International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

http://ijpsat.ijsht-journals.org
Vol. 30 No. 2 January 2022, pp.671-678

Effect of Ficus benjamina L. Ethanol Extract on Pancreatic Cell Histology in Diabetic Mice

Muftiah Yasi Dwi Wahyuni*, Warnety Munir, Djong Hon Tjong

Department of Biology, Faculty of Mathematics and Natural Sciences
Andalas University (UNAND)
Padang 25163, West Sumatra, Indonesia

Abstract— Diabetes is characterized by high blood glucose levels and a progressive change in the structure of Pancreatic Islet histology. Pancreatic β cell damage is one of the causes of increased glucose levels. Pancreatic cell damage can interfere with the function of the cells in the production of insulin. The production of insulin would affect the function of this hormone to regulate blood glucose levels. This study aims to determine the effects of Ficus benjamina L. Leaves ethanol extract on the histopathology of pancreatic β cells. We observed damage to the pancreatic exocrine histological structure and the number of pancreatic β cells. The results showed that the ethanol extract of the leaves of F. benjamina L. had a significant effect on reducing the level of damage to the exocrine tissue and increasing the number of pancreatic β cells. Extract dosage of 250 mg/kg body weight is the dose that gives the best effect compared to other extract doses.

Keywords— Ficus benjamina L., Pancreatic Islet, Sel β

I. INTRODUCTION

Diabetes mellitus is one of the four major non-infectious diseases in the world (Department of Heatlh, 2019). The WHO states that the worldwide prevalence of diabetics has increased from 4.7% (108 million) to 8.50% (422 million people) from 1980 to 2014 (World Health Organization, 2016). The number of patients will increase from 415 to 642 million from 2015 to 2040 (International Diabetes Federation, 2015). The number of diabetics in Indonesia is fourth after India, China and the United States, the number of diabetics is 10 million (World Health Organization, 2018).

Diabetes Mellitus is a disease caused by a disturbance in the body's metabolism, namely hyperglycemia. Chronic hyperglycemia in diabetes is associated with certain complications that result in damage or failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Scobie, 2007). Diabetes mellitus is related to the production of the hormone insulin produced by pancreatic β cells. Insulin deficiency caused by poor or sufficient damage to β cells will increase blood glucose levels (Mescher, 2014).

The use of herbal medicines to treat diabetes mellitus has been developed using secondary metabolites of plant, one of which is flavonoids. Flavonoids are one of the phenolic compounds in plants that have antioxidants and has an important role in preventing cell damage by reactive radicals (oxidative stress) (Redha, 2010). Studiawan and Santosa (2005) reported that the role of flavonoid glycosides in the ethanol extract of *Eugenia polyantha* is to bind hydroxyl radicals to prevent diabetogenic activity from exposure to alloxan, where alloxan is a compound that has diabetogenic effects on experimental animals used. It is also

Corresponding Author: Muftiah Yasi Dwi Wahyuni

known that the flavonoid content of *Curcuma manga* Val. may prevent damage to pancreatic β cells due to alloxan exposure (Hendrikos, 2014).

Ficus benjamina L. is a type of plant that contains flavonoid compounds. Flavonoids found in the ethanol extract of the leaves of *F. benjamina* L. can protect the liver from damage that is more severe caused by exposure to CCl_4 (Kanaujia *et al*., 2011). The flavonoid content of *F benjamina* L. may neutralize radical reactive compounds during normal metabolism due to the effects of diabetic substances or hyperglycemia. It is, therefore, necessary to test the effect of flavonoid compounds found in this plant in pancreatic β-cell histopathology. This research aims to determine the effect of banyan leaf (*F. benjamina* L.) ethanol extract on pancreatic exocrine histology and the number of pancreatic β cells induced by alloxan.

II. Research Methodology

The study was conducted at the Animal Structure and Development Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University.

2.1. Plant Material

F. benjamina L. leaves were collected from around the Andalas University campus. The leaves had been identified and confirmed by the Andalas University Herbarium (ANDA).

