

Potential of Binahong (*Anredera cordifolia* [Tenore] Steen) in Reducing TNF- α Expression on Regeneration of Pancreas β Cells on White Rats (*Rattus norvegicus*) Diabetes Mellitus Models

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Abstract

Diabetes mellitus (DM) is a metabolic disorder characterized by increased blood glucose levels (hyperglycemia) due to impaired insulin secretion and/or increased insulin resistance. Providing binahong leaf steeping (*Anredera cordifolia* [Tenore] Steen) is one alternative treatment for people with diabetes mellitus using medicinal plants. This research was conducted to find out the right concentration by administering binahong leaf steeping to reduce hyperglycemia in STZ induced rat and to analyze administration of binahong leaf steeping in reducing TNF- α expression in pancreatic β cell regeneration. This study used 25 white rats (*Rattus norvegicus*) which were divided into 5 groups: group 1, negative control (K⁻), group 2, positive control (K⁺), groups 3, 4, and 5. With STZ induction, each is given a dose of binahong leaf steeping, 50.4 mg/180ml of water; 100.8 mg/180ml of water and 151.2 mg/180ml of water for 14 days. The results showed that the administration of binahong leaf steeping to STZ induced rat could reduce glucose rats in a row by 130.00, respectively; 117,23 and 112,45 and steeping binahong leaves (*Anredera cordifolia* [Ten] S) significantly influence the reduction in TNF- α expression in pancreatic β cell regeneration. Thus, this study can be concluded that the right dose to reduce hyperglycemic and regeneration of pancreatic β cells produced by STZ induced rats is 151.2 mg/180ml of water and statistically influences in reducing TNF- α expression in pancreatic β cell regeneration.

Keywords

Diabetes Mellitus, (*Anredera cordifolia* [Tenore] Steen), Streptozotocin (STZ), Cell β Pancreas, TNF- α

1. Introduction

One of the plants that have many benefits in treating diseases is binahong plant (*Anredera cordifolia* [Tenore] Steen). According to Rochani, N. 2007 [1], binahong leaves have active compounds of alkaloids, saponins, and flavonoids. Manoi, F. 2009 [2] states that all parts of the binahong plant can be used as medicine, starting from the stem, roots, flowers, and leaves. However, the most commonly used for health as herbal medicine is the leaves. Shabella, R. 2012 [3] states that in the community binahong leaves are used to treat pain, ulcers, and canker sores, and provide extra stamina, blood circulation, and gout. Besides consuming binahong can also overcome swelling and blood clotting, treat diabetes mellitus, lower cholesterol, and heal wounds.

The results of research Kemila, M. 2010 [4] state that the flavonoids contained in the infusion of binahong leaves are thought to act as antioxidants that capture free radicals from diabetes mellitus-inducing substances, namely alloxan. The antioxidant activity is thought to be a mechanism in overcoming the working effects of alloxan and influencing the decrease in blood glucose levels of alloxan-induced rat. Based on research by Rendon, A.J., *et al.*, 2006 [5], the administration of triterpenoid compounds isolated from binahong leaves is also able to stimulate a decrease in blood glucose levels of rat that have hyperglycemia.

Plasma concentrations of acute phase proteins are largely dependent on liver biosynthesis of these proteins, and changes in production are influenced by proinflammatory cytokines such as IL-1, IL-6 and TNF- α . These cytokines are produced during the inflammatory process and are stimulators of acute phase proteins and are markers of chronic inflammation that are often detected in cardiovascular disease, diabetes mellitus, osteoarthritis and rheumatoid arthritis [6] [7]. Diabetes has been shown to be associated with high levels of serum inflammatory cytokines, namely TNF- α and IL-1 β . Other results showed that there was an increase in the expression of TNF- α and IL-1 β in alveolar bone osteoblast cells in a rat model of diabetes induced by Streptozotocin [8]. Increased TNF- α is also associated with poor glycemic control in humans [9]. TNF- α and IL-1 β that increase in diabetes can be a clinical marker of periodontal abnormalities, which is one of the manifestations and complications of diabetes. An *in vitro* study showed that, two major cytokines produced by macrophages, namely IL-1 and TNF- α , cause structural changes of pancreatic β cells and decrease the capacity of insulin secretion. Biochemical changes in the condition of diabetes also seem to influence the increase in TNF- α and IL-1 β . Increased glucose levels in cells cause excessive production of ROS by mitochondria [10].

