



## Test of the Potential of Ethanol Extracts, Simplician Medium and Forest Umbi Juice (*Eleutherine bulbosa* (Factory) Urb.) Against Blood Glucose Levels of Rats, and Histopatology Ratkreas Rat (*Rattus norvegicus*) Hypercolesterolemia Model Diabetic

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### Abstract

This study aims to examine the presence or absence of secondary metabolite compounds in ethanol extract, simplicia steeping and forest onion tuber juice, in male glucose parameters and pancreatic histopathology. Using test animals as many as 30 male white rats divided into 6 groups and each group consisted of 5 rats with group I details as normal controls, group II as negative controls given 0.5% Na-CMC suspension, group III as positive control who were given Metformin 45 mg / kg BB and IV, V, VI as the test group were given ethanol extract, simplicia extract and forest onion tuber juice with each dose of 20 g / kg BW. The results showed that there were secondary metabolites in the ethanol extract of forest onion tubers, namely flavonoids, saponins, alkaloids, steroids and tannins, giving ethanol extract of forest onion bulbs had the effect of reducing blood glucose levels in white mice with a dose of 20g / kgBW with an average of 132 and ethanol extract of forest onion bulbs at a dose of 20g / kg BB with an average of 0.5 can regenerate pancreatic tissue.

**Keywords:** forest onion bulbs (*Eleutherine bulbosa* (Mill) Urb.) Diabetes, pancreatic histopathology

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### ■ Introduction

Diabetes mellitus is a disease of metabolic disorders characterized by increased levels of glucose in the blood due to deficiency or decreased insulin effectiveness. Insulin is a peptide hormone secreted by pancreatic  $\beta$  cells.

This hormone functions in regulating normal blood glucose levels. Abnormalities of secretion or insulin work cause interference in the metabolism of carbohydrates, fats and proteins. Insulin resistance is often associated with disruption of tissue sensitivity to insulin, this is because insulin

is unable to stimulate glucose uptake in insulin targeted tissue, such as muscle and fat[1]

The pancreas is a supporting organ in the digestive system that doubles as an exocrine and endocrine gland and the two parts influence each other's exocrine part of the pancreas consisting of pyramidal cells with an apical portion leading to a small ductal lumen. These exocrine pancreatic cells are zimogenic type, have a nucleus located on the basal and surrounded by oesinophilic cytoplasm with granules containing enzymes in an inactive form. The endocrine part of the pancreas consists of several endocrine cells which form a separate collection called Langerhans Island. Langerhans Island varies in shape and size between the exocrine and pancreatic cells. Langerhans Island secretes various hormones, namely insulin, glucagon, samotostatin and polypeptides[2]. Salah satu tanaman obat yang banyak digunakan oleh masyarakat khususnya masyarakat Sulawesi Tengah sebagai obat adalah Bawang Hutan (*Eleutherine bulbosa* (Mill) Urb).

Previous research conducted by Ramla Alia (2016) stated that Forest onion tuber extract (*Eleutherine bulbosa* (Mill) Urb) at a dose of 500 mg / kg BB was most effective in reducing blood glucose levels in mice[3]. Another researcher conducted the study, Joni Tandi (2017) using ethanol extract of dayak onions with a dose of 100 mg / kg BW can restore cholesterol levels in white mice [4].

Based on the description above, the researcher is interested in conducting further research, to determine the effect of ethanol extract, simplicia steeping and forest onion tuber juice with each dose of 20 g / kg in providing maximum results to reduce cholesterol blood glucose levels and tissue regeneration rat pancreatic model of hypercholesterolemia-diabetes. This research is expected to be a new source of information for the community about the efficacy of forest onion bulbs.

## ■ Experimental

### Tool

Sieve number 40 mesh, maceration vessel, Blender (Cosmos), Porcelain cup, Corongkaca (Pyrex), Spread (Pyrex), Glucometer (Accu check), Glucotest strip test (Accu check), Scissors,

Animal enclosure test, Pumpkin measure (Pyrex ), Lumpang and Alu, Mortar and stamper, Water drop, Pipette drops, Raktabung, Rotary vaccum evaporator (Eyela), Oral Sonde 3 ml (Terumo Syringe), Markers (Snowman), Spuit injection 1 ml, 3 ml (Terumo Syringe), Oral syringe 3 ml, 5 ml (Terumo Syrinege), Spot plate, Reaction (Pyrex), Place for drinking and eating mice, Gram scales, Analytical scales (Ohaus), Container extract, steeping and juice.

