). Determination of the sample size using formula1 with simple random sampling technique, where the total sample of 30 male white rats (*Rattus norvegicus* strain wistar) was obtained

### Group

This study used 30 male white rats which were divided into six treatment groups. The first group (K-) is the group giving BR-1 and taking ad libitum (unrestricted), the second group (K +) is the group giving alloxan with a single dose of 150 mg / kgBW., The third group (P1) is the group giving aloxan single dose 150 mg / kgBW followed by the administration of acarbose 12mg / kgBW, the fourth group (P2) is a group with alloxan administration of a single dose of 150 mg / kgBW followed of 200 mg / 200 grBW matoa bark extract, the fifth group (P3) is the group with the administration of a single dose of alloxan 150 mg / kgBW continued with 300 mg / 200grBW bark extract, the sixth group (P4) was the group with the administration of a single dose of alloxan 150 mg / kgBW followed of 400 mg / 200grBW matoa bark extract.

### Procedure

Adaptation of experimental animals for 7 days with the aim that the rats adjust themselves, later on the 8th day of the animal were induced a single dose of alloxan 150mg / kgBW IP after being fasted for the previous 18 hours. Blood glucose is carried out on the 10th day. Rat blood sugar measurements were performed using a digital glucometer with the Nesco® brand and Nesco® brand test strips. Matoa bark extract is given for 14 days every day by oral sonde in accordance with the group dose. At the end of

experiment, rats were anesthetic using chloroform. After the animal is anesthetized properly, the animal is placed on a candle table by using an oar. Using surgical scissors, surgery is performed abdominal to neck level. Then the pancreatic organ of the caput is taken and fixed with 10% formalin. After confirmed dead rats eat will be collected into one and buried.

Determination of serum blood glucose levels was carried out by the enzyme Glucooxidase (GOD-PAP) method. The serum is taken slowly then the GOD-PAP reagent is added. The principle works is that glucose is oxidized by the enzyme glucose oxidase to produce gluconic acid and H<sub>2</sub>O<sub>2</sub>. Then H<sub>2</sub>O<sub>2</sub> is reacted with amynophenasone and phenol with the help of the enzyme peroxidase to produce quinoneimine. The resulting color was calculated for absorbance at a wavelength of 500 nm using a UV-Vis spectrophotometer, then the glucose concentration was calculated using the formula :

$$\text{Blood glucose level (mg/dL)} = \frac{(A \text{ Sample})}{(A \text{ Standard})} \times C \text{ Standard}$$

Determination of HbA1c levels was done after the experimental animals were treated on day 38. Measurement of rat HbA1c was carried out using the HPLC method. Blood sampling of rats was performed from rat heart ventricles by cardiac puncture. The blood drawn is then inserted into a tube containing EDTA.

Determination of serum MDA levels was carried out by the method of thiobarbituric acid reactive substances (TBARS). The blood that has been taken is collected in a vacutainer with a clot activator and centrifuged at a speed of 3000 rpm for 10 minutes. Blood serum was taken and MDA concentration analyzed. 200 ml of serum was added with 1 ml of 20% TCA and 1 ml of 1% TBA. The solution is mixed until it is homogeneous by being heated on a water bath for 10 minutes. After it cold, then centrifuged at 3000 rpm for 10 minutes. The pink filtrate was measured for absorption and read the absorbance at a wavelength of 532 nm using a UV-Vis spectrophotometer. MDA levels are calculated using the MDA standard curve (Sunarmi et al., 2007).

The mean number of endocrine cells of Langerhans Island is calculated by calculating the

average number of endocrine cells of Langerhans Island of the pancreas every five Langerhans islands of pancreas each rat in each treatment group using HE staining using a 400x magnification light microscope under the supervision of an anatomist pathologist.

#### Data Analysis

In this study blood glucose, HbA1c and MDA levels, and average number of endocrine cells of pancreatic Langerhans island every five pancreatic Langerhans islands, each rat in each treatment group was processed using SPSS 24 and tested by Independent T test, One-Way ANOVA Test, Post- Hoc Test, and Linear Regression Test.

