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Research Article,

Pancreas Histopathology Changes After the Administration of the Extracts of Paitan Leaves (*Thitonia Diversifolia*) In Diabetic Mice (Mus Musculus)

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Abstract:

Diabetes mellitus (DM) is a metabolism disease marked by the hyperglycemia that occurs because of insulin disorders. The failures of pancreatic beta cells and insulin resistance in peripheral tissues are the main reasons for DM disorder. A Paitan Leaf (Thitonia *diversifolia*) has flavonoid compounds that can lower the production of glucose and regenerate damaged pancreatic islets of Langerhans cells. The purpose of this study was to investigate the impacts of giving the extracts of Paitan leaves (*T. diversifolia*) orally to repair the histopathology description of pancreas in diabetic mice (*Mus musculus*). A total of 25 samples divided into 5 groups (control negative, control positive, treatment 1, treatment 2, and treatment 3). Before giving the treatment, all samples were checked for their glucose blood rate. The Pancreas of diabetic mice were then observed by using a microscope with 400x magnification. Histological parameters assessed were degrees of damaged pancreatic islets of Langerhans cells to figure out damage structure and infiltration cell inflammation. The successful parameter of induction was the increase of the rate of blood glucose. The result of this study using the analysis of the Chi-Square test showed the significant difference by giving the extract of Paitan leaves with description histopathology value P = 0.012 ($p < 0.05$). Giving the extract of Paitan leaves (*T. diversifolia*) reduces damages of pancreatic islets of Langerhans cells

Keywords: Diabetes mellitus, the extracts of Paitan leaves (*Thitonia diversifolia*), levels of blood glucose, structure of histopathology pancreas of mice (Mus musculus).

Introduction:

Diabetes mellitus (DM) is a group of metabolic diseases with characteristics of hyperglycemia due to abnormal insulin secretion, insulin action, or both (Soelistijo, 2020). Diabetes mellitus is divided into 4 types namely type 1 DM, type 2 DM, gestational DM, and other types of DM. Diabetes mellitus is one of the global health threats. Various studies of epidemiology show an increase of the incidence and prevalence of type 2 DM in the world. Indonesia occupies the third rank of type 2 DM in Southeast Asia with prevalence by 11.3%. *The International Diabetes Federation* (IDF) estimates there were 463 people aged 20-79 years suffering from DM in 2019. This predictive figure will keep increasing to 578 million in 2030. Indonesia was in the seventh rank of DM sufferers in 2019 in the world, that is 10.7 million. Of top ten DM sufferers in the world, Indonesia is the only Southeast Asian country that could be predicted to have a contribution to prevalence of DM cases in Southeast Asia (Federation, 2019).

Two defects of main metabolic of type 2 DM are the failure of pancreatic beta cells to produce the insulin production and resistance of insulin in muscle skeleton, adipose tissue and liver. The resistance of insulin is compensated through hyperfunction of pancreatic beta cells and hyperinsulinemia in the stage of early diabetes.

The beta cells then fail to adapt due to the increase of the need of secretory for maintaining the circumstances of euglycemic which causes chronic hyperglycemia. The changes that occur in pancreas decline islets of Langerhans. In addition, there is an amyloid deposition in islets of Langerhans Island and around vessels of blood (Maitra, 2015). The DM treatment is intended for repairing disturbance of pathogenesis not only for lowering HbA1c, but also for combining treatment based on drug performance in accordance with pathophysiology of type 2 DM. Furthermore, the treatment must start as early as possible to prevent or slow down the progress of damaging beta cells (Soelistijo, 2020).

