

The effect of *Mezzetiaparviflora* Becc. extract on cell β regeneration in wistar rats' pancreas induced by streptozotocin

¹Jangga,²Ajeng Kurniati Roddu,³A. Indrawati

¹Faculty of Pharmacy,
¹Universitas Indonesia Timur, Makassar, Indonesia

Abstract—This research aims to know the effects of *Mezzetiaparviflora* Becc. extracts to the regeneration of Wistar rats pancreatic β cells that were induced by streptozotocin. This research uses an experimental design of randomized controlled group design. Data analysis was performed using a unpaired T-test to compare the number of rat pancreatic β cells between groups. The results showed that the *M. parviflora* extract affects to the regeneration of pancreatic β cells, there is no difference in the effect of dosing stratified *M. parviflora* extract toward pancreatic β cell regeneration, and there is no difference in treatment effect before and after induction of streptozotocin (STZ) against pancreatic β cell regeneration.

Index Terms—*M. parviflora*, pancreatic β cells, streptozotocin.

I. INTRODUCTION

Indonesia is one country that has quite high natural wealth, both flora and fauna. One of the potential flora that has been widely used by the community since ancient times is the use of plants as a medicine. Until now, the traditional use of herbs by the community in Indonesia is a genetic heritage. It is also supported by the natural wealth of Indonesia, also due to the diversity of the nation's cultural heritage, so it is necessary to develop the cultivation of medicinal plants that can be accounted for medically. For that research efforts should be encouraged so that the potential of drugs derived from natural materials can be disclosed, so that traditional medicine can be utilized by the community with more efficient to succeed.

Today, the topic of free radicals and antioxidants become a critical discussion of the medical because the presence of excessive oxidation reactions in the body initiate most diseases. The oxidation reaction occurs at any time and triggers the formation of free radicals that can damage the structure and function of cells. However, antioxidants may inhibit the reactivity of free radicals such as polyphenols.

Prevalence of diabetes in the world is increasing rapidly. In 2015, there were estimated 1.6 million deaths were directly caused by diabetes. The survey predicted that diabetes will be the seventh leading cause of death in 2030, with potential areas in Asia and Africa. Based on the survey ranked, Indonesia as the 4th largest DM patient in the world after India, China and the United States (Ministry of Health of the Republic of Indonesia, 2013) and in South Sulawesi DM in 2013 by 7% (Provincial Health Office of South Sulawesi, 2014).

Polyphenols as of the focus in this research have the effect of antiatherogenic, antithrombotic, anti-inflammatory, antihyperglycemic, can modulate the immune system, antimicrobial, analgesic, anti-cancer and may prevent cardiovascular disease [1][2]. Some plants contain polyphenol compounds and are used as DM drugs by people in Indonesia.. Ongkea (*Mezzetia parviflora* Becc.), Mangosteen (*Gracinia mangostana* Linn.), Lidah Buaya (*Aloe vera* Linn.), Brotowali (*Tinospora crispa* Linn.) and Mengkudu (*Morinda citrifolia* Linn.). With polyphenol content of 20.24% each, 16.21%, 5.62%, 4.34%, and 1.5%.

One of the plants containing polyphenols is *Mezzetiaparviflora*. Bau-Bau People in Buton Regency have used the bark decoction of *M. parviflora* as cholesterol-lowering drugs, slimming, a diabetes drug, and tumor drug.

Considering the potential and the high content of polyphenol compounds on *M. parviflora*, this research developed *M. parviflora* extract be standardized herbal medicine. The drug effect as antihyperglycemic and based on the pancreatic β cell regeneration on male Wistar rats. This animal sample was induced by STZ 40 mg/kg rats weight in a single dose. *M. parviflora* extract given in the form of a suspension with a dose of 100 mg/kg rats weight and 300 mg/kg rats weight.

II. MATERIAL AND METHODS

The research was conducted in the laboratory of Faculty of Pharmacy, Universitas Indonesia Timur from September to December 2016. This research uses an experimental design of randomized controlled group design. The study used the following tools: Analytical balance (Sartorius), mikropipet (Ependorf), a set of minor surgical instruments, *histopathologic* examination of *Haematoxyllin Eosin routine*, Uv-Vis (Hewlett Packard) spectrophotometer and binocular microscope.

Materials Research

Ingredients used in the study are Clinical ongkea, Wistarwistar white rats, Folin-Ciocalteu reagents, pH 4.5 buffer, STZ ALX-350-010 from ALEXIS Corporation, Hematoxylin Eosin histopathologic material.