2.2. Preparation of Plant Extract

The *F. benjamina* L. leaves that have been dried were mashed with a grinder. About 1000 g of leaves powder was extracted by maceration with 96% ethanol (2,5 l) in a dark bottle. The sample was immersed in a place protected from light for 5 days while stirring repeatedly. The extract was filtered off and the residue was macerated again until the resulting mass was clear. The filtrate was concentrated using a rotary evaporator (Hasti *et al*., 2014).

2.3. Chemical and Reagents

Alloxan was obtained from Pharmaceutical Laboratory Andalas University, Padang. All other reagents and chemicals have been commercially procured and have been of analytical grade.

2.4. Animals

We used male DDY (*Deutsch Democratic Yokohama*) mice (*Mus musculus*), aged 2.5-3 months, weighing 25-30 g and without losing weight more than 10%. Animals have been obtained from the Agam Regency Veterinary Center in West Sumatra. Animals were acclimatized for a week to experiment in animal rearing cages and fed with standard pellets and drinking water *ad libitum*.

2.5. Diabetes Melllitus Induction

In Experimental animals, alloxan monohydrate has been used intraperitoneally at a dose 150 mg/kg BW to induce diabetes. Three days after alloxan mohydrate induction, Nesco Multicheck was used to measure fasting glucose levels. Mice with glucose levels >200 mg/dl were considered to have diabetes.

2.6. Experimental Procedures

The mice were divided into five groups (five mice per group). All the extract treatment were administered orally.

Group 1 : normal control and no treatment given

Group 2 : diabetes control and induction of 0.1 ml of alloxan monohydrate was administered

Group 3 : alloxan monohydrate + ethanol extract of *F. benjamina* L. leaves 125 mg/kg BW

Group 4 : alloxan monohydrate + ethanol extract *F. benjamina* L. leaves 250 mg/kg BW

Group 5 : alloxan monohydrate + ethanol extract of *F. benjamina* L. leaves 500 mg/kg BW

2.7. Pancreatic Histology Preparation

Mice in all groups were killed by cervical dislocation and the pancreas was quickly dissected (dropped by Bouin's solution to prevent damage when removed). The pancreas was set with bouin solution for 18-20 hours, followed by a process of dehydration with graded alcohol (70 to 100 %). Then use xylol for clearing and embedding in paraffin wax. The tissues were then cut to a thickness of 5 µm using a rotary microtome and treated with Hematoxylin-Eosin dye for histological observation (McManus and Mowry, 1960).

2.8. Observation

Pancreatic exocrine histological conditions were determined based on cell damage categories (Table 1) and the number of pancreatic β cells was calculated on each Langerhans island found.

Table 1. Scoring Categories of Pancreatic Exocrine Histology Damage (Dharma, Berata, and Samsuri, 2015)

Score	Damage Category
0	No Necrosis
1	A quarter of the Pancreas is necrotic
2	A half of the Pancreas is necrotic
3	Three-quarters of the Pancreas is necrotic
4	All parts of the pancreas undergo necrosis

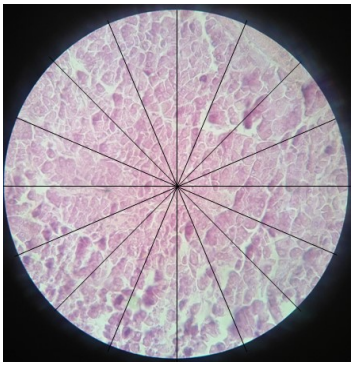

Fig. 1. Division of the Pancreatic Exocrine Histological Observation part

2.9. Data Analysis

Pancreatic exocrine histology data were analyzed by the Kruskal-Wallis test and if the test were found to be significantly different then proceed with the Mann-Whitney Test. Pancreatic β cell data were analyzed by ANOVA and if significantly different it will be followed by the DNMRT test (Duncan New Multiple Range Test).

III. Result and Discussion

3.1. Pancreatic Exocrine Histology

From the observation of pancreatic histology, we found several types of damage to the pancreatic exocrine consisting of necrosis, cell degeneration, and fibrosis (Figure 2). The worst damage was found in group G_2 (alloxan 150 mg/kg body weight) consisting of necrosis, cell degeneration, and fibrosis. The same damage type was also found in groups G_3 (125 mg/kg body weight) and G_5 (500 mg/kg body weight) with a lower damage level. In the G_4 group (250 mg/kg body weight) only found necrosis and cell degeneration damage type.