DM sufferers usually look for alternative medication for economical, available, and easy medicine. Generally, a society chooses to utilize potential plants that have the ability to lower the rate of blood glucose. One of them is Paitan leaves (*T. diversifolia*) (Rinawati et al., 2019). Paitan leaves (*T. diversifolia*) have flavonoid compounds that have effects like insulin, that is, to lower production of glucose in hepatocytes. The ability of flavonoids in lowering the rate of blood glucose works by regenerating the damaged pancreas and protecting pancreatic beta cells from damage as well as stimulating insulin release (Ramadhani et al., 2020). The study have been done Yuneldi et. al (2018) on Flavonoids in three extracts of Paitan leaves (*T. diversifolia*) have proven that they could lower the rate of glucose in blood. The findings also proved that extracts of Paitan leaves could increase the insulin sensitivity. In addition to antioxidant effects, flavonoids can stimulate regeneration of pancreatic β cells (Yuneldi et al., 2018). Setiomulyo, M.K.S (2016) also proved that the decoction Paitan leaves (*T. diversifolia*) can lower the rate of blood glucose of diabetic mice. The dose of extract Paitan leaves was used 3 mg/ kg BW of a mouse rat.

Based on the using Paitan leaves (*T. diversifolia*) have been used to decrease of the rate of blood glucose traditionally, and some studies have proven that this plant can recovery pancreatic damages, however, it is still a question whether the decrease of the rate of blood glucose is in accordance with the regeneration of islets of Langerhans cells. Therefore, the researcher is interested in knowing the effects of giving the extract of Paitan leaves (*T. diversifolia*) to repair the description of histopathology pancreas in diabetic mice (*Mus musculus*).

Method Study:

This experiment study was carried out in the laboratory Pharmacology Faculty Andalas University Pharmacy and Laboratory Pathology Anatomy of Siti Rahmah Hospital. This study used 25 laboratory mice (*Mus musculus*) aged 2-3 months, body weight 20-30 grams as many as 25 mice as test animals. The laboratory animals were grouped into 5, each consisting 5 mice as in the following:

1. Group 1 as control negative
2. Group 2 as control positive induced by alloxan 150 mg/ kg BW
3. Group 3 (P1) as treatment group induced by alloxan 150 mg/ kg BW and given the extract of Paitan leaves with the dose of 200 mg/ kg BW
4. Group 4 (P2) as as treatment group induced by alloxan 150 mg/ kg BW and given the extract of Paitan leaves with dose of 300 mg/ kg BW
5. Group 5 (P3) as treatment group induced by alloxan 150 mg/ kg BW and given the extract of Paitan leaves with dose of 400 mg/ kg BW

Making the Extract of Paitan Leaves *(Thitonia Diversifolia)*

The Extract was made from powder dry of Paitan leaves (*T. diversifolia*) with a maceration method. The Paitan leaves (*T. diversifolia*) were put into a container or a dark colored bottle and then soaked with 96% of ethanol. The Paitan leaves were soaked for 3 days and stirred twice a day. After 3 days maceration, the solution was separated by filter and filtrate 1 was taken. The dregs remaining in the bottle were soaked again in ethanol as in the first step and filtrate 2 was produced. Filtrate 1 and filtrate 2 were dissolved. The distillation was then conducted to evaporate ethanol in the solution by using a rotary evaporator aparatus. The result was the solution of the extract of Paitan leaves (*T. diversifolia*).

Test Animal Treatment:

All the test animals were acclimatized with standard food (Bio Rat) for 7 days, and then the rates of their glucose were checked. On the eighth

day, group positive and group treatment were induced with alloxan 150 mg/ kg BW for 7 days. The Successful parameter of the induction was the increase of the rate of fasting blood glucose more of 126 mg/dl. On the fifteenth day, each group treatment was given orally the extract of Paitan leaves (*T. diversifolia*) 200 mg/ kg BW, 300 mg/ kg weight and 400 mg/ kg BW for 15 days. On the 30th day, pancreatic organs of the mice were taken.

The Description of Histopathological Pancreas:

After pancreas were taken, there were 4 processes to get the description of histopathological pancreas**.** First, the pancreases were dissolved into 10% of buffered formalin solution for at least 24 hours. Second, they were then dehydrated with different levels of alcohol concentration. Third, they were processed by the clearing process using xylol, impregnating and manufacturing block paraffin. The blocks were cut 5 µm thick with a microtome. Fourth, the pancreases were colored by Hematoxylin-Eosin (HE). Microscopic pancreases were observed with microscope light with 400x magnification. The observation of the description of histopathology pancreas was examined based on change or injury Langerhans islets as in score following scores:

Score 0 is normal or no there is damage island Langerhans.

