



Histopathological Pancreas Analysis of *Hylocereus polyrhizus* Peel Ethanolic Extract on Alloxan Induced Diabetic Mice

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Abstract

Diabetes is a metabolic disorder due to the disruption of the pancreas in producing insulin. Alloxan is a compound that has diabetogenic properties and is toxic, especially to pancreatic beta cells. When given to experimental animals, namely mice, it will cause them to become diabetic. Mice were separated into five groups consisting of 6 mice. Mice were divided into a normal control group without alloxan, a diabetic control group with alloxan, an extract treatment group of 100 mg/kg and 300mg/kg *Hylocereus polyrhizus* peel extract, and a positive control group with 600 µg/kg Glibenclamide. All treatments were given orally for 14 days. Neck dislocations were performed on all groups of mice on the 14th day after extract administration to retrieve pancreatic tissue. In this study, dragon fruit peel extract improved pancreatic beta cell damage compared to the diabetes control group. Giving ethanol extract of red dragon fruit peel (*H. polyrhizus*) can improve the histopathology of the pancreas in mice with diabetes mellitus. Ethanol extract of red dragon fruit peel (*H. polyrhizus*) at a dose of 300 mg/kg BW is the optimum dose for repairing pancreatic beta cell damage in mice with diabetes mellitus.

Keywords: Alloxan, Diabetes, Histopathological pancreas, *Hylocereus polyrhizus* peel extract, Mice

INTRODUCTION

Diabetes mellitus is a metabolic disease caused by various factors, including genetic factors, age, diet and lack of physical activity ¹. Diabetes mellitus, commonly called people with diabetes, is a metabolic disorder due to the disruption of the pancreas in producing insulin ². International Diabetes Federation (IDF) states that the prevalence of diabetes mellitus in the world is 1.9%, making DM the seventh leading cause of death worldwide. In contrast, in 2012, the incidence of diabetes mellitus worldwide was 371 million people ³.

Alloxan is a compound that has diabetogenic properties and is toxic, especially to pancreatic beta cells. When given to experimental animals, namely mice, it will cause them to become diabetic. The mechanism of action of alloxan that causes damage to pancreatic beta cells is to enter and be absorbed into pancreatic beta cells first and then absorbed by pancreatic beta cells. The ability to absorb substances from alloxan by pancreatic beta cells will determine the toxicity level and diabetogenic properties. After absorption of substances occurs, pancreatic beta cells will be damaged through several simultaneous processes, namely by oxidation of sulfhydryl groups and forming free radicals ^{1,2}.

Alloxan causes damage to pancreatic beta cells by activating reactive oxygen (ROS), which is initiated by the reduction

reaction of alloxan. Alloxan has high activity against cellular compounds containing -SH groups, cysteine, and protein-bound sulfhydryl compounds. The result of alloxan reduction is dialuric acid which is later reoxidized to alloxan as before and will form a redox reaction cycle which will produce superoxide radicals. The superoxide radicals will undergo dismutation to become hydrogen peroxide. The target of ROS is DNA from the islets of Langerhans cells in the pancreas. Alloxan can also increase the concentration of systolic-free calcium ions in beta cells. Alloxan causes damage to pancreatic beta cells by activating reactive oxygen (ROS) initiated by the reduction reaction of alloxan. Alloxan has high activity against cellular compounds containing -SH groups, cysteine, and protein-bound sulfhydryl compounds. The result of alloxan reduction is dialuric acid which is later reoxidized to alloxan as before and will form a redox reaction cycle which will produce superoxide radicals. The superoxide radicals will undergo dismutation to become hydrogen peroxide. The target of ROS is DNA from the islets of Langerhans cells in the pancreas. Alloxan can also increase the concentration of systolic-free calcium ions in pancreatic beta cells, which will depolarise pancreatic beta cells. Necrosis in pancreatic beta cells is thought to occur due to the depolarization of the pancreatic beta cell membrane as a result of alloxan induction. Damage to the membrane will cause damage to pancreatic beta cells easily ³⁻⁵.

Changes that occur in the pancreas as a result of alloxan induction are the shrinkage of the size of the islets of Langerhans and the nucleus of the cells undergoes pyknosis which indicates the occurrence of necrosis. Necrosis causes lytic changes that involve the cytoplasm of cells which have a characteristic that there is a vacuole formation, but the most obvious changes occur in the cell nucleus, namely cell death which is usually seen to shrink, the boundaries are not clear and dark in color or commonly referred to as pyknosis.⁶

Currently, the clinical treatment of diabetes is mainly oral and insulin injection. Until now, there is currently no effective medicine and treatment must be done on a daily basis. Oral medications such as glibenclamide were still used as antidiabetic, but the medicine's side effects are solid and fast. Continuous medicine usage will be chemical dependent, inhibiting Langerhans cells' islets from regenerating. The community's desire for plants used in traditional medicine formulations is also rising as a result of research showing that medications made from plants are healthier and have fewer adverse effects than those made from chemicals⁷⁻¹⁰.

