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EFFECT OF ETHANOL EXTRACT GENDOLA LEAF LEAF (*Basella alba* L.) ON DECREASING BLOOD GLUCOSE CONDITION AND HISTOPATOLOGY PANKREAS WHITE RATS (*Rattus norvegicus*) INDICATED STREPTOZOTOCIN

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ABSTRACT

This study aims to examine the content of secondary metabolites in red gendola leaf ethanol extract, the effect of ethanol extract of leaf of red gendola on decreasing in blood glucose levels, the effect of graded doses to decrease blood glucose levels and show illustration histopathology pancreas of the rats induced diabetes mellitus streptozotocin.
The research was a laboratory experiment using a modified posttest study design randomized controlled groups design. A total of 24 rats were divided into six groups, each group consisted of four rats with details of group I, II, and III as a control group, and groups IV, V, and VI as the experimental group. Group I: normal control given CMC Na 0,5%; Group II: negative controls were given CMC Na 0,5% and streptozotocin induced 40 mg/kg intraperitoneally rats; Group III: positive controls were given a dose of glibenclamide 0.45 mg/kg and streptozotocin induced 40 mg/kg intraperitoneally rats; each experimental group was given ethanol extract of leaf of red gendola at a dose of 100 mg/kg, 200 mg/kg, 400 mg/kg and streptozotocin induced 40 mg/kg of rat intraperitoneally.The results show that: there are secondary metabolites in red gendola leaf ethanol extract namely alkaloids, flavonoids, tannins, saponins and phenols ; the ethanol extract of leaf of red gendola at a dose of 100 mg/kg effective in lowering blood glucose levels in male rats and at a dose of 400 mg / kg effective in regenerating pancreatic tissue; ethanol extract of leaf of red gendola dose of 200 mg/kg and 400 mg/kg did not give full effect to the decrease in blood glucose levels in male rats and ethanol extract of leaf of red gendola at doses of 100 and 200 mg / kg did not give full effect to the regeneration of pancreatic tissue streptozotocin-induced rats induced streptozotocin.

Keywords: Streptozotocin, gendola red (Basella alba L.), blood glucose levels, histopathology pancreas.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia or a condition in which blood glucose levels are higher than normal values associated with abnormalities of carbohydrate, fat, and protein metabolism caused by decreased insulin secretion, decreased insulin sensitivity causing multiple microvascular and macrovascular complications (Sukandar et al., 2013).

Histopathology is a branch of biology that studies the condition and function of tissues in relation to disease. Histopathology is very important in relation to the diagnosis of disease because one of the considerations in the diagnosis is through observation of the allegedly disturbed tissue. Analysis of organ / tissue histological conditions is seen from changes in morphology, structure and indications of damage / infection / mutation due to disease, toxic or other mutagenisis (Gerrit, B. 1998) effects.

World Health Organitation (WHO) 2016 states generally high blood glucose causes deaths of about 7% in men aged 20-69 years and 8% in women aged 20-69 years. International Diabetes Federation (IDF) 2015 mentions that around 415 million adults worldwide suffer from DM. IDF estimates that 318 people have impaired glucose tolerance. The IDF also estimates that by 2015 five million people die from DM and by 2040 there will be 642 million people living with DM, up more than 50% from the previous figure. Indonesia ranks 7th with 10 million cases of DM disease

A variety of plants can be used as natural remedies, because the side effects are very small if used properly. One plant that can lower blood sugar levels is a red gendola leaf (Basella alba L.) family basellaceae. Red gendola plants have antioxidant properties and are able to ward off free radicals in the body that are believed to cause various degenerative diseases. These antioxidant properties are associated with chemical compounds contained in red gendola plants such as alkaloids, saponins, phenols, terpenoids, tannins, steroids and flavonoids (Kumar, V. et al. 2011; A. Nantia, et al.)

The objective of the study was to investigate the effect of red gendola ethanol extract (Basella alba L.) at doses of 100 mg / kg BW, 200 mg / kg BW, and 400 mg / kg BW of decreased glucose regeneration levels of pancreatic β male rat (Rattus norvegicus) induced streptozotocin. Determine effective doses in lowering blood glucose levels and regeneration of pancreatic β male white male (Rattus norvegicus) β-induced streptozotocin cells (Rattus norvegicus) induced streptozotocin.