Research Methods

There are Two groups were the control group and experimental group. The control group consists of group I (healthy control), group II (pain control), and group III (drug control) and experimental groups consist of groups IV, V, and VI.

Group I, day 1, the rats are fastened first and observed four tails, then given Na colloidal solution. CMC 1% orally daily for 28 days as healthy control, at day 7, day 14, day 21 and day 28, rats were fastened first and then observed each four tails.

Group II, day 1, the rats are fastened first and observed 4 tails, then induced STZ 40 mg/kg body weight of rats with single dose i.p. as sick control, on day 7, day 14, day 21 and day 28, the rats were fastened first and then observed each of 4 tails.

Group III, day 1, the rats are fastened first and observed four tails, then induced STZ 40 mg/kg body weight in single-dose rats on i.p. as a positive comparison. Starting the seventh day is given oral galvus daily for 21 days, and before being given the galvus on day 7, day 14, day 21 and day 28, the rats were fastened first then observed each four tails.

Group IV, day 1, the rats are fastened first and observed four tails, then induced STZ 40 mg/kg body weight of single-dose rats on i.p. Starting on the seventh day was given a suspension of 100 mg/kg body weight extract daily per day for 21 days, before being given polyphenol klikaongkea on day 7, day 14, day 21, and day 28, the rats were fastened first and then observed each of 4 tails.

Group V, day 1, the rats are fastened first and observed four tails, then induced STZ 40 mg/kg body weight in single-dose rats on i.p. Starting on the seventh day was given a suspension of 300 mg/kg body weight extract daily orally every day for 21 days, and before being given the extract of klikaongkea on day 7, day 14, day 21, and day 28, rats were fastened first and then observed each of 4 tails.

Group VI, starting on day minus 2, rats were given polyphenolic suspension onkea 300 mg/kg of body weight of oral rats every day for 30 days, on the first-day rats were fastened first and observed four tails, then induced STZ 40 mg/kg body weight single dose rats on i.p. Before giving the extract of klikaongkea on.

Data Analysis

Data analysis was performed using unpaired t-test to compare the number of rat pancreatic β cells between groups.

III. RESULT AND DISCUSSION

The observation of pancreatic β cell Wistar rats with Hematoxylin-eosin staining of as follows:

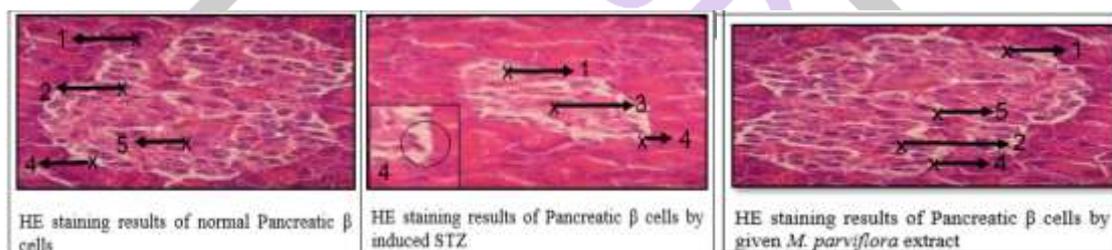


Figure 1. 1) The acinar lobules, 2) pancreatic β cells, 3) Atrophy of the islets of Langerhans 4) septum connective tissue, 5) Capillary

Fig 1 shows that after STZ induced, it can damage pancreatic endocrine cells especially β cells so that insulin secretion into the blood vessel decreases. The occurrence of necrosis and degeneration of the Langerhans islet is marked by the empty spaces in the middle of the island of Langerhans (3rd arrow). The empty spaces of the Langerhans islets due to the β cell necrosis. Necrosis is cell death due to fatal damage characterized by complete cell structure and cell damage followed by cell lysis and tissue inflammation, decreasing the number of pancreatic β cells indicates a disturbance of insulin metabolism in the pancreas.

The number of pancreatic β cells in the treatment group on day 7 decreased after STZ induced. In the control group, there was a decrease in the number of pancreatic β cells from 7th to 28th day with a mean value of 86.45 ± 5.09 to 50.07 ± 3.30 . According to STZ is a substance that can lead to alkylation that directly methylates DNA, causing DNA strand breaks, unscheduled DNA synthesis, DNA addition, chromosomal aberration, micronuclei, sister chromatid exchanges and pancreatic β cell death.[3][4].