Score 1 is for mild damage (degeneration and necrosis less than 25% of Langerhans islets). Score 2 is for moderate damage (degeneration and necrosis Langerhans islets 25%-75%) and score 3 is for heavy damage (more than 75%) (Widyatmaka & Ismail, 2021).

Data Analysis

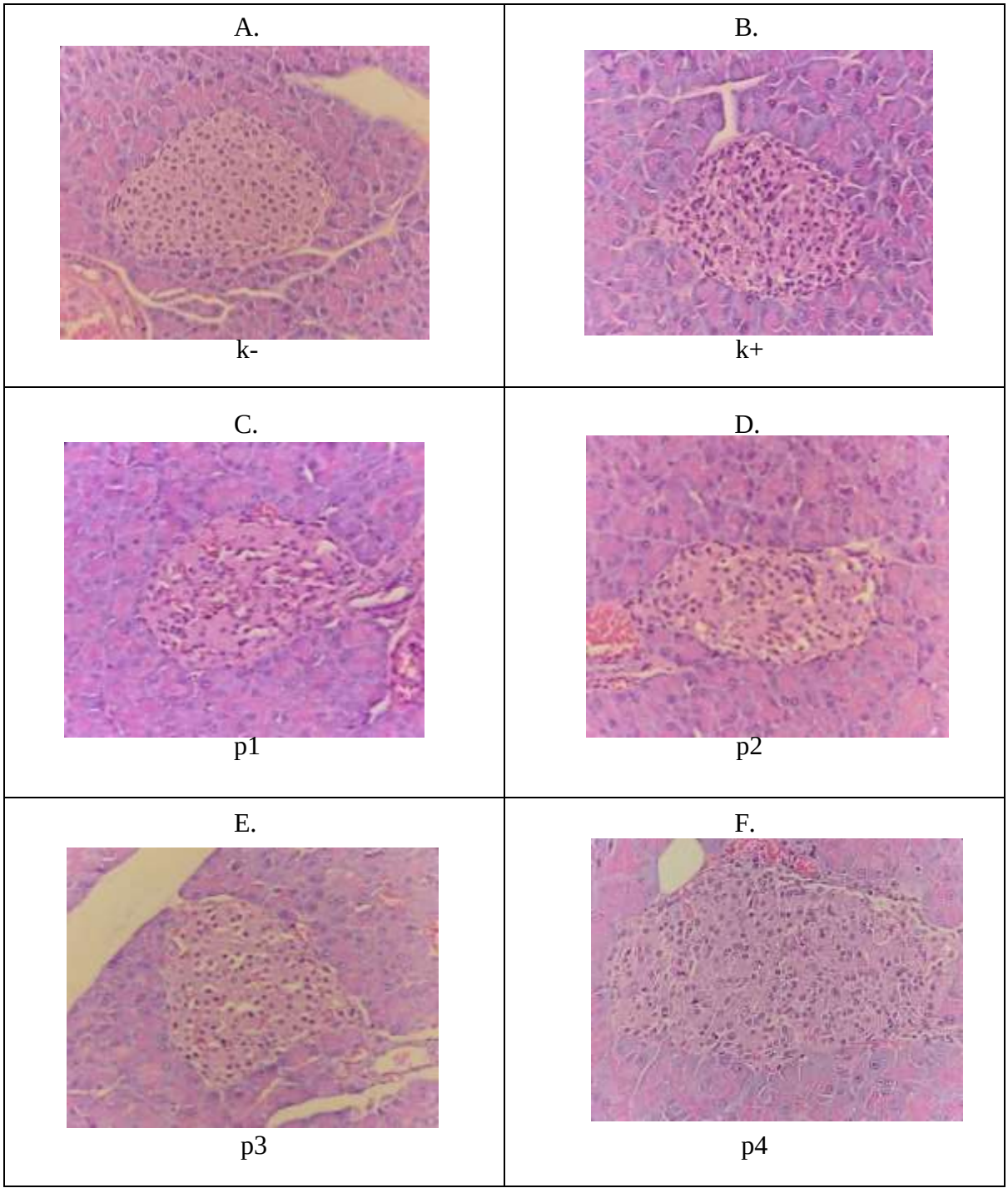
The data were analyzed by using the SPSS 25. Chi-Square test was used to find out the significant difference in the change in histopathology pancreas description after giving orally the extracts of Paitan leaves with $\alpha = 0.05$.