RESEARCH METHODS

Tools

Glass tools, mesh sieve no. 40, maseration vessels, blenders (kirin), bottles of drinking rats, porcelain cups, glukometer (accu chek), glukotest strips (accu chek), surgical scissors (Smics), rat enclosures, duster napkins, microscopes (Olympus Bx-51) mortar and stamper, scalpel (Smics), tube rack, rotary vaccum evaporator, injection syringe (1 mL, 3 mL), oral syringe, marker (snowman), sterofom, test tube, analytical scales (precisa), feeding rats, mouse scales (cook master), waterbath.

Material

Leaves of red gendola (Basella alba L.) taken from around Palu city of Central Sulawesi, white male rats wistar strains obtained from the city of Palu Central Sulawesi, distilled water, Alcohol 70%,

100% alcohol, 95% alcohol, 90% alcohol, aqua pro-injection, hydrochloric acid, iron (III) chloride, citric buffer saline (citric acid, sodium citrate), dragendolf LP, ethanol 96%, ether, 10% formalin, glibenclamide (indofarma), henskun (sensi), cotton, , filter paper, duct tape, lieberman-burchard, mask, standard feed, magnesium powder, Na CMC, streptozotocin, tissue.

Preparation of Streptozotocin Induction Solution

Streptozotocin weighed as much as 0.32 grams and then dissolved using saline citrate-buffer with pH 4.5 and then induced in rats via intraperitoneal (i.p). The dose of streptozotocin is 40 mg / kg BW.

Preparation of Glibenclamide Suspension 0.45 mg / kg BW

Glibenclamide dose in adult humans is 5 mg per day, if converted in rats weighing 200 grams is 0.018 then the dose of glibenclamide for rats is 0.45 mg / kg body weight. Weighed glibenclamide tablet powder equivalent to 0.036 mg then suspended in NaCC 0.5% to 50 ml then shaken until homogeneous.

Making red gendola leaf extract

The 1061 gram glycated red gelsole leaves were extracted using maceration with 96% ethanol solvent for 3 days, the extract was then filtered using filter paper and then obtained by filtrate. The filtrate was concentrated using a rotary vaccum evaporator at 60 ° C and continued evaporation by using waterbath until a viscous extract was obtained.

Data analysis

Data obtained were blood glucose levels of male white rat (Rattus norvegicus) before induced streptozotocin and after induced streptozotocin. Data analysis was performed by using one way ANOVA statistic test at 95% confidence level. This test is used to determine the differences between treatment groups and continued with post hoc Least Significant Difference (LSD). Further data of microscopic examination results obtained in the form of data scoring the level of damage to white male rats pancreas. Further analyzed using Kruskall Wallis nonparametric test to know the significant difference between treatment group and control group with p value <0,05 selected as the level of significance. If there are significant differences, then Mann Whitney test to see the meaningful difference each group.Data analyzed using SPSS 23 program

Table 1. Phytochemical Test Result of Leaf Red Ganola Ethanol Extract

Testing	Results
Alkaloid Test	Positif (+)
Flavonoid Test	Positif (+)
Phenol Test	Positif (+)
Saponin test	Positif (+)
Test Tanin	Positif (+)

Information :
(+) : contains a class of compounds tested

Table 2. Mean Blood Glucose Level of Male Rats in the 0th, 7th, 14th, 21st and 28th days.

	Mean ± SD Blood Glucose Level (mg / dL)						
Days To	Normal Control	Negative Control	Positive Control(Glibenklamid)	Dose 100 mg/Kg BW	Dose 200 mg/Kg BW	Dose 400 mg/Kg BW	p

0	83,25 ± 12,89	83,5 ± 5,67	88 ± 5,70	82± 2,82	89± 12,70	85,5± 6,83	0,897
7	84,5 ± 6,38	304,25 ± 41,45	268,25± 63,88	321,75± 91,09	249,5± 38,09	372,5± 112,55	0,002
14	89,75 ± 6,09	214,25 ± 59,13	273,75± 174,88	178,25± 34,32	207± 86,32	172,25± 57,93	0,259
21	88,25 ± 6,37	246,5 ± 42,62	140,75± 35,96	154,75± 62,53	132,5± 13,00	126,5± 20,5	0,001
28	80,75 ± 6,68	273,25 ± 28,481	101,25± 10,15	124,5± 41,05	113,75± 13,60	120,5± 11,52	0,000