Dragon fruit (*Hylocereus costaricensis*) is one of the plants from the Cactaceae or cactus family. There are four types of dragon fruit plants: red flesh dragon fruit, white flesh dragon fruit, super red flesh dragon fruit, and yellow flesh dragon fruit. Red dragon fruit weighs up to 350 – 550 g. Red flesh dragon fruit has a higher antioxidant content than the white type and has many benefits for the body. The fruit has positive benefits for the body, but the skin of the red dragon fruit (*Hylocereus polyrhizus*) also has benefits for diseases such as heart disease and diabetes. The content of flavonoids and tannins in the skin of red dragon fruit (*H. polyrhizus*) is an active compound that has anti-diabetic properties. One of the flavonoids in dragon fruit skin is anthocyanins. Anthocyanin levels range from 8.8 mg/100g of dragon fruit. Anthocyanin compounds give a red color to the skin, flower crown and roots of dragon fruit. Anthocyanin dyes are unstable and easily degraded. Stability is affected by pH, storage temperature, light, enzymes, oxygenation, structural differences, and concentrations of anthocyanins.

Flavonoid compounds in the skin of red dragon fruit (*H. polyrhizus*) have the ability to prevent pancreatic beta cell damage due to the antioxidant activity contained in it, namely by capturing or neutralizing free radicals associated with phenolic OH groups so that they can improve the state of damaged tissue. Flavonoid compounds can repair damage to pancreatic beta cells through various mechanisms, one of which is increasing the catalase enzyme, which will later break down hydrogen peroxide into oxygen and water, which are harmless to cells and the growth of these cells. Flavonoid compounds in cells can also reduce the amount of ROS, thereby helping cell integrity and also the viability of these cells. Tannins can capture free radicals that can cause damage to pancreatic beta cells and inhibit damage from pancreatic beta cells so that pancreatic beta cells can function properly. These antioxidants also function to keep the number of pancreatic beta cells normal^{5,11}.

Several previous studies stated that the red dragon fruit plant has the potential to improve the histopathology of the liver and kidneys of mice, among others, the research conducted by Sari et al. (2016)¹² which showed that administration of red dragon fruit juice at a dose of 250 mg/kg/day for 30 days could reduce the effect of saccharin on the microanatomical structure of the liver and kidneys. The results of research conducted by Putra et al. (2019)¹¹ also showed that this showed that administration of red dragon fruit juice at a dose of 8 grams/2.5 ml could significantly improve the brain tissue of diabetic rats from necrosis with a p value = 0.000 ($\alpha < 0.05$).

Based on the description above, the researcher wants to innovate by researching the effect of giving red dragon fruit peel extract (*H. polyrhizus*), which contains important compounds such as flavonoids and tannins, which are expected to be able to improve the damage to the histopathological picture of the pancreas in male white mice (*Mus musculus*) induced by alloxan.

MATERIALS AND METHODS

Animals

Adult male mice (*Mus musculus L.*), aged approximately three months and weighing between 25-35 grams, were utilized in this study. Mice were put in a plastic cage covered with wire mesh. Every two days, a layer of 1 cm husk was changed to cover the cage's bottom. The intensity of light, humidity, and room temperature were adjusted. Ethical codes, institutional, and national regulations of live animals were rigorously followed. The Health Research Ethical Clearance, Faculty of Dental Medicine, Universitas Airlangga, granted ethical clearance for the animals used in this enquiry (Reg No. 453/HRECC.FODM/VII/2021).

Concoction of extract

H. polyrhizus peels were dried in the oven at 60°C. The peel was then made into powder with a grinder. A mix of 1:7 *H. polyrhizus* peel powder was mixed in 96% ethanol and set at room temperature for 2 hours. Subsequently, the mix was filtered, and macerations separated and re-macerated with 96% ethanol with a ratio of 1:4. All maceration was evaporated at 60°C after being filtered till it became a thick extract. The solvent used to dilute the extract into desired concentration was Carboxyl Methyl Cellulose (CMC).

Histopathological pancreas test

Five groups of six mice each were created by dividing the mice. Alloxan was used to produce type 1 diabetes in animals. All groups, with the exception of the normal control group, received intraperitoneal injections of 150 mg/kg of alloxan that had been dissolved in 0.9% NaCl. Blood glucose levels in mice were assessed using a glucose kit five days after injection. This work employed diabetic mice with fasting glucose levels of 200 mg/dL or above. The mice were separated into four groups: a positive control group receiving 600 µg/kg of glibenclamide, a diabetic control group receiving 100 mg/kg and 300 mg/kg of *H. polyrhizus* peel extract, and a normal control group receiving no alloxan. For 14 days, all therapies were taken orally.