Diabetes with a longer duration will cause a significant increase in ROS compared with a shorter duration of diabetes. Increased ROS will cause excessive oxidants to form and can cause PARP activation through DNA breakdown. This PARP activation will result in inhibition of GAPDH and result in an increase in the polyoldane and hexosamine pathways [10]. Increased pathway can cause an increase in non-enzymatic glycation, excessive AGEs production, oxidative stress, and synthesis of diacylglycerol (DAG), which in turn activates protein kinase C (PKC). This activated PKC will activate NF κ B to stimulate pro-inflammatory genes to release inflammatory mediators, such as TNF- α and IL-1 β [11]. Not being separated also the condition of prolonged hyperglycemia will cause the accumulation of AGEs, where the accumulation of AGEs will make endothelial cells and monocytes more easily stimulated, which will make these cells produce inflammatory mediators in large numbers [12].

Based on the descriptions above, it is suspected that binahong plants have a phytopharmaca effect. This can prevent the active NF κ B from stimulating pro-inflammatory genes to release inflammatory mediators, such as TNF- α due to streptozotocin-induced. Therefore, it is necessary to conduct research on binahong so that it can be used by the community as an alternative medicine in preventing or treating pancreatic beta cell damage due to diabetes mellitus.

2. Materials and Methods

2.1. Research Design, Location, and Research Time

This research is an experimental study using pre and posttest control group design [13]. The study was conducted at the Zoology Laboratory and Material Laboratory of the Faculty of Mathematics and Natural Sciences, Ambon-Maluku Pattimura University, and histopathological observations and immunohistochemistry tests were carried out at the Pathology laboratory, Faculty of Medicine, Gajah Mada University, Jogjakarta and this research was carried out for three months starting in June-August 2017.

2.2. Experimental Animal

The population of this study was 25 white rats (*Rattus norvegicus*). In this study, rats were divided into two control groups and three treatment groups, with the number of samples per group of 5, so that the total sample was 25 rats. This study uses a purposive sampling technique and simple random sampling grouping. The sample size in this study was calculated using the Federer formula to determine how many rats would be used [14]. This research was conducted using 25 male white rats weighing $\pm 150 - 300$ grams and aged $\pm 2 - 3$ months. This white rat is kept in the Zoology Laboratory of the Faculty of Mathematics and Natural Sciences, Pattimura University, which is well ventilated under standard conditions of humidity, temperature and light. These white rats are handled according to experimental animal rearing standards. Each white rat is fed a standard (standard feed) and given an ad libitum drink. Before treatment, all animals

try to acclimatize for 2 weeks. Try animals adapted to the environment \pm for 1 week. All animals were kept in the same way and before treatment; all rats were left for 8 - 12 hours. Before giving treatment all experimental animals were weighed in advance to calculate the dosage setting.

The working procedure in rat is carried out as follows: 1) Rats fasted for 8 - 12 hours, while still being given a drink, 2) Rat induced STZ 186.9 mg/kg BW in intraperitoneal, 3) Glucose levels were observed on the third day to find experimental animals which has diabetes mellitus. Rats with blood sugar levels above 200 mg/dl are used in the study, 4) Rats were weighed and grouped into 5 groups (2 control groups and 3 test groups) where each group consisted of 5 rats. 5) Then rats with diabetes were treated according to their treatment group within 1 day, 6) Blood glucose in rat was measured at 0, 2, 4, 6, & 24 hour intervals. Blood samples are taken by injuring the tail tip of the rat, and then checked with a glucometer (Easy Touch® GCU) with data written as mean \pm SD.

2.3. Plant Preparation (Extraction)

Simplisia dried binahong leaf (*Anredera cordifolia* [Ten] S) was cleaned, then put in a maserator, maceration was carried out with 70% ethanol solvent and allowed to stand for \pm 30 minutes. Maserat is separated by filtration. The search process is repeated at least twice with the same amount and type of solvent until the solvent is clear. All maserates are collected, the solvent is evaporated with a vacuum or low pressure evaporator (rotary evaporator) until a thick extract is obtained. After the heavy viscous extract constant, microcell and corn starch dryers were added in a ratio of 60:40.