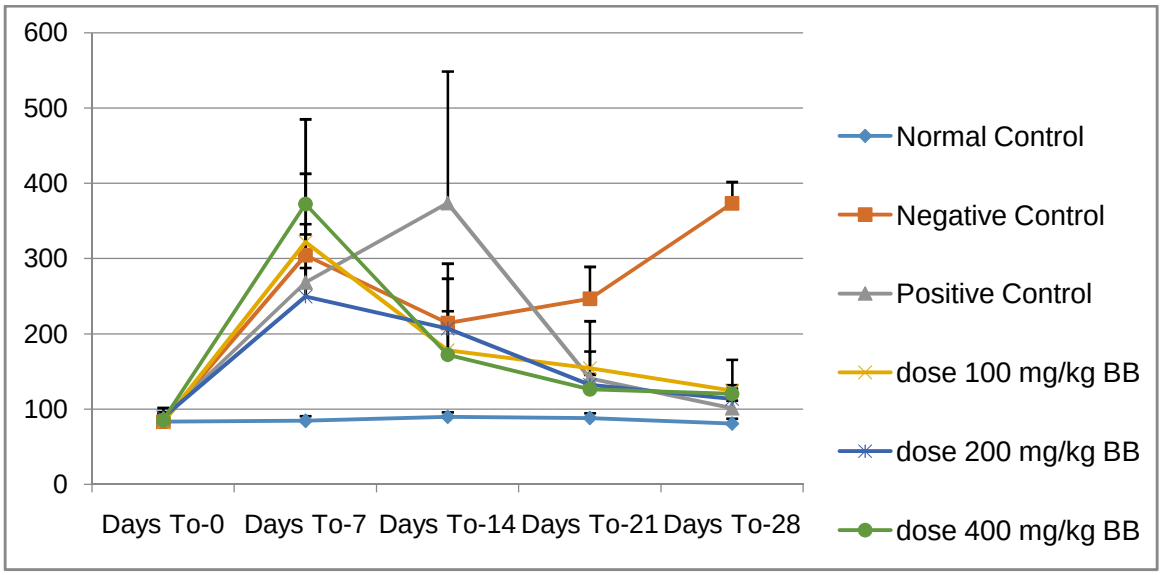

Figure 1. Graph of Blood Glucose Levels Before Treatment, After Induction, and During Treatment