### Material

Distilled water, Aluminum foil, Aqua pro injection (Otsuka), Concentrated hydrochloric acid P (Merck), Sulfuric acid (Merck), Anhydrous acetic acid (Merck), Citric acid, Iron (III) chloride (Merck), Extracts, steeping simplicia and garlic tuber juice, Ethanol 96% (Merck), Ether, Handskun, Test animals, Filter paper, Liebermann-Burchard (Merck), Sodium chloride, Sodium citrate, Sodium Carboxymethyle Cellulose (Bioworld), High cholesterol feed (Standard 80 feed %, 15% lard, duck egg 5%), Dragendorff reagent, PTU (Propiltiourasil), magnesium P powder, Streptozotocin (Bioworld USA) and Metformin Tablet.

### Extraction

The making of forest onion tuber extracts was carried out using maceration method by means of simplicia powder weighed as much as 3000 grams then put into a maceration vessel using ethanol as much as 5 L, then left for 3 x 24 hours protected from light while stirring occasionally. The extract is filtered using filter paper and the filtrate is obtained. Furthermore, the filtrate was evaporated using a rotary vacuum evaporator at a temperature of 70 °C and continued with thickening carried out using a temperature of 60 °C until a thick extract was obtained.

### Simplisia steeping

Making simplicia steeping of forest onion bulbs (*Eleutherine bulbosa* (Mill) Urb) is done by taking 20 grams of simplicia of forest onion bulbs (*Eleutherine bulbosa* (Mill) Urb) which has been mashed and dissolved in warm water before boiling water, then let stand for a moment, so the temperature drops to a temperature of 70-80 °C

which is the temperature in brewing as much as 200 ml [12].

### Juice

The making of forest onion tuber juice (*Eleutherine bulbosa* (Mill) Urb.) Is done by taking 20 grams of forest onion bulbs (fresh *Eleutherine bulbosa* (Mill) Urb) which has been chopped, then blended with 200 ml of water then waits for a while until the water is reddish, strain and set aside the pulp.

### Making 0.5% CMC Choloidal Na Solution

Sodium carboxymethyl cellulose (Na CMC) weighed 0.5 grams sprinkled in a mortar containing 10 ml of heated distilled water, left for 15 minutes until a transparent mass was obtained, then mixed until homogeneous. Na CMC solution was transferred into a 100 ml volumetric flask. The volume is sufficient with distilled water up to 100 ml.

### Making a Metformin Suspension

The dose of metformin in adult humans is 500 mg per day, if converted to mice weighing 200 grams is 0.018, the dose of metformin for mice is 45 mg / kg BW. Weighed metformin tablet powder equivalent to 388 mg then suspended in 0.5% Na CMC to 100 ml.

### Making Simvastatin Suspension

Streptozotocin was weighed as much as 0.24 grams and then dissolved using saline citrate-buffer with a pH of 4.5 and then induced in rats via intraperitoneal (ip). Streptozotocin dose is 30 mg / kg BB.

### Manufacture of fatty food

High cholesterol feed used was standard feed (80%), lard (15%), and duck egg yolk (5%). Feed is made in the following way: heating pork fat by heating until the lard becomes oil. Eggs are boiled until cooked, separated by egg yolks with egg white. The eggs are cooked, the yellow is taken and crushed until smooth. Standard feed is crushed until smooth and mixed with lard oil and egg yolk. The mixture is stirred until homogeneous is then formed into pellets and put into the oven to dry. The maximum amount of food consumed per day is 20 grams / mouse and given for 4 weeks.

### Data analysis

Data obtained in the form of blood glucose levels and cholesterol levels were analyzed statistically using one way ANOVA test at a 95% confidence level. Furthermore, further testing of the Post Hoc Least Significant Difference (LSD) was carried out. To find out effective sedian gives maximum results. Scoring data on pancreatic Langerhans island damage were analyzed statistically using the Kruskal-Wallis test followed by the Mann-Whitney test to determine differences between all treatment groups. Processing data using the SPSS 23 program.