### DISCUSSION

The blood sugar, HbA1c, MDA levels and the average sum of endocrine cells of pancreatic Langerhans islets can be seen in Table 1, 2, 3, 4 and Figure 1 respectively. The results showed significant differences in all parameters between all treatment groups. This is consistent with the research by Elya et al. which shows that the matoa bark extract has an inhibitory activity of α-glucosidase enzymes in vitro with an IC 50 value of 17.12 ppm while Longan has an IC 50 of 247.21 ppm (Elya et al., 2015; Hilma et al., 2016). So it can be said that matoa has better inhibitory activity because it has a lower IC50 value than longan. Smaller the IC 50 value means that the extract's activity as an α-glucosidase inhibitor is higher (Sebaugh, 2017).

The administration of matoa bark extract gave a significant difference in serum MDA levels at the treatment dose of 300mg / 200grBB and 400mg / grBB. Sarangarajan et al wrote in their research that the intake of exogenous antioxidants is directly proportional to the oxidative stress process that occurs in the body of experimental animals (Sarangarajan et al., 2017). Matoa bark extracts have many types of phytochemicals such as polyphenols, alkaloids and flavonoids which are known to have various antidiabetic effects and are a powerful class of antioxidants (Elya et al., 2015; Aba & Asuzu, 2018).

Table1. Blood Glucose Levels Examination Results

Group	Blood glucose levels				Average
	1	2	3	4	
K-	79	112	127	82	100
K+	410	441	472	498	455
P1	111	87	197	201	149
P2	109	213	236	240	199
P3	238	231	194	-	221
P4	223	230	213	-	223

Table 2. HbA1c Examination Results

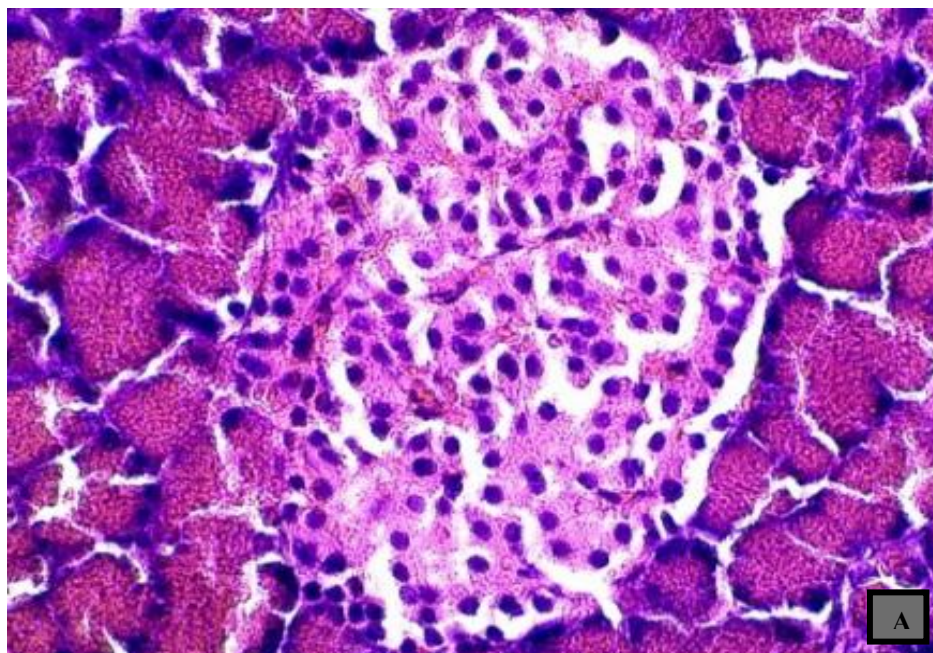
Group	HbA1c				Average
	1	2	3	4	
K-	4	3.4	3.8	-	3.7
K+	10.8	10.7	13.7	12.1	11.8
P1	3.7	3.7	3.4	3.5	3.5
P2	3.1	3.2	3.2	3.3	3.2
P3	3.6	3.2	3.5	-	3.4
P4	3.5	3.7	3.6	-	3.6