Results

Results of univariate data analysis indicated that 100% test mice which were not given the extracts of Paitan leaves (*T. diversifolia*) had histopathology description category 3. Laboratory mice given orally the extracts of Paitan leaves (*T. diversifolia*) with 200 mg/ kg BW showed 40% of the description of histopathology category 2 and 60% with the description of histology category 3. The mice given orally extracts of Paitan leaves 300 mg/ kg BW showed 80% of description histology category 2 and 20% with description histology category 3. The mice given 400 mg/ kg BW yielded 40% with description histology category 1 and 60% with description histology category 2 while on the overview histopathology 3 became 0%.

Table 1:-

The Extract of Paitan leaves	Histopathological Overview			P value
	1	2	3	0.012
Not given Paitan leaves	0 (0%)	0 (0%)	5 (100%)	
dose of 200 mg/ kg BW	0 (0%)	2 (40%)	3 (60%)	
dose of 300 mg/ kg BW	0 (0%)	4 (80%)	1 (20%)	
dose of 400 mg/ kg BW	2 (40%)	3 (60%)	0 (0%)	



The description of the pictures above is as in the following:

- Picture A. group control negative, normal pancreas without damages.
- Picture B. group control positive, damaged islets of Langerhans > 75%, cell pyknotic nuclei. The borders between cells are not clear.
- Picture C group P1, damage islets of Langerhans > 75 %, the borders between cells are not clear. There are a lot of capillaries.
- Picture D. group P2, the damages of Langerhans islets are medium. It looks like there is a degeneration and pyknotic nuclei.
- Picture E. group P3, the damages of Langerhans islets are medium (25%-75%), the degeneration is reduced and some pycnotic cores appear.
- Picture F. Group P4, the damages of Langerhans islets are mild (<25%) cytoplasm granular is seen clearly. The border cells are almost clear. There are a few pyknotic nuclei.

The Results of bivariate data analysis using the Chi-Square test proved that there is a significant relationship of giving the extracts of Paitan leaves in the description histopathology with value P = 0.012 (p <0.05). It is clear that there are significantly different results from the groups which were not given the extracts of Paitan leaves with the groups that were given the extracts of Paitan leaves 200 mg/ kg BW, 300 mg/ kg BW and 400 mg/ kg BW.

Discussion:

This research proved that alloxan induction caused damage to the islets of Langerhans in the form of degeneration of beta cells of Langerhans islet. The damage to pancreatic β cells impacts on the increase of blood glucose and insulin dependent diabetes mellitus occurs to experiment animals. Mechanism of β cells damage was to enable oxygen reactivation (Reactive *Oxygen Species* /ROS) that started with reaction alloxan reduction. Alloxan has high activity to cellular compounds containing SH group, cysteine, and sulfhydryl compounds protein bound. The result of the reduction process of alloxan is sour dilaurate which is deoxidized to become alloxan again and shape cycle of redox reaction resulting in redox radical superoxide (Nugroho, 2006).

One of the targets of ROS is DNA in cells of islets of Langerhans pancreas. Alloxan is able to reach pancreas because insulin receptors are in the pancreas. The process is that the alloxan with pancreatic beta cells damages insulin receptors accompanied with damage of the pancreatic islet of Langerhans β cells (Nugroho, 2006). The consequences from damage to insulin receptors and damage to pancreatic β cells are that insulin cannot be produced normally. The blood glucose cannot enter into cells and cannot be utilized to transform into energy. Therefore, glucose rate in the blood becomes high (Putri, DKSC, Hermanto, B., & Wardani, 2014).