2.4. Experimental Design

In the activity test binahong leaf extract (*Anredera cordifolia* [Ten] S) begins by inducing rat with STZ, then given binahong leaf extract (*Anredera cordifolia* [Ten] S) by administering an oral dose once a day. In this study, there were 5 groups in the treatment, namely group 1, negative control (K-) was given distilled water (aquades); group 2, positive control (K+) given glibenclamide dose 3 mg/200g body weight of rat; groups 3, 4 and 5, the results of STZ induction, were given a dose of steeping binahong leaves each 50.4 mg/180ml of water; 100.8 mg/180ml of water and 151.2 mg/180ml of water for 14 days. All experimental protocols for experimental animals are in accordance with animal welfare guidelines. Ethical clearance of research has been obtained from the Health Research Ethics Commission (HREC), Faculty of Medicine, Diponegoro University and Dr. General Central Hospital Kariadi Semarang, dated April 16, 2010.

2.5. Immunochemical Procedure

The preparations are immersed in xylol 2 times, sequential alcohol (96%, 90%, 80%, and 70%) for the hydration process; Washed in PBS pH 7.4 3 times each

for 5 minutes; Soaked in 3% hydrogen peroxide (in destilate water) for 20 minutes; Washed in PBS pH 7.4 for 3 × 5 minutes. Soak in 1% BSA for 10 - 30 minutes at room temperature; Washed in PBS pH 7.4 for 3 × 5 minutes. Primary antibodies are added for 1 hour at room temperature; then incubated overnight; then washed in PBS pH 7.4 for 3 × 5 minutes. Then added a secondary antibody labeled Strep avidin horseradish peroxidase (SA-HRP) for 1 hour at room temperature; Washed in PBS pH 7.4 for 3 × 5 minutes. Preparations are added chromogen DAB (3,3-diaminobenzidine tetrahydrochloride) for 10 - 20 minutes at room temperature; Wash in distilled water for 3 × 5 minutes; Counterstained with hematoxylin for 5 minutes at room temperature; Washed in distilled water for 3 × 5 minutes; Mounting with insert; Observation using an optical microscope at magnification of 100 and 400 times.

2.6. Histological Analysis

After 14 day, all rats were deuterped and neutralized. Organs are removed and examined for further processing in the preparation of histopathological preparations. The liver is removed and cut into 1 × 1 × 1 cm size, then fixed in 10% NBF for 24 hours. After streaming, it is then put into each tissue cassette according to the treatment group. Tissue cassettes are inserted into the tissue processor for the stages of dehydration, clearing, and embedding. Meanwhile, the blocking stage is carried out with a paraffin block, then cutting with a microtome in a thickness of 5 - 6 μ. Furthermore, the preparations were stained with Hematoxylin & Eosin (HE) staining. After drying and covered with a glass cover, the preparations are ready to be examined under a microscope.

2.7. Statistical Analysis

Descriptive Data: Histopathological evaluation was carried out on changes and population of pancreatic endocrine cells in the island of Langerhans using Hematoxylin-Eosin (HE) staining as well as immunohistochemical staining with anti-insulin antibodies.

Inferential Data: The effectiveness of decreased blood glucose by binahong leaf (*Anredera cordifolia* [Ten] S) compared with negative controls, was processed as mean ± SD. Mean differences from each group were statistically analyzed using One Way ANOVA. To assess the statistical hypothesis, the calculated p price is determined to be compared with a 95% confidence level ($\alpha = 0.05$).

3. Result

3.1. Measurement of Rat Blood Sugar Levels

The administration of binahong (*Anredera cordifolia* [Ten] S) steeping to white rat suffering from diabetes mellitus can affect blood sugar levels. The following is the average data from the measurement of blood sugar levels of diabetes mellitus rats before and after administration of binahong steeping.

From **Figure 1** it can be seen that after STZ administration, post STZ blood glucose values increased above the normal range of 70 - 110 mg/dl, compared with negative control blood glucose (K-). An increase in blood glucose in each treatment group due to a diabetogenic drug that is STZ monohydrate from the results of the measurement of blood glucose levels day 0 glucose levels in 5 groups under normal conditions namely group K(-) 97.67 mg/dl, K(+) 125.67 mg/dl, P1 (dose 50.4 mg/180ml water) 119.33 mg/dl, P2 (dose 100.8 mg/180ml water) 115.67 mg/dl, P3 (151.2 mg/180ml water) 108.33 mg/dl. Then from 4 groups STZ was induced in group K(+) and treatment group. From the induction results, the mean data in group K(+) increased by 285.00, (P1) 278.33 mg/dl, (P2) 249.33 mg/dl, (P3) 219 mg/dl.