Neck dislocations were performed on all groups of mice on the 14th day after extract administration to retrieve pancreatic tissue. The pancreas was then rinsed in normal saline, fixed in a 10% formalin buffer solution, dehydrated in 70% alcohol for 2 hours, and washed in stages with 90%, 95% alcohol, absolute ethanol (3 times), and xylol (3 times), each one for 20 minutes. The infiltration process was then completed by applying paraffin three times for 30 minutes before embedding. Furthermore, a microtome with a thickness of 5 mm was used for the cutting. The paraffinization stage followed, with the preparation being introduced to xylol twice for 5 minutes each time. The slides were stained for 10 minutes with hematoxylin and eosin (HE). A picture microscope (Zeiss Axioscope 2 plus, USA) with a Canon ZoomBrowser EX digital camera was used to assess the stained sections qualitatively¹³.

Data analysis

All data in this study were analyzed with SPSS ver. 22. Two-way ANOVA was used to investigate its significance. Differences were deemed significant if *P* value < 0.05.

RESULTS

Pancreatic histopathology

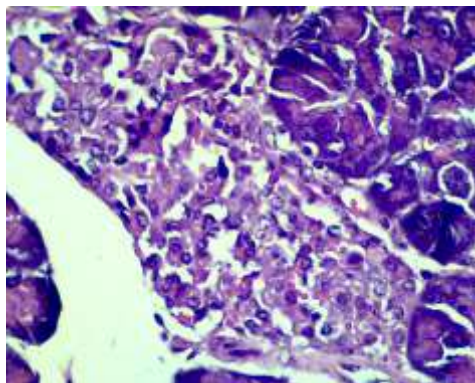


Figure 1: Histopathology of normal control group pancreatic islet cells with HE staining and 400x magnification

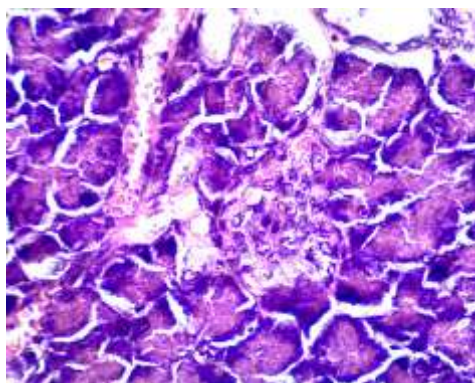


Figure 2: Histopathology of pancreatic islet cells in the diabetes control group by HE staining and 400x magnification

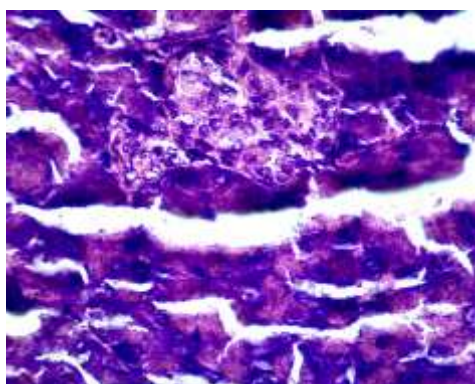


Figure 3: Photomicrographs of histopathological changes in pancreatic islet cells treated with dragon fruit peel dose 150 mg/kg BW dengan pewarnaan HE dan perbesaran 400x

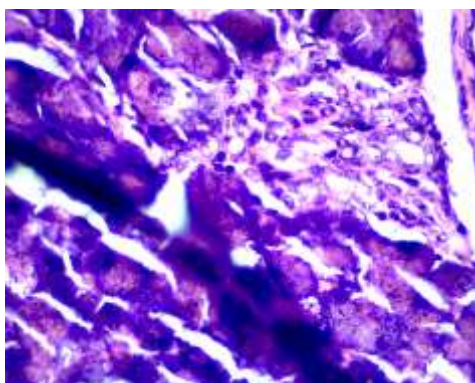


Figure 4: Photomicrographs of histopathological changes in pancreatic islet cells treated with dragon fruit peel dose 300 mg/kg BW with HE staining and 400x magnification