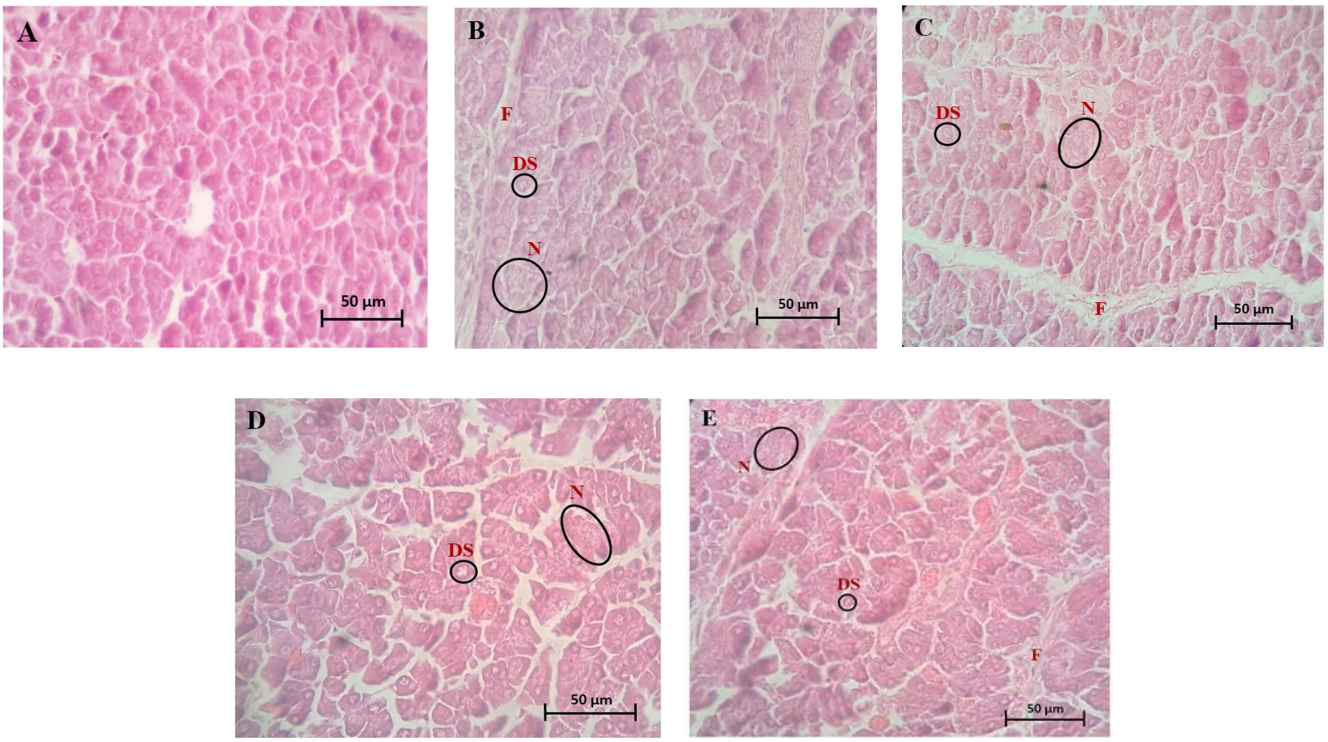

Fig. 2. Pancreas exocrine histology of *Mus musculus*; (A) G_1, (B) G_2, (C) G_3, (D) G_4, (E) G_5. Hematoksilin-Eosin Stain. (**F** : Fibrosis, **DS** : Cell Degeneration, **N** : Necrosis)

Cell damages in the exocrine pancreatic histology were found in all treatments, except controls. Based on observations, we found that most of the exocrine pancreas experienced karyolysis, cell nucleus broke, and some cells did not have a nucleus because of the lysis. Cell damage is suspected caused by the induction of alloxan which initiates hyperglycemic conditions in test animals. Hyperglycemia can worsen cell damage. This is because hyperglycemia tends to increase the formation of free radicals through glucose metabolism pathways (Robertson *et al*., 2004).