Table 3. MDA Level Examination Results

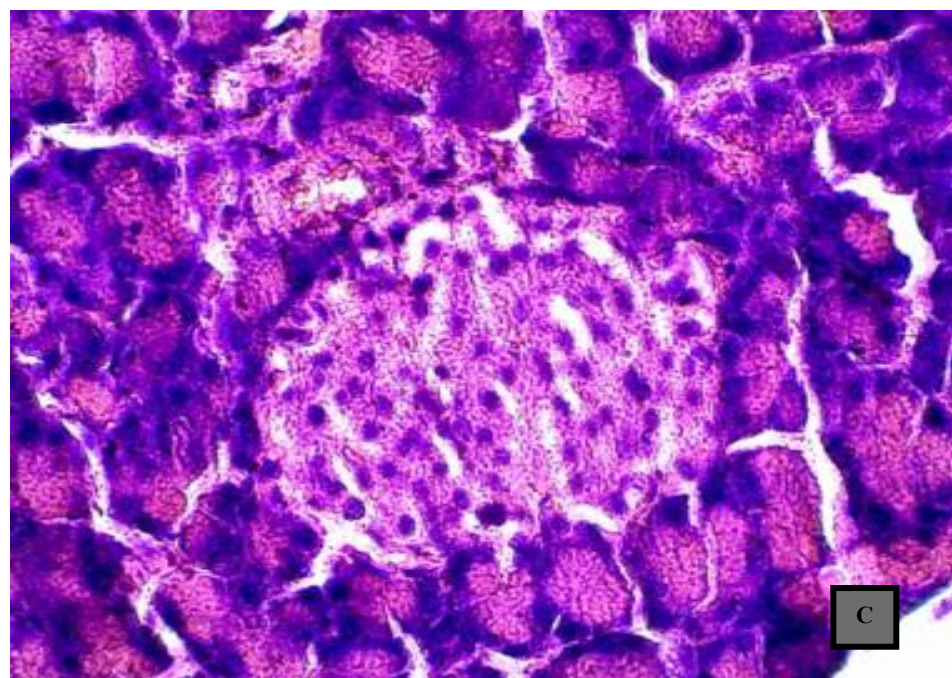
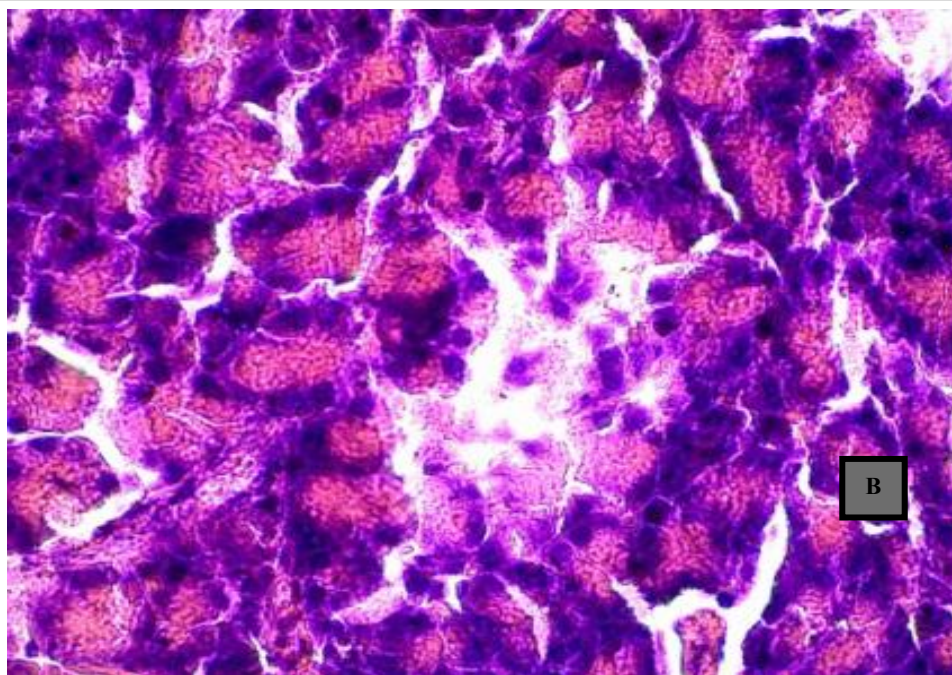
Group	MDA Levels				Average
	1	2	3	4	
K-	2.23	1.26	1.66	1.12	1.34
K+	3.09	2.71	2.74	3.01	-
P1	3.04	2.79	2.23	2.61	-
P2	2.42	1.85	2.23	-	-
P3	1.45	1.48	1.45	-	-