### ■ Results and Discussion

This study used the test material for forest onion bulbs (*Eleutherine bulbosa* (Mill) Urb.) Obtained from Tipo Village, Ulujadi District, Provention, Central Sulawesi. Before conducting the research, the plant was identified in the UPT. Biological Resources of Tadulako University, Central Sulawesi. The results of the identification prove that the forest onion bulbs used in the study were of the *Eleutherine bulbosa* (Mill) species Urb.

This study used 30 animal test rats (male *Rattus norvegicus*). The use of male white mice as a test animal because it can provide more stable research results because it is not influenced by the estrus cycle and pregnancy as in female white rats. Male white mice also have faster drug metabolic rate and the body's biological condition is more stable than female mice [5].

Diabetic male white rat hypercolestroemia was treated for 14 days, in group 1 was normal control, group 2 rats were given 0.5% Na CMC colloidal solution as a negative control, group 3 rats were given metformin suspension as a positive control. Metformin is a positive control because metformin has the main effect of reducing liver glucose production (gluconeogenesis), and also improves peripheral glucose uptake. Especially used for people with fat diabetes. Metformin works by lowering blood glucose through its influence on the workings of insulin at the cellular level and decreasing the blood glucose production of the liver. Metformin does not have a stimulating effect on pancreatic beta cells so it does not cause hypoglycemia and weight gain [6]. Group 4, rats

were given ethanol extract, group 5 rats were given simplicia steeping and group 6, rats were given juice then blood glucose levels were measured on the 42nd day and 49th day.

The results of measurements of blood glucose levels on day 0 of male white rats (*Rattus norvegicus*) were still in the normal range, ranging from 83-110 mg/dL. After 28 days of high cholesterol feeding and 1 week after streptozotocin induction. The results of measurements of blood glucose levels in mice after induction experienced a significant increase between 214-509 mg/dL which showed rats had a diabetic condition (rats were declared diabetic if blood glucose levels were > 200 mg/dL)[7].

Results of measurements of blood glucose levels of male white mice (*Rattus norvegicus*) on day 35 after administration of high cholesterol feed and streptozotocin induced dose of 30 mg / kg BB for normal control, negative control, positive control, ethanol extract, steeping simplicia and successive juices 90.2, 408.2, 287, 252.6, 431.4 and 416.8 respectively. This shows that all treatment groups had hyperglycemia characterized by blood glucose levels > 200 mg / dL, except for normal controls.

The results of the one way Anova statistical test on the 35th day showed significant results with a value of  $P = 0,000$  ( $P < 0.05$ ) which means that there were significant differences in all treatment groups. This shows the effect of giving streptozotocin, so that it was followed by the LSD Post Hoc test to see significant differences between the treatment groups. Post Hoc LSD further test results showed that normal controls differed significantly from all groups, while negative controls differed not significantly from steeping and juice groups but differed significantly from normal controls, positive controls and ethanol extract groups. Positive controls differed insignificantly from the extract group. ethanol but was significantly different from normal controls, negative controls, steeping and juice groups. This is caused by the condition of mice that are physiologically different so that the increase in blood glucose levels is different in responding to an increase in blood glucose levels after streptozotocin induction dose of 30 mg/kg[8]

The results of measurement of blood glucose levels on day 42 for normal control, negative

control, positive control, ethanol extract group, steeping simplicia and juices were 103.8, 355, 184.8, 189.2, 203.6 and 229.4 respectively. This showed that there was a decrease in blood glucose levels in normal controls, positive controls and extract groups while negative controls, steeping and juice groups showed a decrease in blood glucose levels but had not reached normal blood glucose levels.

The results of the one way Anova statistical test on the 42nd day showed significant results with a value of  $P = 0,000$  ( $P < 0.05$ ) which means that there were significant differences in all treatment groups. This shows the antidiabetic effect of the variation of forest onion tuber preparations, so it was followed by the LSD Post Hoc test to see significant differences between the treatment groups.