On day 21 to day 28, the number of pancreatic β cells in the treatment group increased significantly compared to pain control ($p < 0.05$) due to pancreatic β cell regeneration (more β cell count). This is shown in the picture given ekstraklikaongkea. Pancreatic β cells are stable cell groups capable of proliferating throughout their lives, so that pancreatic β cells re-synthesize insulin.[5]

In the control group, the pain shows the occurrence of necrosis and degeneration is indicated by the decreasing number of pancreatic β cells. This is because in this group did not get the extract of klikaongkea. While in the treatment group the number of pancreatic β cells increased on day-to-14, day 21 and day-28.

The results in table 1 showed that the treatment 21 days indicated the mean number of pancreatic β cell Wistar rats between Group I and Group II was significantly different. The statistical value refer to $p = 0.015 < 0.05$. The Group I to Group III, IV, V and VI were not significantly different with p-value 0.932 respectively; 0.481; 0.281 and 0.253 > 0.05 . While the group II to Group III, IV, V and VI significantly different where the p-value 0.000 respectively; 0,002; 0,000 and 0,000 $< 0,05$. Between-Group III to Group IV, V, and VI were not significantly different with p-value 0.311 respectively; 0.056 and 0.054 > 0.05 . Meanwhile, the Group IV to Group V and Group VI were not significantly where the p-value respectively 0.669 and 0.600 > 0.05 and between the Group V to Group VI was also not significantly different with $p = 0.890 > 0.05$. After 21 days treatment, the *M. parviflora* extract affected the increasing number of cell β pancreas, There is no difference in the influence of dosing multilevel against an increasing

number of β cell pancreas, and there is no difference in the influence of the treatment before and after the induction of STZ against pancreatic β cell regeneration.

Table 1. The Observation of The Average Number of Pancreatic β Cells Per Group

Days to	The mean of pancreatic β cells number					
	Group I	Group II	Group III	Group IV	Group V	Group VI
1	87,35 \pm 5,50	87,75 \pm 5,21	87,77 \pm 8,43	88,07 \pm 4,67	88,10 \pm 5,33	89,40 \pm 9,02
7	87,12 \pm 5,57	86,45 \pm 5,09	84,62 \pm 4,17	84,72 \pm 5,02	84,52 \pm 5,07	84,85 \pm 4,96
14	88,20 \pm 5,19	79,57 \pm 6,80	84,55 \pm 6,17	85,57 \pm 1,36	86,02 \pm 3,79	87,97 \pm 4,59
21	83,50 \pm 10,48	64,07 \pm 4,97	84,00 \pm 2,59	88,30 \pm 7,32	90,20 \pm 4,20	90,62 \pm 4,10
28	89,10 \pm 5,62	50,07 \pm 3,30	86,05 \pm 2,19	89,70 \pm 5,65	92,45 \pm 3,27	93,70 \pm 3,98

The number of pancreatic β cells Group III, Group IV, Group V and Group VI on the 7th day decreased after STZ induced. In the group of Group II (pain control) decrease, the number of pancreatic β cells started 7th, up to day 28 with a mean value of 86.45 \pm 50.07 \pm 5.09 becomes 3.30.

STZ is a substance that can lead directly methylation alkylating DNA, causing DNA strand breaks, unscheduled DNA synthesis, the addition of DNA, chromosomal aberrations, micronuclei, sister chromatid exchanges and pancreatic β cell death (Muhilal, 1991).

On day 21 and all 28 the number of pancreatic β cells in Group III, Group IV, Group V and Group VI has increased significantly compared to the Group II ($p < 0.05$) due to the regeneration of pancreatic β cells (the number of β cells more). Pancreatic β cell is a stable cell type capable of proliferating throughout his life, so the pancreatic β cells back to synthesize insulin (McGavin and Zachary, 2007). Statistically, the number of pancreatic β cells at Group I, Group III, Group IV, Group V and Group VI showed no significant differences with Group II ($p < 0.05$). The average number of pancreatic β cells showed an increase in Group I, Group III, Group IV, Group V and Group VI with mean of each group is 89.10 \pm 5.62; 86.05 \pm 2.19; 89.70 \pm 5.65; 92.45 \pm 3.27 and 93.70 \pm 3.98 compared with KII with the average of 50.07 \pm 3.30.

Repair pancreatic β -cell function characterized by an increase in the mean number of β cells in the pancreas *M. parviflora* extract, starting on day 14. Neogenesis of cells can occur as a result of the normalization of blood glucose-mediated insulin. Two types of precursor cells will appear in regenerating islets of Langerhans cells. One type of express glucose transporter-2 (Glut 2) and other types express insulin and somatostatin. Both of these cells then become monospecific cells containing insulin and fill in the islets of Langerhans are damaged / empty[6].