3.2. Cell β Histology Overview Langerhans Island Pancreas Rat Given Steeping Binahong Leaves

The following is a description of β cell histology Langerhans Island Pancreas rat given steeping binahong leaves, as follows:

Histological picture of pancreatic organs of white rat with Hematoxylin-Eosin (HE) staining showed that in the positive control group rats suffered β cell damage in the island of langerhans (**Figure 2(b)**). In the group of rat fed binahong leaf steeping with a dose of 50.4 mg/180ml of water (**Figure 2(c)**) showed the regeneration of cells in the island of Langerhans although there was still visible damage in the form of necrosis, edema, atrophy and β cell nuclei undergoing karyopicnotis.

Whereas in the group of rats given binahong leaf steeping (**Figure 2(d)**) dose of 100.8 mg/180ml of water and 151.2 mg/180ml of water (**Figure 2(e)**) shows the regeneration of cells that can be seen from the number of cells that are stained on the island of Langerhans so that it can be seen the lack of damage that occurs in the histological picture of the pancreas can be compared with the histological picture of the pancreas in positive control rat.

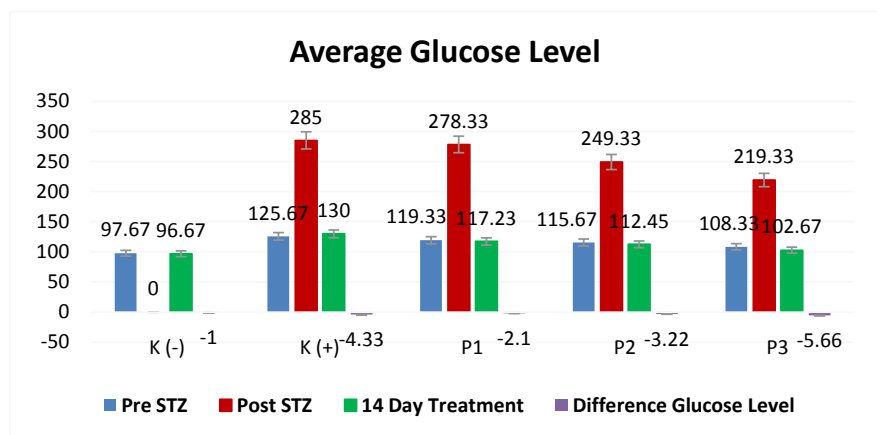


Figure 1. A mean diagram of steeping binahong leaves on a decrease in rat blood sugar levels.

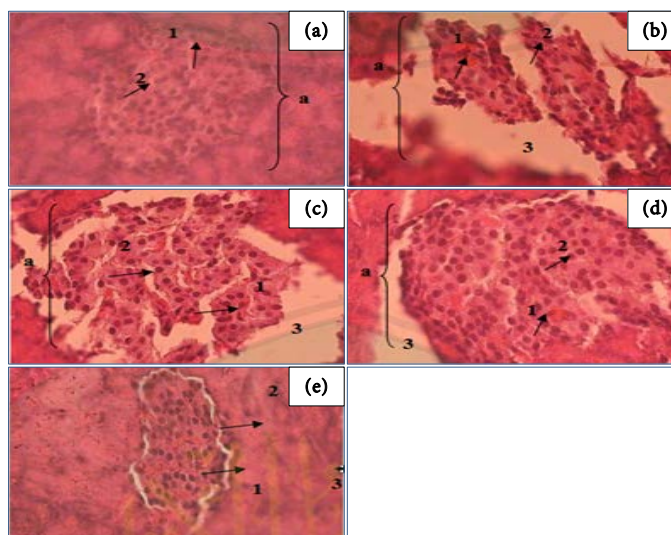


Figure 2. Histology of white rat pancreas. (a) Rat Group K (+), (b) Rat Group K (-), (c) Group P1 (d) Group P2, (e) Group P3. Langerhans island; 1 = alpha cell (irregular nucleus); 2 = beta cells (large and round nucleus); 3 = empty space due to necrosis. Note: (a) 200× magnification; (b, c, d) and (e) 400× magnification. The darker color of the Langerhans Island indicates the presence of insulin secreted by pancreatic β cells in the Langerhans Island. The black arrow is the center of the Langerhans Island. Magnification 40×.