There are significant differences between the various treatments given to the level of pancreatic exocrine damage. The highest level of damage was observed in G_2 treatment, i.e. 150 mg/kg BW alloxan-induced and there is no *F. benjamina* ethanol extract given. G_4 treatment group (250 mg/kg BW) had a significant effect on reducing the level of pancreatic exocrine damage compared with other extract treatments (Table 2).

Alloxan induction can cause necrosis in consisting of pyknosis, karyorrhexis, and karyolysis. According to Boudreau et al. (2006), alloxan causes karyolysis of the nucleus cell, the disintegrates of cytoplasmic components, the cell boundaries become unclear, and debris mass containing fragments of lysis of the cell nucleus were found. The similar report according to Singh and Gupta (2007), alloxan induction can cause pancreatic damage consisting of clots, degranulation, hydropic degeneration, necrosis, and fibrosis.

Table 2. Scoring the level of pancreatic exocrine tissue damage based on cells undergoing necrosis

Treatment	Average Scoring ± SD	Damage Category
G_1	$0 \pm 0{,}00^{a}$	No Necrosis
G_2	$2{,}6 \pm 0{,}55^{d}$	¾ part of pancreas cells were necrosis
G_3	$2{,}4 \pm 0{,}55^{cd}$	½ part of pancreas cells were necrosis
G_4	$1 \pm 0{,}00^{b}$	¼ part of pancreas cells were necrosis
G_5	$1{,}8 \pm 0{,}45^{c}$	½ part of pancreas cells were necrosis

Numbers with different lowercase letters show significantly different in the Mann-Whitney Sig test. <0.05

Ethanol extract of *F. benjamina* L. leaf could reduce the cell damage level that occurs due to the induction of alloxan. Flavonoid compounds contained in the extract are probably could prevent or repair damaged cells that occur. Flavonoids can bind and neutralize ROS or act as natural antioxidants. Sandhar et al. (2011) reported that flavonoid compounds have an antidiabetic activity that can regenerate damaged cells. Prameswari and Simon (2014) stated that the flavonoids contained in water extracts of fragrant Pandanus leaf showed activity to repair cell damage caused by the induction of alloxan through cell regeneration.

From the observations, G_3 (125 mg/kg BW) and G_5 (500 mg/kg BW) groups did not have a significantly different effect on the increase of pancreatic cells average number. The G_3 group probably has a low flavonoid compound content so that the antioxidant activity caused is also low. While the G_5 group with the highest extract dose had a high flavonoid content so it had high antioxidant activity. Higher concentrations of flavonoids can reduce or eliminate the antioxidant activity in an extract thereby reducing the ability to cure damage due to exposure to toxic substances (2010). The ethanol extract of *F. benjamina* L leaves at a dose of 250 mg/kg BW had a better impact on repairing damage to pancreatic exocrine cells than other doses. This is presumably because the antioxidant activity at these doses can work effectively in repairing cell damage. Antioxidant activity is proportional to the levels of antioxidants present in a substance, but at certain levels, the antioxidant activity will reach a stable period (2007).

3.2. Pancreatic β Cell Counts

Average number of pancreatic β cells indicate that each treatment has a significantly different effect. The G_4 treatment group (250 mg/kg body weight) had an higher average pancreatic β cell than other treatments (Table 3). The average number of pancreatic β cells in the G_3 and G_5 groups was lower than the G_4 group. Pancreatic β cells are characterized by a more concentrated color than other cells (Figure 3). The color density of pancreatic cells is caused by the color absorption of these cells is greater than other cells.

Table 3. The effect of *F.benjamina* L. leaves ethanol extract on the average number of pancreatic β cells

Treatment	Average Number of Cells β
G_1	$36,4^{b}$
G_2	$13,8^{a}$
G_3	$12,6^{a}$
G_4	$25,2^{ab}$
G_5	19^{a}

Numbers with different lowercase letters show significantly different in the 5% DNMRT test

From the microscopic observation, the average number of pancreatic β cells in the treatments G_2, G_3, G_4, and G_5 is less than the control (G_1). This occurs because the induction of alloxan given to test animals causes the hyperglycemic condition. According to Nugroho and Purwaningsih (2006), induced-alloxan would destruct essential substances in pancreatic β cells, cause decreasing insulin-carrying granules in pancreatic β cells. Decrease of insulin granules cause impaired glucose metabolism so that blood glucose levels will increase.