Table 4. The Average of Endocrine Cell Examination Results on Langerhans Islet

Group	Number Of Endocrine Cells of The Pancreatic Langerhans islets				Average
	1	2	3	4	
K-	172	178	201,2	160,4	177,9
K+	59,4	59,4	49,6	52,6	55.25
P1	120,8	133	136,6	110,6	125,25
P2	130	161	149	-	146,66
P3	259,2	144,4	202,8	-	202,13









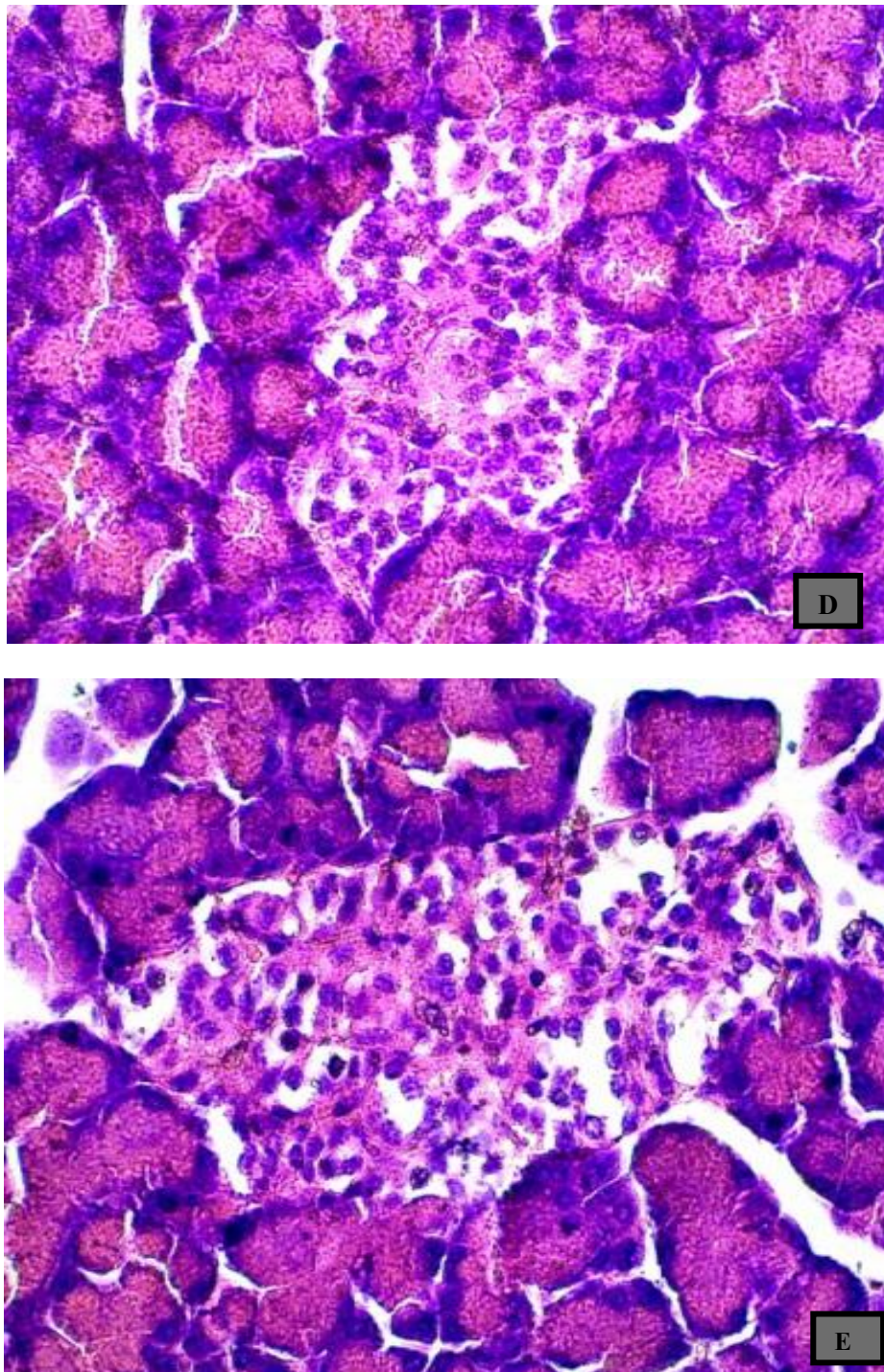


Figure 1. Histopathological picture of Langerhans Islet endocrine cells, 400x magnification in white rats (*Rattus norvegicus strain wistar*) as shown in Figure 1. The group (E) matoa bark extract 400mg/200gBW showed the highest number of endocrine cells in Langerhans islet followed by the negative group (A), the 300mg / 200gBW (D) matoa bark extract group and the 200mg/200gBW (C) matoa bark extract group; while the alloxan-induced group (B) showed the least number of endocrine cells in the islets of Langerhans.

Aside from being an antioxidant, the polyphenol content in matoa stem bark is can also prevent hyperglycemia (Abbas et al., 2017). The stem bark of matoa has the highest polyphenol content compared to the fruit and leaf (Kawamura et al., 2010). Polyphenols can reduce oxidative stress by inhibiting the chain reaction of superoxide turning into hydrogen superoxide by donating hydrogen atoms to bind to free radicals