3.3. TNF- α Expression in β -Pancreatic Cell Photomicrographs through Immunohistochemical Testing

Tumor Necrosis Factor (TNF- α) expression in white rat pancreatic histology with immunohistochemical staining before treatment, after being given STZ and after being treated with steeping binahong leaves showed significantly different results.

The group K(-) showed less TNF- α expression compared to the group K(+) had decreased TNF- α expression. This shows the expression of TNF- α appears under normal conditions needed to defend the condition of homeostasis in the immune system. In **Figures 3(c)-(e)** an increase in TNF- α expression is indicated by the number of pancreatic beta cells that have increased. TNF- α expression in rat pancreatic cells is shown by the presence of brown in the immunohistochemical (IHC) picture of pancreatic cells.

The administration of binahong (*Anredera cordifolia* [Ten] S) leaves in white rat (*Rattus norvegicus*) gives an effect in the form of an increase in pancreatic β cells in rat pancreatic cells with an average increase in β cells in the dose group of 50.4 mg/180ml, 100.8 mg/180ml of water and 151.2 mg/180ml of water when compared to the group that was only given STZ (positive control). The number of rat pancreatic cells expressed by TNF- α can be seen in the following **Table 1**.

Based on research data binahong steeping leaves significantly ($p < 0.05$) can increase pancreatic β regeneration in pancreatic damage in rat-induced STZ. Seen in **Table 1** of the three treatment doses of steeping binahong leaves, a dose

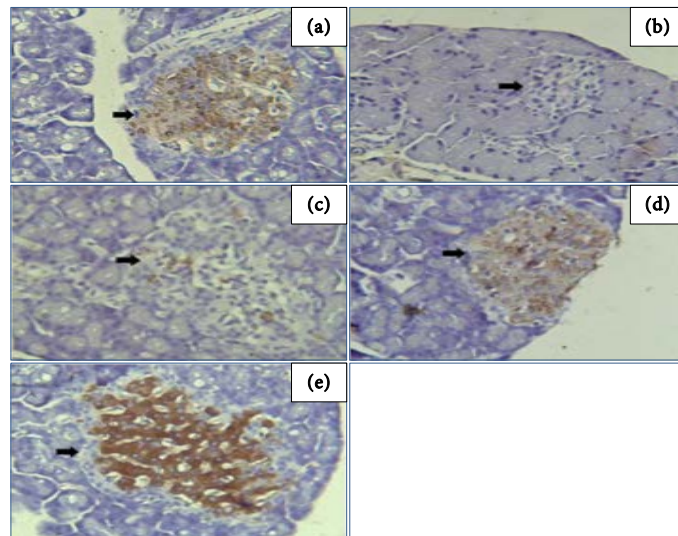


Figure 3. Histology of white rat pancreatic cells expressed by TNF- α . (a) Langerhans island rat control (-); (b) Langerhans island control (+), (c) Langerhans island rats were given steeping binahong leaves at a dose of 50.4 mg/180ml; (d) Langerhans island rats were given steeping binahong leaves 100.8 mg/180ml of water; (e) Langerhans island rat was given steeping binahong leaves 151.2 mg/180ml of water. The black arrows: β cells express TNF- α with brown spots. 400 \times magnification.

Table 1. Number of rat pancreatic cells expressed by TNF- α .

Group	Mean cell β Pancreas (\pm SD)
Control (-)	33.00 \pm 1.528a
Control (+)	12.33 \pm 2.517b
P1	25.00 \pm 5.000c
P2	30.33 \pm 2.517d
P3	32.67 \pm 1.155d

Information: Superscript with the same letter, shows no significant difference ($p < 0.05$).

of 151.2 mg/180ml is the dose that shows the results closest to the negative control rat seen from a decrease in blood glucose levels and repair of pancreatic tissue. At a dose of 151.2 mg/180ml it is better than a dose of 50.4 mg/180ml. This is probably related to the large content of compounds and active ingredients present in the complex leaves of binahong steeping, each of which can work non-specifically on rats induced by STZ.