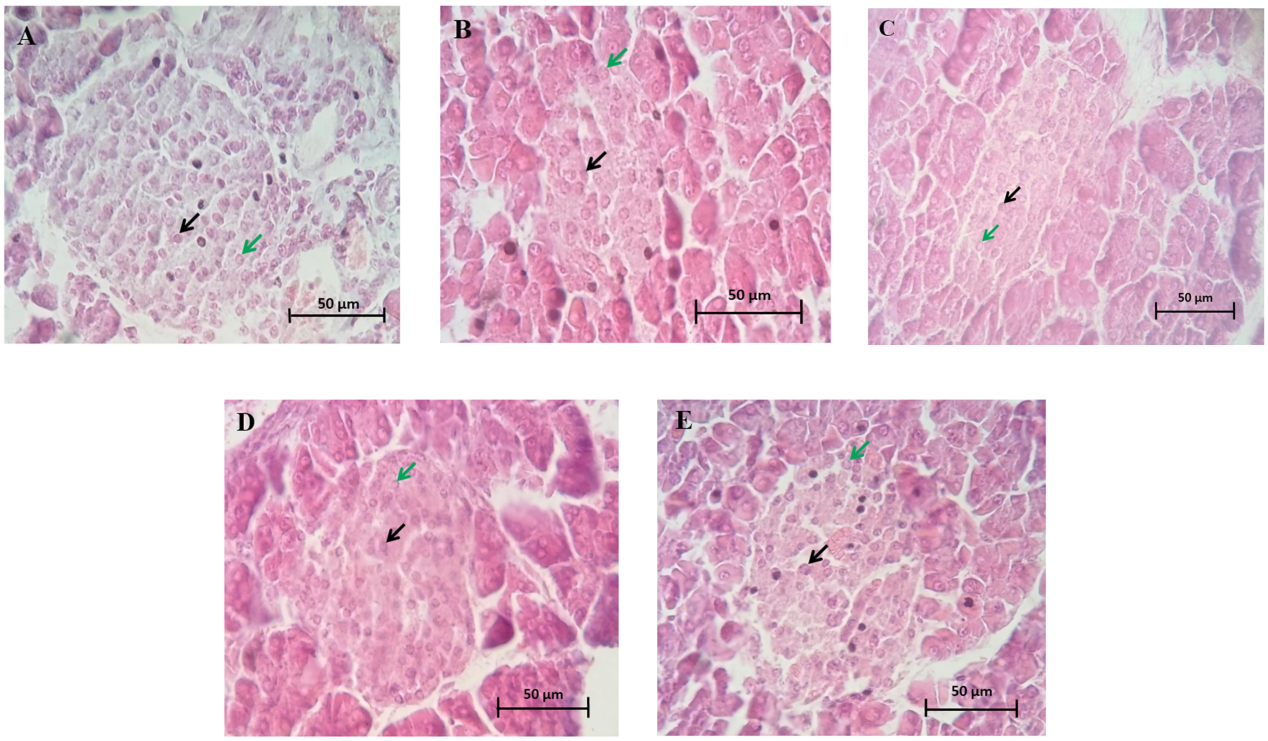

Fig. 3. Histology of the Langerhans Island Pancreas of *Mus musculus*; (A) G_1 (Control), (B) G_2, (C) G_3, (D) G_4, (E) G_5. Hematoxylin-Eosin staining. 400x magnification. (Green Arrow: Cell α, Black Arrow: Cell β)

Alloxan induction also causes necrosis of pancreatic β cells and would change the cytoplasm and nucleus structure. Apoptosis (death of the cell) will occur in the nucleus, the nucleus will shrink and the nucleus boundaries become unclear. This condition causes reduction of the β cell granules in the pancreas (2014). Kunharjito (2018) reported the induction of alloxan given to experimental animals causes degeneration of cells in the pancreas. Changes in alloxan-exposed cells may occur either quantitatively (reduction in number or size of cells) or qualitatively (necrosis, amyloidosis, and degeneration).