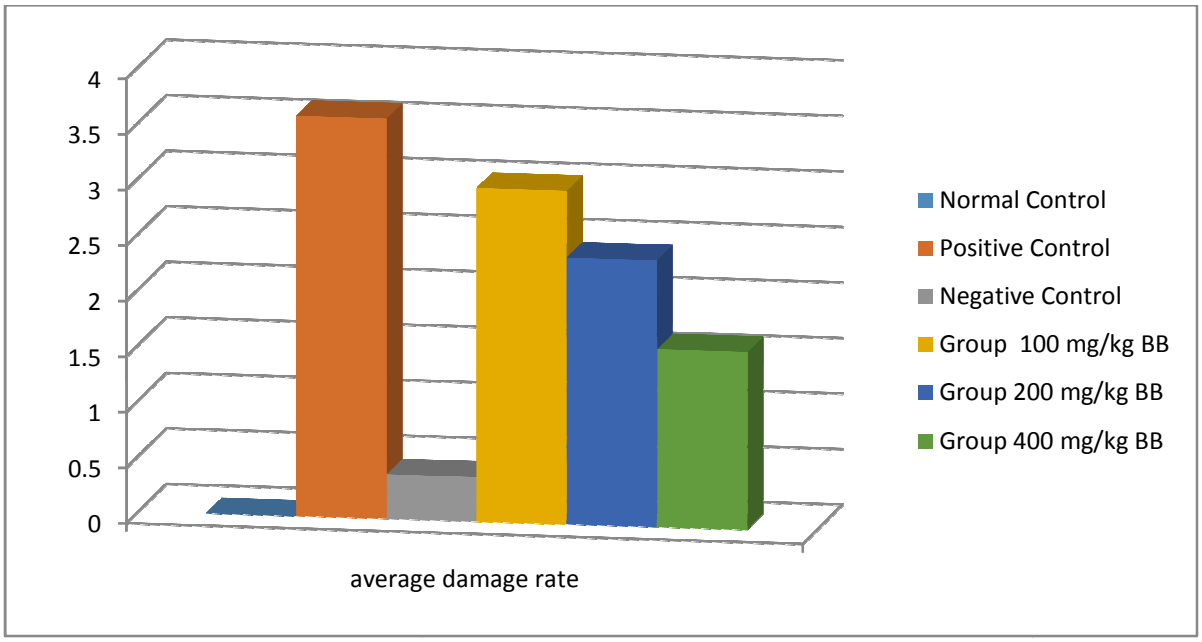

Figure 2. Average rate of pancreatic damage

Table 2. Scoring the extent of damage to rat pancreas

Rats	Treatment Group					
	Normal Control	Negative Control	Positive Control (glibenklamid)	Dose 100 mg/kg BW	Dose 200 mg/kg BW	Dose 400 mg/kg BW
1	0	3	0	3	3	2
2	0	4	1	3	2	2
3	0	4	1	3	2	2
4	0	4	0	3	2	1
5	0	3	0	3	3	1
Average	**0**	**3,6**	**0,4**	**3**	**2,4**	**1,6**
SD	**0**	**0,49**	**0,49**	**0**	**0,49**	**0,49**

Information :

Score 0 = no inflammatory cells (normal)
Score 1 = inflammatory cell 1/3 part (normal cell shape)
Score 2 = cell inflammation 1/2 part (partially necrotic cell shape)
Score 3 = inflammatory cell 2/3 parts (many cell forms are necrotic)
Score 4 = most necrotic cells.