The damage of Langerhans islets is in several forms. It can be the forms of degeneration cells and necrosis B cells. Cell nucleus experiences pyknosis. Vacuolation of cytoplasm was caused by initial stages of apoptosis due to increasing production of the oxidative stress produced by high activity from reticulum endoplasmic in β cells as a marker of insulin resistance (Edem, 2009). This proves that alloxan could damage cell endocrine pancreas, especially β cells, so it made the insulin secretion into blood vessels decreased. The reduction of insulin secretion affected the increase of rate of glucose in blood. In line with findings, the increase was at an average of 300mg.

The extracts of Paitan leaves (Thitonia Diversifolia) given orally to the induced mice (Mus Musculus) were capable of lowering blood sugar levels and repairing some damage of pancreas as shown in the picture histopathology group P1-P3. The P3 group showed the significant results of this study that inducing 400 mg/ kg BW of the extract of Paitan leaves revealed mild damage, no heavy damage to the pancreases of the mice.

Thitonia Diversifolia contains two major components which role as antidiabetic, that is, flavonoids and sesquiterpenes lactones found on the leaves. Flavonoids functions to increase absorption of glucose because it can inhibit α-glucosidase and α- amylase, whereas sesquiterpene lactone functions as a protein inhibitor for stress oxidation (Sasmita et al., 2017). Flavonoid compounds are antioxidants that can protect pancreas cells from oxidation. The processes that occur through inhibition were chain reaction of lipid peroxidation, radical binding, and the increase of glutathione peroxidase activity (Thongsom et al., 2013). Inhibition reaction of oxidation was conducted through a mechanism of radical free capture by donating one unpaired electron on free radicals, so free radicals are induced (Pasaribu et al., 2015) . Glutathione peroxidase is an antioxidant enzyme functioning to bind peroxidation (H2O2 or lipid peroxidation). The periodical decrease of free radicals can repair damaged pancreas cells so that pancreas can secrete insulin. Normal insulin secretion decreases the rate of blood glucose.

Other studies proved that flavonoid compounds can repair damage to pancreatic beta cells through increased catalase enzymes which will then break hydrogen peroxide into oxygen and water that are not dangerous for cells as well as cell growth. Flavonoid compounds in cells can also reduce the amount of ROS to help the integrity of cells and viability of the cells. (Patel, 2010)

The latest studies conducted by Chunudom proved that the extracts of Paitan leaves became a food supplement useful for treatment and management

of diabetes mellitus through the glucose transporter 2 (GLUT2). Diabetic mice given the extracts of Paitan leaves (*T. diversifolia*) orally significantly decreased blood glucose and increased insulin levels highly correlated with hepatic mRNA and GLUT2 protein expression (Chunudom et al., 2020). GLUT2 is especially expressed in the liver, pancreatic β cells, and tubular basolateral membrane proximal kidneys which play a role in glucose homeostasis. GLUT2 is a main glucose transporter in hepatocytes, and is required for absorption of the heart glucose. The absorption of glucose is triggered by binding the insulin into the surface cell receptor which increases the transmission of glucose in hepatocytes through GLUT2 (Thorens, 2015).

Another Study carried out by Rondius Solfain proved that TD extracts contain flavonoid compounds which are antioxidant under circumstances of hyperglycemia to prevent formation of oxidant and oxidative stress as the consequence of induction of alloxan on the liver and pancreas. Coloring results of immunohistochemistry of pancreatic tissues labeled by monoclonal antibody (Abm) IL-1β showed the increase IL-1β marked by expression aggregate color browning of the langerhans pancreas cells in the control positive group and treatment group Giving TD extracts lower the significant IL-b expression compared to control positive group It is the fact that cytokine is an important factor in dysfunction of diabetic cells in type 2. Langerhans pancreas is the target of inflammation and experiences a local inflammatory process. IL-1β influences insulin secretion and induces apoptosis. The increase of expression of IL-1β and IL1b receptors occurs in type 2 diabetes-induced mice (Solfaine et al., 2022).

Conclusion:

Giving the extracts of Paitan leaves (*T. diversifolia*) reduces the damaged islets of Langerhans pancreas in diabetic mice (Mus Musculus) induced by alloxan. Further Studies are crucial to investigate compounds responsible for its effects and its mechanism in the pancreas**.**

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