4. Discussion

The increase in glucose in group K(+) and treatment caused by STZ diabetogenic compounds, which are caused due to pancreatic β cell damage. The workings of STZ damage cells which are analogs that accumulate in pancreatic β cells through the process of glucose GLUT2 into the cytosol which will generate reactive oxygen species (ROS) with a reaction cycle that produces a dilauric acid reac-

tion that will experience a redox cycle, the redox cycle then forms radicals superoxide which mutates to produce hydrogen peroxide and the final stage will undergo an iron catalyst reaction process to form hydroxyl radical compounds. Hydroxy radicals will have an impact on damage to pancreatic β cells resulting in the occurrence of insulin dependent diabetes mellitus [15].

The treatment by administering binahong steeping in 3 groups, showed the results of a decrease in blood glucose levels which were initially hyperglycemic. In group P1 to 115.00 mg/dl, P2 to 116.00 mg/dl, P3 to 102.67 mg/dl while in group K(+) there was an increase in glucose to 130.00 mg/dl. Based on the results of the Analysis of Variants (ANOVA) using the SPSS 17 program, it was shown that binahong leaf steeping influenced the decrease in blood sugar levels of diabetic rat mellitus. Furthermore, based on the results of further tests showed in each treatment group with a dose of 50.4 mg/180ml of water, 100.8 mg/180ml of water and 151.2 mg/180ml of water were significantly different between the control and each treatment group. A dose of 151.2 mg/180ml of water was significant in the reduction of STZ induced hyperglycemic rat.

This can be seen from the percentage of endocrine cells experiencing relatively reduced necrosis (indicated by the reduction of empty space due to necrosis) and the presence of endocrine cells that remain in normal conditions. Qualitatively, this shows an increase in the number of more cells, especially β cells. This condition indicates the endocrine cell regeneration process although there are still some endocrine cells that have degenerated but the amount is less than the diabetic group without steeping. Repair of pancreatic β cells is related to the presence of bioactive compounds contained in the leaves of binahong steeping namely the content of flavonoids which are included in the group of polyphenol compounds which are proven to have antioxidant activity. According to Suryani, C.L., Tamaroh, S. 2015 [16] antioxidant activity is able to capture free radicals causing pancreatic β cell damage and inhibit pancreatic β cell damage so that the remaining β cells still function. Antioxidants are thought to be able to protect a number of β cells that remain normal so as to enable the regeneration of β cells that still exist through the process of mitosis or through the formation of new islets by means of endocrine proliferation and differentiation from ductal and ductular cells. An improvement in insulin-producing β cells, an increase in the amount of insulin in the body that can facilitate the entry of blood glucose into cells so that there is a decrease in blood glucose levels in the body.

Brown cell expression was found most in the group of rat given treatment group 3 (P3). TNF- α expression on white rat pancreas β cells after being given steeping binahong leaves showed the highest increase in number of pancreatic β cells with treatment group 3 (P3), treatment 2 (P2) and the lowest decrease in treatment group 1 (P1). The emergence of the brown color is caused in the process of staining Immunohistochemistry of antigens in pancreatic cells bound to the primary antibody then labeled by secondary antibodies (Goat Anti Ratbiotin labeled), after all binding the addition of a diaminobenzidine substrate (DAB) which aims to produce a brown color on cytokines (TNF- α is labeled), after all

binding the addition of a diaminobenzidine substrate (DAB) which aims to produce a brown color in cytokines (TNF- α) [17].

Binahong leaf steeping contains compounds that have antioxidant activity as free radical scavengers. Capture of free radicals by compounds contained in binahong leaves causes reduced damage to the pancreatic tissue, so that infiltration of mononuclear cells into the pancreatic tissue in the process of phagocytosis of damaged β cells is also reduced. This situation causes a decrease in the inflammatory process so that TNF- α production also rises and pancreas β cells repair so as to produce insulin.

Regeneration that occurs in the treatment group is gradually thought to be due to the presence of flavonoid bioactive compounds. This opinion is supported by the statement of Prameswari, O.M., Widjanarko, S.B. 2014 [18] stating that Flavonoids have antidiabetic activity that is able to regenerate cells on the island of Langerhans. Research conducted by Ifridah, Y.L., 2014 [19] also suggests that flavonoids can play a role in regulating blood sugar reduction and increasing the improvement of the distribution of insulin-producing β cells in Langerhans Island through Hematoxylin-Eosin (HE) staining. Furthermore Arjadi F. 2010 [20] stated that flavonoids can cause the regeneration of islets of Langerhans repairing β cells, stimulating insulin release and/or as insulin-like compounds.