(Prameswari and Widjanarko, 2014). The role of polyphenols as antioxidants is thought to protect pancreatic  $\beta$  cells from the toxic effects of free radicals produced under conditions of chronic hyperglycemia (Avila et al., 2017). Polyphenols also have antidiabetic potency by increasing the secretion of GLP-1 (Glucagon like peptide-1) which indirectly stimulates pancreatic langerhans islets to regenerate bew  $\beta$  cells

(Vinayagam et al., 2015; Avila et al., 2017). Flavonoids can also increase the antioxidant capacity of beta cells through both enzymatic and non-enzymatic pathways. The inhibited oxidation process prevents the formation of ROS and lipid peroxidation which can cause autophagy, apoptosis and necroptosis as well as provide opportunities for normal  $\beta$  cells to carry out its physiological functions, namely producing insulin which plays a role in the uptake of blood glucose into body cells (Ghorbani et al., 2019). Matoa bark extract (*Pometia pinnata*) also shows weak inhibition of DPP-IV enzymes during in vitro test (Elya et al., 2015). Inhibition of the DPP-IV

enzyme can prevent the metabolism of incretin, an insulinotropic hormone that can increase postprandial insulin activity and response, enabling the control of ideal blood sugar levels (Krentz, 2018). Aba & Asuzu showed that a strategy for controlling blood sugar levels through preventing the increase in blood sugar levels while increasing insulin performance shows better blood sugar and HbA1c levels (Aba & Asuzu, 2018). In addition, increased levels of incretin, specifically GLP-1, can stimulate proliferation and provide antiapoptotic effects on the pancreatic islets of Langerhans (Miranda et al., 2018).