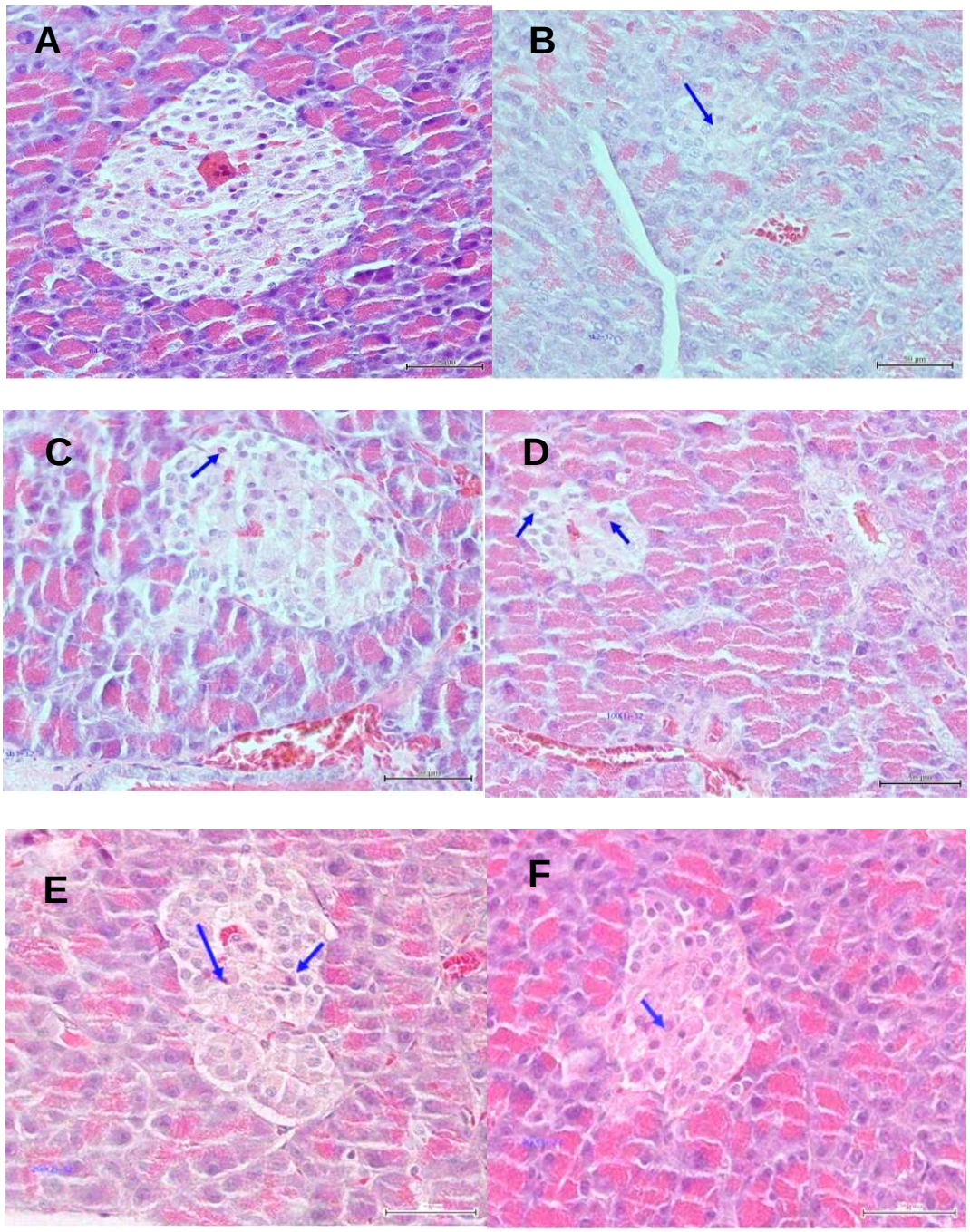

Figure 3. Histology of Mouse Pancreas With HE Staining Magnification 400x (A. Normal Control, B. Negative Control / Streptozotocin induction, C. Positive Control / given Glibenclamide, D. Dose 100 mg/kg BW, E. Dose 200 mg/kg BW, F. Dose 400 mg / kg BW)

Information = → Pancreatic cells are necrotic

DISCUSSION

In this study used animal rat white male test (Rattus norvegicus) as a test animal. The results of measurement of blood glucose level on day 0 of male white rat (Rattus norvegicus) to 5 treatment groups that is around 67 mg / dL - 109 mg / dL. Based on the result of measurement of blood glucose level of the tested animal, it is stated that the blood glucose level of the tested animal is normal because it is still in the range of 50 mg / dL - 135 mg / dL (Sari, D.P. 2016)

The result of measurement of blood glucose level of male white rat (Rattus norvegicus) on day 7 after induced streptozotocin dose 40 mg / kg BB that is at 201 mg / dL - 554 mg / dL and high blood glucose level can be seen from profile of blood glucose level (Figure 1). If the blood glucose level exceeds 200 mg / dL, then the mouse is considered hyperglycemic (Sari, D.P. 2016). Increased blood glucose levels caused by streptozotocin dose 40 mg / kg BB in i.p. The mechanism of action of streptozotocin leads to hyperglycaemia by streptozotocin entering pancreatic β cells via glucose transporter (GLUT2) and causing DNA alkylation. Alkylation or inclusion of methyl groups from streptozotocin into this DNA molecule will cause damage to DNA fragmentation (Ocktarini, R. 2010). DNA damage will trigger the production of poly (ADP-ribose) synthase, an enzyme needed to repair DNA damage. This enzyme requires NAD (nicotinamide adenine dinucleotide) as its substrate, so that the NAD + content in the cell decreases. Decreased levels of NAD + cellular also cause a decrease in the amount of ATP so that synthesis and insulin secretion can be inhibited which causes blood glucose levels to rise. (Suryani, N. et al., 2013).

Based on the statistical calculation of the 14th day blood glucose level showed that there was no significant difference marked with value (P□0,05) P value = 0,259, so no need for further post hoc LSD test and profile of blood glucose level can be seen in (figure 1). This is because the natural ingredients have not had an effect on lowering blood glucose levels because of some internal factors of the male white rat test animal (Rattus norvegicus) as the pancreas condition is still damaged. Therefore, the treatment of extract continued until 21st day.

Day 21 statistical results of one way ANOVA showed that there was significant difference marked with value (P <0,05) value P = 0,001, so did further post hoc LSD test to see the significant difference between treatment group and profile of decreasing glucose level blood can be seen in (figure 1). Treatment day 21 The dose group extract 100 mg / kg BW was significantly different with negative control, normal and different control was not significant with positive control. The dose group of extract 200 mg / kg BW was significantly different with negative control and differed not significant with normal control, positive control. Group doses of 400 mg / kg BW differ significantly with negative controls and differed not significantly with normal controls, positive controls. There was a decrease of blood glucose level on the 21st day marked with negative control significantly different with normal control group, positive control and ethanol extract treatment group of red gendola leaves dose 100 mg / kg BW, 200 mg / kg BW, 400 mg / kg BW . Blood glucose levels have not been seen to be normal because some internal factors of male white rat test (Rattus norvegicus), such

as poor pancreatic state, cause the amount of insulin in male rats (Rattus norvegicus) is still lacking. So the treatment of extracts continued until day 28 to see the long-term effects of using natural ingredients in lowering blood glucose levels.