Improvements islets of Langerhans in ongkea Klika extract treatment followed by a regeneration of the cells in the islets of Langerhans which is characterized by increasing the number of pancreatic β cells. Extract treatment Klika ongkea 100 mg/kg body weight of mice, 300 mg/kg rat, and 300 mg/kg treated mice on day minus-2 indicates an increase in the number of cell β pancreas is higher than with Group I (control) and with Group III by drug galvus 0.9 mg / 200 g BB rats. Increasing the number of pancreatic β cells suspected to be affected by the increasing number of bioactive compounds along with increasing dose. Increasing doses resulted in an increased number of bioactive compounds contained in the extract.

Increasing the number of pancreatic β cells in the treatment Group IV, Group V and Group VI suspected because of the bioactive compounds in the extract group Klika ongkea as polyphenols (flavonoids, tannins) and alkaloids; these bioactive compounds can act as antioxidants. Antioxidants are involved in the repair process of damaged cells. Cell damage caused by the free radicals can be overcome by the presence of antioxidants which serves as an oxidizing agent before lowering and lowering the damage the cell so that the cell damage can be reduced[7] Polyphenols are known to play a role capturing free radicals or function as natural antioxidants [8]. The antioxidant activity allows the polyphenols to capture or neutralize free radicals (such as ROS or RNS) associated with phenolic OH groups to improve the situation of damaged tissue, in other words, the inflammatory process can be inhibited. Flavonoids may play a role in pancreatic tissue repair damage caused by alkylating DNA induced by STZ, as a result, can repair rat pancreatic morphology. Flavonoids are reported to have an antidiabetic activity that can regenerate islets of Langerhans cells[9].

IV. CONCLUSIONS

Based on the results of research and discussion it can be concluded that the *M. parviflora* extract effect on cell regeneration β pancreas. There is no difference effect of dosing stratified *M. parviflora* extract against cell regeneration β pancreas. Furthermore, there is no difference in treatment effect before and after induction of STZ to regenerate cells pancreatic β .

REFERENCES

- [1] P. Yin *et al.*, "Hypoglycemic and hypolipidemic effects of polyphenols from burs of *Castanea mollissima* Blume," *Molecules*, vol. 16, no. 11, pp. 9764–9774, 2011.
- [2] C. L. Keen, R. R. Holt, P. I. Oteiza, C. G. Fraga, and H. H. Schmitz, "Cocoa antioxidants and cardiovascular health," *Am. J. Clin. Nutr.*, vol. 81, no. 1, p. 298S–303S, 2005.
- [3] A. M. A. Ahmed, F. Sharmen, A. Mannan, and M. A. Rahman, "Journal of Traditional and Complementary Medicine," *J. Tradit. Complement. Med.* xxx, p. 1e10, 2016.
- [4] M. Islam and Q. R. Code, "Streptozotocin is more convenient than Alloxan for the induction of Type 2 diabetes," *IJPR*, vol. 7, no. 1, 2017.

- [5] I. Matias *et al.*, “Regulation, function, and dysregulation of endocannabinoids in models of adipose and β -pancreatic cells and in obesity and hyperglycemia,” *J. Clin. Endocrinol. Metab.*, vol. 91, no. 8, pp. 3171–3180, 2006.
- [6] Y. Guz, I. Nasir, and G. Teitelman, “Regeneration of pancreatic β cells from intra-islet precursor cells in an experimental model of diabetes,” *Endocrinology*, vol. 142, no. 11, pp. 4956–4968, 2001.
- [7] D.-F. Dai *et al.*, “Mitochondrial targeted antioxidant peptide ameliorates hypertensive cardiomyopathy,” *J. Am. Coll. Cardiol.*, vol. 58, no. 1, pp. 73–82, 2011.
- [8] A. Lugasi, J. Hóvári, K. V Sági, and L. Bíró, “The role of antioxidant phytonutrients in the prevention of diseases,” *Acta Biol. Szeged.*, vol. 47, no. 1–4, pp. 119–125, 2003.
- [9] H. K. Sandhar, B. Kumar, S. Prasher, P. Tiwari, M. Salhan, and P. Sharma, “A review of phytochemistry and pharmacology of flavonoids,” *Int. Pharm. Sci.*, vol. 1, no. 1, pp. 25–41, 2011.