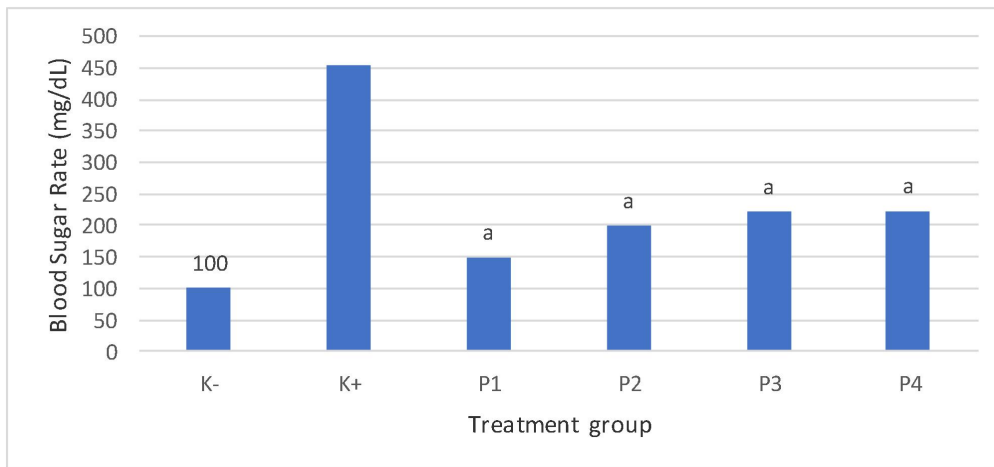


Figure 1. Graph of average of blood glucose levels, a = significant against the K+ group