Based on the analysis of research data, binahong leaf steeping significantly ($p < 0.05$) can increase pancreatic β regeneration in pancreatic damage in rat-induced STZ. Seen in **Table 1** of the three treatment doses of steeping binahong leaves, a dose of 151.2 mg/180ml is the dose that shows the closest result to a negative control rat. That is because there is a decrease in blood glucose levels and repair of pancreatic tissue. At a dose of 151.2 mg/180ml it is better than a dose of 50, 4 mg/180ml. This is due to the high content of compounds and active ingredients present in the complex leaves of binahong steeping, each of which can work non-specifically on rats induced by STZ.

Decreased degree of insulin can occur due to repair of damage to pancreatic tissue. Pancreatic β cell regeneration, presumably due to flavonoid compounds contained in the leaves of binahong acts as inhibitors so as to prevent the production of TNF- α [2]. Flavonoids stimulate a 16% increase in insulin secretion from pancreatic β cells. The action is obtained through the regulation of peroxisome proliferators activated receptors (PPAR α and PPAR γ). The action of beneficial flavonoids in the condition of diabetes mellitus is through its ability to avoid glucose absorption or improve glucose tolerance. Furthermore, flavonoids stimulate glucose uptake in peripheral tissues, regulate the activity and expression of enzymes involved in carbohydrate metabolic pathways and act like insulin, by influencing the signaling mechanism of insulin [21].

Flavonoids are able to regenerate pancreatic β cells and help stimulate insulin secretion [22]. Other mechanisms of flavonoids that show hypoglycemic effects are reducing glucose absorption and regulating the activity of the expression of enzymes involved in carbohydrate metabolism [23]. There are several mechan-

isms of action of oral hypoglycemic drugs, namely increasing insulin secretion (sulfonylureas), increasing insulin receptor sensitivity so that glucose absorption in peripheral tissue increases, increasing insulin sensitivity of muscle tissue, fat and liver tissue, and inhibiting the breakdown of polysaccharides into monosaccharides. Flavonoids have the same mechanism as oral hypoglycemic drugs in the sulfonylurea group in reducing rat blood glucose levels by increasing insulin secretion in pancreatic organs [24].

Flavonoids can prevent complications or progression of diabetes mellitus by cleaning up excessive free radicals, breaking the chain of free radical reactions, binding of metal ions (chelating) and blocking the polyol pathway by inhibiting the enzyme aldose reductase. Flavonoids also have an inhibitory effect on the α -glucosidase enzyme through hydroxylation bonds and substitution in the β ring. The principle of inhibition is similar to acarbose which has been used as a drug for the treatment of diabetes mellitus, which results in a delay in the hydrolysis of carbohydrates, disaccharides and glucose absorption and inhibits the metabolism of sucrose into glucose and fructose [25]. Flavonoids are powerful inhibitors of the α -amylase enzyme that function for the breakdown of carbohydrates. The inhibitory power of this enzyme causes the process of breaking down and absorption of carbohydrates will be disrupted, so that blood sugar levels can be reduced [26].

Some of the conditions that must be met from the immunohistochemical method are that the active ingredient must be able to form antibodies that are specific to the active ingredient to be included. The active ingredient must also accumulate in sufficient quantities in cells or tissues so that it can be bound by specific antibodies and can be visualized [27]. In pancreatic histopathological preparations, white rats can be observed by immunohistochemical methods. In pancreatic histopathological tissues, rat pancreas can be brown in color. The brown color is the result of interactions between antigens that bind with primary antibodies (anti-30 kDa and anti-whole protein and secondary antibodies and substrate diaminobenzidine (DAB). Chromogen DAB (3,3-diaminobenzidine tetrahydrochloride) contains H_2O_2 peroxide as a signaling substance that will form a complex with the peroxidase enzyme. The complex formed from DAB chromogen will form a dark brown color; this chromogen has a very strong bond with peroxide so that the dehydration and clearing process will not change color.

5. Conclusion

Based on the results of research that has been done, it can be concluded that (a) the right concentration of steeping binahong leaves (*Anredera cordifolia* [Ten] S) in reducing hyperglycemia in STZ induced rat is a dose of 151.2 mg/180ml of water, and (b) administration of steeping binahong leaves (*Anredera cordifolia* [Ten] S) has a statistically significant effect in reducing TNF- α expression in rat pancreatic β cell regeneration.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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