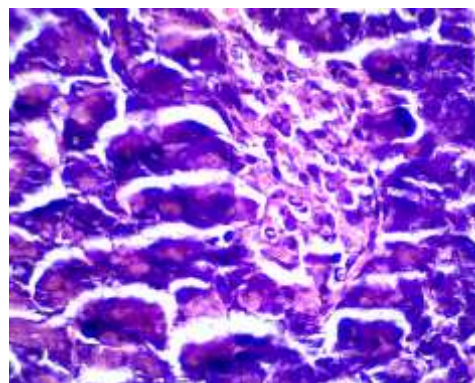


Figure 5: Histopathology of pancreatic islet cells positive control group with HE staining and 400x magnification

DISCUSSION

In this study, diabetes was induced in mice with the alloxan. The oxygenated pyrimidine derivative alloxan (2,4,5,6-tetraoxypyrimidine;2,4,5,6-pyrimidinetetrone) is aqueous as an alloxan hydrate. Alloxan has been used to create experimental diabetes by specifically damaging the insulin-producing beta-islets in the pancreas¹⁴ and established a diabetic experimental animal model which damaged the pancreatic or islets of Langerhans cells of the animal using numerous methods. The use of chemical agents can affect the performance of the pancreas, especially in the Langerhans islets. Injections of chemicals that affect insulin will cause a hyperglycemic effect.

In this study, dragon fruit peel extract was chosen to repair damage to the function of pancreatic β -cells. The sugar test shows that dragon fruit peel contains less sugar than dragon fruit flesh. Glucose in dragon fruit peel was measured at 30 mg/100 g and fructose at 10 mg/100 g. In contrast, the glucose of dragon fruit flesh is 193.33 mg/100 g and fructose 56.67 mg/100 g. Dragon fruit peel extract continues to be researched and developed as an antidiabetic because it is made from natural ingredients compared to antidiabetic drugs already on the market, such as glibenclamide which is the derivative of sulfonylurea. Glibenclamide works by stimulating insulin secretion by pancreatic beta cells. However, these treatment methods can cause side effects such as nausea, abdominal discomfort and anorexia¹⁵.

Glibenclamide(5-chloro-N-[2-[4-(cyclohexylcarbamoyl)sulfamoyl]phenyl]ethyl]-2-methoxybenzamide), also referred to as glyburide, is a new sulphonylurea oral hypoglycaemic drug for the treatment of non-ketotic maturity-onset diabetes mellitus¹⁶. It is a β -cell stimulant in the pancreas, but on the other hand, it increases insulin sensitivity or the degree to which it binds with target cells. It differs in that it is furthermore easier to endure. The hypoglycemic effect becomes apparent at significantly lower dosages than in first-generation drugs¹⁷.

Changes in the islets of Langerhans can be observed quantitatively, such as a decrease in beta cells and the size of the islets of Langerhans. Furthermore, qualitative observations showed necrosis, amyloidosis, and degeneration. Histopathology observation revealed that the diabetic mice induced by alloxan showed the tiny measurement of Langerhans islet and had pyknotic cells. Progressive changes in Langerhans's islets cause hyperglycemia, indicating diabetes has occurred. Langerhans islet has a central core of Insulin-containing B cells and a surrounding mantle of glucagon-containing A cells, the pancreatic polypeptide has the substance of PP cells, and somatostatin has a substance of D cells¹⁸. The diabetic Langerhans islet has vacuolizations and degeneration of pancreatic tissue besides degradation of B cells number. The size and constitution of the diabetic islet are

influenced by these two factors ¹⁹. Some previous studies showed histopathology Langerhans islet in diabetes that the effect of alloxan on beta cells causes necrosis and degeneration has even been reported to be 40-50% of beta cell necrosis ²⁰. Karyolytic beta cell nucleus, cytoplasmic components disintegration, cell boundaries are unclear, and a mass of debris containing nuclear fragments and necrosis ²¹. Amyloid deposition saw around 60-70% in the beta cells of the islets of Langerhans ²². In addition, the detachment of islets of Langerhans is accompanied by the replacement of exocrine cells by connective tissue (fibrosis). Treatment with glibenclamide (600 µg/kg BW) significantly reduced HbA1c levels, raised Hb levels, and enhanced the activity of enzymes involved in the metabolism of glucose, liver damage enzymes, and glycogen ²³. On the other hand, flavonoid chemicals in dragon fruit peel extract increase plasma insulin because their mechanism involves blocking phosphodiesterase, which causes cAMP levels in pancreatic cells to rise. This, in turn, causes insulin from Ca routes to be stimulated ²⁴.

CONCLUSION

Giving ethanol extract of red dragon fruit peel (*H. polyrhizus*) can improve the histopathology of the pancreas in mice with diabetes mellitus. Ethanol extract of red dragon fruit peel (*H. polyrhizus*) at a dose of 300 mg/kg BW is the optimum dose for repairing pancreatic cell damage in mice with diabetes mellitus.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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