Ethanol extract of leaves of *F. benjamina* L. at a dose of 250 mg/kg BW (G_4) has the best effect on the pancreatic β cell repairment. This is presumably due to the flavonoid compound contained in the extract. Sukadana (2011) reported that flavonoids compound in the ethanol extract of leaves of *F. benjamina* L. has antioxidant activity that plays a role in cell protection and repairment.

The extract dose of 125 mg/kg body weight (G_3) and 500 mg/kg body weight (G_5) has no significant effect on increasing the number of pancreatic β cells. The small average number of β cells was found in G_3 treatment. this is probably caused by the flavonoid content in the extract dose is lower than other doses so it does not have a significant effect. The G_5 treatment also has a small pancreatic β cell's average number. This is probably because the extract dose given was high and it causes negative feedback on the pancreatic β cell's average number.

Ramadhani (2017) reported that the water extract of Surian leaves (*Toona sinensis*) with the highest dose (400 mg/kg BW) was not effective in reducing blood glucose levels. Higher doses have higher flavonoid content and cause decreasing antihyperglycemic activity because it can eliminate antioxidant activity.

IV. Conclusion

Ethanol extract of *F. benjamina* L. leaves at a dose of 250 mg/kg BW has the best effect on repairing damage in pancreatic exocrine cells and increasing the average number of pancreatic β cells.

ACKNOWLEDGMENT

Author would like to thank Dr. Warnety Munir and Dr. Djong Hon Tjong as supervisor who guided and provided advice during the study period. Thanks to the research partner in the Animal Structure and Development Laboratory for the support.We also appreciatethe reviewers' suggestions to this article and we would like to say thanks to the Biology Department of Andalas University.

REFERENCES

[1] Akrom, P.D. Harjanti, and T. Armansyah. 2014. Efek Hipoglikemik Ekstrak Etanol Umbi Ketela Rambat (*Ipomea batatas* P.) pada Mencit Swiss yang Diinduksi Aloksan. *Pharmaciana.* vol 4 (1): 65-76. doi https://10.12928/pharmaciana.v4i1.400

[2] Bouayed, J. and T. Bohn. 2010. Exogenous Antioxidants-Double-Edged Swords in Cellular Redox State. *Oxidative Medicine and Cellular Longevity* Vol. 3 (4): 228-237. doi : 10.4161/oxim.3.4.12858.

[3] Boudreau, M.D., H.W. Taylor, D.G. Baker, and C.M. Jay. 2006. Dietary exposure to 2-aminoanthracene induces morphological and immunocytochemical changes in pancreatic tissues of fisher-344 rats. *Toxicol. Sci*. vol 93: 50-61. doi https:// 10.1093/toxsci/kfl033.

[4] Department of Health. 2019. Infodatin Ministry of Health. Hari Diabetes Sedunia Tahun 2018. Jakarta : Kemenkes RI.pp 1-8.

[5] Dewi, J.R., E. Teti, and S.M. Erni. 2007. Antioxidant Activity of Extract Obtained by Applying Various Solvents to the Local Brown Sorghum (*Sorghum bicolor*) Bran. *Jurnal Teknologi Pertanian* vol. 8 (3): 188-197.

[6] Hasti, S., M. Enda, U. Rahayu, and U.Y. Lisni. 2014. Sub-Chronic Toxicity of *Ficus benjamina* L. Leaves Ethanol Extract on The Liver Function of White Mice. *Elsevier Procedia Chemistry.* vol 13: 204-208. doi : 10.1016/j.proche.2014.12.028.

[7] Hendrikos, R. 2014. Efek Ekstrak Etanol Rimpang Temu Mangga (*Curcuma mangga Val*.) terhadap Kerusakan Sel β Pankreas Mencit Putih yang Diinduksi Aloksan secara Histologis. [Undergraduate Thesis]. Padang : Andalas University.

[8] International Diabetes Federation. 2015. IDF Diabetes Atlas Seventh Edition 2015.