The 28th day of one way ANOVA statistical results showed that there was significant difference in all treatments with P (0,05) value P = 0,000, so that LSD post hoc further test was done to see the significant difference between treatment group and profile decreased blood glucose levels can be seen in (figure 1). Further post hoc LSD assay results showed the Group dose of extract 100 mg / kg BW was significantly different with normal control, negative control and different was not significant with positive control. The dose group of extract 200 mg / kg BW was significantly different with negative control and differed not significant with normal control, positive control. The dose group of extract 400 mg / kg BW was significantly different with normal control, negative control and different was not significant with positive control. This shows the extract of red gendola leaf ethanol (Basella alba L.) has an effect in lowering blood glucose level of male white rat test (Rattus norvegicus).

In the positive control group glibenclamide given due to the mechanism of action of glibenclamide which stimulates the secretion of insulin from the granules of pancreatic Langerhans β cells. Its stimulation through its interaction with ATP-sensitive K channels on the membrane of β cells that lead to membrane depolarization and this state will open the Ca channel. Opening of Ca canal so Ca ++ will enter β cells, stimulate granules containing insulin and will occur insulin secretion with amount equivalent to peptide C. (Tanu, I. 2007). Glibenclamide may increase insulin release and stimulates GLUT4 release in streptozotocin-induced diabetic rats so that GLUT 4 can lead to muscle, adipose and liver tissue to reduce 8-OhdG levels. This condition is supported by the presence of antioxidant enzyme endongen superoksid dismutase (SOD), catalase (CAT), gluthathion peroksidase (GPx) which serves as the first line of defense against free radical acavenger, as detoxification H2O2 and prevent damage macromolecular component of the cell. (Tandi, J. 2016).

Reduced blood glucose levels are caused by the presence of bioactive compounds found in red gendola extract (Basella alba L.) such as alkaloids, flavonoids, tannins and saponins. Alkaloid compounds are believed to have the ability to regenerate damaged pancreatic beta cells. Antioxidant activity is capable of capturing free radicals that cause improvements in the destruction of pancreatic beta cells of DM causes. There is improvement in pancreatic tissue, then there is an increase in the amount of insulin in the body so that blood glucose will enter the cell and there is a decrease in blood glucose levels. Flavonoids increase insulin secretion, increase glucose uptake in peripheral tissues, and inhibit gluconeogenesis. Flavonoids are known to prevent the destruction of pancreatic beta cells because they have antioxidant activity by capturing or neutralizing free radicals with phenolic OH groups so as to improve the state of damaged tissue (Sari, D.P. 2016). Tanin has hypoglycemic activity that is by increasing glycogenesis. Tanin also serves as an astringent or chelating agent that can wrinkle the small intestine epithelial

membrane, thus reducing the absorption of the juice, inhibiting sugar intake and increasing the rate of blood sugar (Prameswari, O.M; Widjanarko, S.B. 2014). Saponin lowers blood glucose levels by inhibiting glucose transport in the gastrointestinal tract and stimulating insulin secretion in pancreatic beta cells (Sari, D.P. 2016).

The result of observation of histology of rat pancreas using Olympus Bx-51 microscope with 400x magnification obtained data of pancreatic histology damage scores analyzed by Kruskal-Wallis test to obtain p = 0,00 (p <0,05) treatment with the control group. To see a significant difference in each group, the Mann-Whitney test was performed.