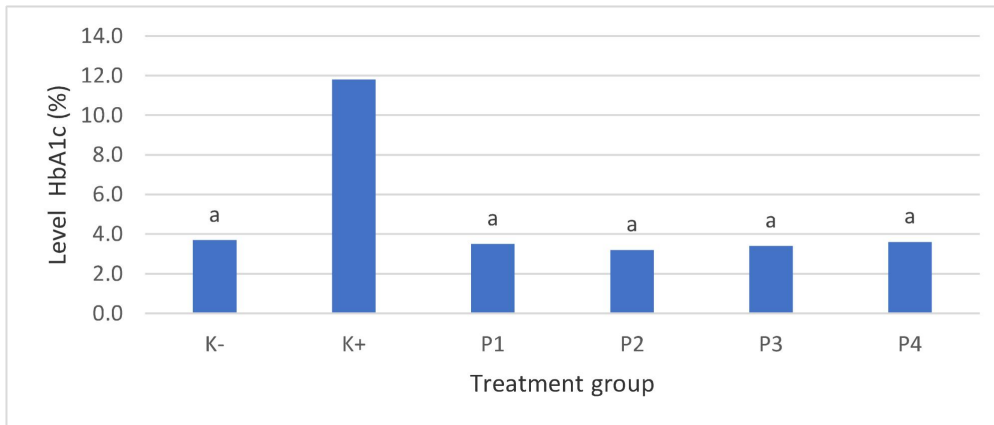


Figure 2. Graph of average of HbA1c, a = significant against the K+ group

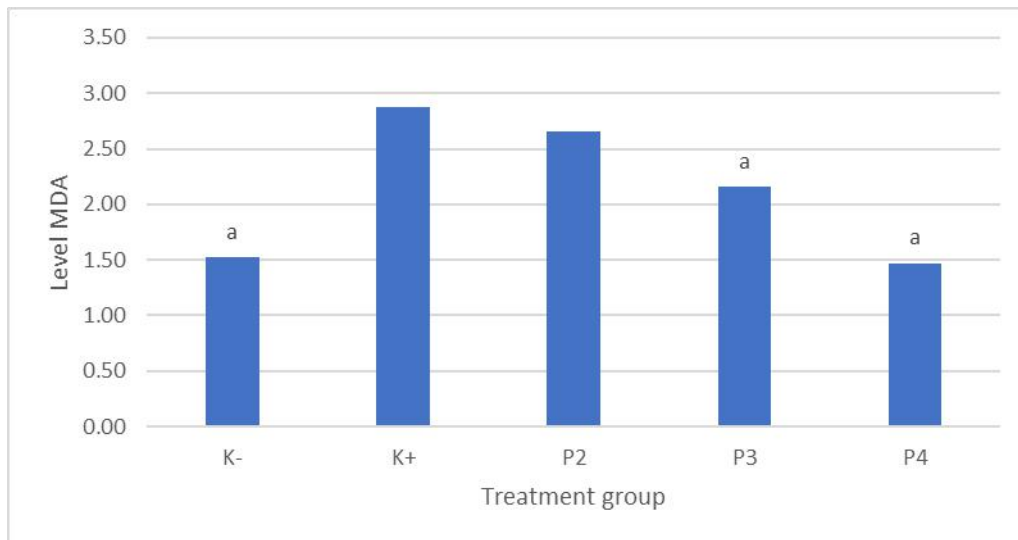


Figure 3. Grape of average of MDA level, a = significant against the K+ group

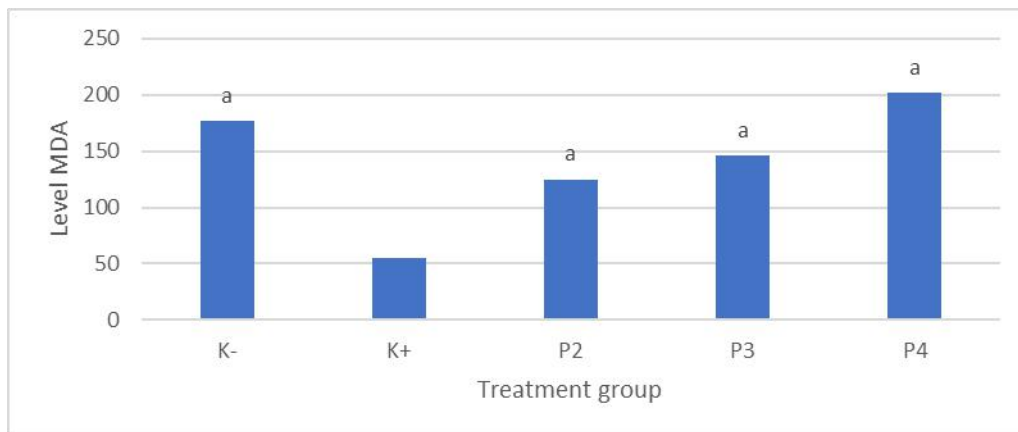


Figure 4. Grape of average number of endocrine cells of the pancreatic Langerhans islets graph, a = significant against the K+ group

In this study, administration of matoa bark extract showed improvements in non-clinical parameters such as the number of islets of Langerhans and MDA levels as markers of oxidative stress with a linear dose-effect curve pattern. Whereas clinical parameters such as HbA1c and blood sugar show a dose pattern of homeostatic effect; the higher the dose, the smaller the effect (Polisak and Milisav, 2012). Researchers hypothesize that duration of treatment plays an important role in the results obtained. Alloxan, which is a potent inducer of diabetes mellitus can induce diabetes in experimental animals within 2x24 hours (Rohilia and Shahjad, 2012). However, administration of herbal extracts that mainly work as antioxidants requires a variety of time to effect on clinical parameters. Studies conducted by Golbidi, Alireza & Laher show that the administration of exogenous antioxidants begins to have an effect at 4 weeks of treatment; treatment of less than 4 weeks does not have a significant clinical effect (Golbidi et al., 2011). The effects of beta cell repair as well as the reduction in oxidative stress

levels in experimental animals may have begun to work in a shorter time but clinical improvement may take longer. In addition, the structural improvements of beta cells observed are not necessarily accompanied by functional improvements.

### CONCLUSION

Matoa bark extract has antidiabetic activity against male wistar strain induced by alloxan by decreasing blood glucose, HbA1c, MDA levels and increasing the average number of endocrine cells of the pancreatic Langerhans islets. The doses that begin to provide significant antioxidant and antidiabetic effects are 300mg/200gBW.

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