[9] Kanaujia, V.K., R. Irchhaiya, H.K., Singh K. Deepak, V. Mohini, D.Y. Rahul, and S. Dileep. 2011. Evaluation oh Hepatoprotective Activity on te Leaves of *Ficus benjamina* Linn. *Scholar Research Library.* vol 1(3) : 59-69.

[10] Kunharjito, W.A.C., M. Avesina, D.P. Anggriyawanti, and E.R. Purnama. 2018. Pemanfaatan Daun Kelor (*Moringa oleifera*) terhadap Pemulihan Struktur Pankreas Mencit Diabetik. *BIOTROPIC The Journal of Tropical Biology.* vol. 2(2) : 85-92. doi https:// https://doi.org/10.29080/biotropic.2018.2.2.85-92

[11] McManus, J.F.A. and R.W. Mowry. 1960. Staining Methods: Histologic and Histochemical. Alabama : Paul B. Hoeber Inc. pp 423.

[12] Mescher, A.L. 2014. Histologi Dasar Junqueira Text & Atlas. Jakarta : McGraw-Hill Companies Inc. and EGC Medical Publisher. pp 641.

[13] Nugroho, B.A. and E. Puwaningsih. 2006. Perbedaan Diet Ekstrak Rumput Laut (*Eucheuma* sp.) dan Insulin dalam Menurunkan Kadar Glukosa Darah Tikus Putih (*Rattus norvegicus*) Hiperglikemik. *Media Medika Indonesia.* vol. 41 (1): 23-30.

[14] Prameswari, O.M. and B.W. Simon. 2014. Uji Efek Ekstrak Air Daun Pandan Wangi terhadap Penurunan Kadar Glukosa Darah dan Histopatologi Tikus Diabetes Mellitus. *Jurnal Pangan dan Agroindustri* Vol. 2 (2) : 16-27.

[15] Ramadhani, Y. 2017. Aktivitas Antihiperglikemia Ekstrak Air Daun Surian (*Toona sinensis*) pada Tikus Putih (Sprague-Dawley) yang Diinduksi Streptozotocin. [Undergraduate Thesis]. Padang : Andalas University.

[16] Redha, A. 2010. Flavonoid : Struktur, Sifat Antioksidatif, dan Peranannya dalam Sistem Biologis. *Jurnal Belian* vol 9(2): 196-202.

[17] Robertson, R.P., H. Jamie, O.T.T. Phuong, and P. Vincent. 2004. β-Cell Glucose Toxicity, Lipotoxicity, and Chronic Oxidative Stress in Type 2 Diabetes. *Diabetes.* vol 53(1): 119-124. doi https://10.2337/diabetes.53.2007.s119

[18] Sandhar, H.K., K. Bimlesh, P. Sunil, T. Prashant, S. Manoj, and S. Pardeep. 2011. A Review Of Phytochemistry And Pharmacology Of Flavonoids. *Internationale Pharmaceutica Sciencia.* vol. 1 (1) : 25-41.

[19] Scobie, I.N. 2007. Atlas of Diabetes Mellitus Third Edition. United Kingdom : Informa Healthcare. pp 138.

[20] Singh, N. dan Gupta, M. 2007. Regeneration of β Cells in Islets of Langerhans of Pancreas of Alloxan Diabetic Rats by Acetone Extract of *Momordica caharantia* (Linn.) (Bitter Gourd) Fruits. *Indian Journal of Experimental Biology.* vol 45 : 1055-1062. PMID: 18254212

[21] Studiawan, H. and Santosa, M.H. 2005. Uji Aktivitas Penurun Kadar Glukosa Darah Ekstrak Daun *Eugenia polyantha* pada Mencit yang Diinduksi Aloksan. *Media Kedokteran Hewan.* vol 21(2): 64-65.

[22] Sukadana, I.M. 2011. Kandungan Senyawa Steroid-Alkaloid pada Ekstrak n-Heksana Daun Beringin (*Ficus benjamina* L.). *Jurnal Kimia.* vol 5 (2): 164-179.

[23] World Health Organization. 2016. Global Report on Diabetes. Switzerland : WHO Press.

[24] World Health Organization. 2018. Global Report on Diabetes. Switzerland : WHO Press.