Based on Mann-Whitney test results showed that there were significant differences between normal group with negative control and treatment group doses 100, 200, and 400 mg / kg BW (p <0.05), but different was not significant with positive control (p > 0.05). The negative control group differed significantly with normal control, positive control and all treatment groups (p <0.05). The positive control group differed significantly with the negative control and all treatment groups (p <0.05), but differed not significantly with normal controls (p> 0.05). The treatment group of 100 mg / kg BW was significantly different with normal control, negative control, positive control, treatment group of 200 mg / kg BW and 400 mg / kg BW (p <0.05). The treatment group dose 200 mg / kg BW was significantly different with normal control, negative control, positive control, treatment group dose 100 mg / kg BW and dose 400 mg / kg BW (p <0,05). The treatment group of 400 mg / kg BW was significantly different with normal control, negative control, positive control, treatment group 100 mg / kg BW and 200 mg / kg BW (p <0.05).

Based on data of pancreatic histology damage scores on normal control given NaCC 0.5% histology picture of pancreatic mouse with average value 0 did not appear to change, where the morphology of langerhans island still looks normal and β cells in it do not experience necrosis. In the negative control induced streptozotocin and given 0.5 cmC Na CM histologic images of rat pancreas with an average value of 3.6 had severe pancreatic β cell necrosis. The occurrence of this necrosis is characterized by a cavity on the island of Langerhans. Necrosis is cell death due to fatal damage characterized by complete cell structure and cell damage followed by cell lysis and tissue inflammation (Zubaidah, E. 2016). This suggests that giving 0.5% CMC Na can not regenerate pancreatic β cells. Streptozotocin with its cytotoxic action works directly on pancreatic β cells, by entering pancreatic β cells via glucose transport (GLUT 2) and will lead to DNA alkylation. Alkylation or the inclusion of methyl groups from streptozotocin into this DNA molecule will cause DNA damage (Baqarizky, F. 2015). In positive controls given glibenclamide, the histologic features of rat pancreas with an average value of 0.4 show the presence of decreased pancreatic β cell necrosis. This is because glibenclamide has an effect in stimulating pancreatic β cells to secrete insulin (Nugroho, 2012). At doses of 100 mg / kg BW, 200 mg / kg BW and 400 mg / kg BW of pancreatic histologic findings with mean values of 3, 2.4 and 1.6 respectively showed a better morphology of Langerhans island than the negative control

and pancreatic β cells with relatively reduced necrosis. This suggests that administration of red gendola leaf extract may regenerate pancreatic β cells. Regeneration of pancreatic β cells occurs because of the compounds contained in the red gendola leaf one of which flavonoid can prevent damage to beta cells of the pancreas because it has antioxidant activity by capturing or neutralizing free radicals associated with phenolic OH groups so as to improve the state of damaged tissue (Arifin, H. et al., 2006).

From the observation and analysis of rat pancreatic histological preparations were done, it is evident that the ethanol extract of leaves of red gendola (Basella alba L.) had an effect on the regeneration of β cells by observing the pancreatic histopathology description white male rats (Rattus norvegicus) induced by streptozotocin. Ethanol extract of leaves of red gendola at a dose of 100 mg / kg, 200 mg / kg, and 400 mg / kg has had an effect on the regeneration of pancreatic β cells white male rats. However, at a dose of 100 mg / kg and 200 mg / kg of red gendola leaf extract effect in regenerating pancreatic β cells are not very effective, when compared with a dose of 400 mg / kg is more effective in regenerating pancreatic β cells. This is because of differences in dose-rise, so that the active substances contained in the leaf extract of red gendola will be different at each dose. The effect obtained from the active compound content of the leaf extract of red gendola namely alkaloids, flavonoids, phenols, saponins, and tannins. These compounds function to regenerate damaged pancreatic β cells and prevent pancreatic β cell damage, because it has antioxidant activity that can capture free radicals (Arifin, H. et al. 2006) and stimulates the activity of glutathione reductase, catalase and superoxide dismutase which contributes to prevent the formation of free radicals and function to repair damage to cells and tissues caused by free radicals (Nantia, EA et al., 2013).

CONCLUSION

Based on the results of research and discussion it can be concluded that :

1. Extract of red gendola lean ethanol (Basella alba L.) at doses of 100 mg / kg BW, 200 mg / kg BW, and 400 mg / kg BW have an effect on lowering blood glucose levels of male rats (Rattus norvegicus) induced streptozotocin .

2. Dose of red gendola ethanol extract (Basella alba L.) effectively decrease blood glucose level of male white rat (Rattus norvegicus) that is at dose extract 100 mg / kg BB. And at doses of 400 mg / kg BW is effective against regeneration of pancreatic tissue.

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