Data from blood glucose level measurements on day 49 for normal control, negative control, positive control, ethanol extract group, simplicia and juice steeping were 113, 355.2, 124.2, 133.6, 140.8 and 167.8 respectively. This shows that there is a decrease in blood glucose levels that are close to normal values in positive controls, ethanol extract and simplicia steeping, while the juice group shows a decrease in blood glucose levels but has not reached normal blood glucose levels which are characterized by high blood glucose levels.

The results of the one way Anova statistical test on the 49th day showed significant results with a value of  $P = 0,000$  ( $P < 0.05$ ) which means that there were significant differences in all treatment groups. This shows the antidiabetic effect of the variation of forest onion tubers given, so it was followed by the LSD Post Hoc test to see significant differences between the treatment groups. Post Hoc LSD further test results showed that the negative control group was significantly different from all treatment groups. Normal controls were not significantly different from positive controls, ethanol extract groups and simplicia steeping but differed significantly from negative controls and juice groups. This was due to ethanol extract and Simplicia steeping can reduce blood glucose levels in male white mice comparable to positive controls.

The treatment group of the ethanol extract of forest onion bulbs with various extracts, steeping and tuber juice had an effect on the reduction of

blood glucose levels of hypercholesterolemic male-Diabetes white rats. Based on the maximum results to reduce blood glucose levels between ethanol extract, steeping and forest onion tuber juice (*Eleutherine bulbosa* (Mill) Urb) in male hypercholesterolemic white rats - ethanol extract because it gives the effect of decreasing blood glucose levels closer to normal controls. This shows that the extracts are thick preparations obtained by extracting the active substance from vegetable simplicia or animal simplicia using suitable solvents, then using the extraction method with the process of withdrawing soluble chemical content from a simplicia powder, so that it is separated from the material which cannot late [9]. While juice is a liquid obtained from parts of edible fruit that is washed, crushed, purified (if needed), with or without pasteurization and packaged to be consumed directly. Steeping is a way of providing traditional medicine by watering the medicinal ingredients with boiling water, after being left for a while, which is a bit cold just drunk [10].

Based on the results of rat pancreatic histopathology preparations and statistics, it can be seen that the extract, simplisisa steeping and forest onion tuber juice dose of 20 g/kg BW have had an influence on regeneration of pancreatic tissue, with an average scoring of 0.5 while in steeping scoring the average of 1.5 and 1.25 tuber onion juice of the effect in regenerating pancreatic  $\beta$  cells is not very good compared to the ethanol extract of forest onion bulbs. This shows that the extracts are thick preparations obtained by extracting the active substance from vegetable simplicia or animal simplicia using suitable solvents, then using the extraction method with the process of withdrawing soluble chemical content from a simplicia powder, so that it is separated

from the material which cannot late[11]. While juice is a liquid obtained from parts of edible fruit that is washed, crushed, purified (if needed), with or without pasteurization and packaged to be consumed directly. Steeping is a way of providing traditional medicine by watering the medicinal ingredients with boiling water, after being left for a while, which is a bit cold just drunk [9] .

Table 1. Phytochemical Test Results Ethanol Extract, simplicia steeping and onion bulb juice Forest

Testing	Result
Flavonoid Test	+
Saponin Test	+
Alkaloid Test	+
Steroid Test	+
Tanin Test	+

These data (Table 1) indicate that the root juice contains secondary metabolites, namely flavonoids, saponins, alkaloids, steroids, and tannins.

Table 2 shows that on day 0 there is no significant difference in each group which is indicated by a P value > 0.05 (P value = 0.719). On days 35, 42, 49 showed a significant difference in each group which was indicated by a P value < 0.05

Table 3 shows that on day 0 it shows that there is no significant difference in each group which is marked with a P value > 0.05, on days 35, 42, 49 shows a significant difference in each group which is indicated by P value < 0.05

Table 2. Average Results of Measurement of Blood Glucose Levels

Days To-	Mean $\pm$ SD Blood Glucose Level (mg / dL)						P
	Normal Control	Negative Control	Positive Control (Metformin)	Ethanol extract	Steeping	Juice	
0	98.8 $\pm$ 7.32	94.4 $\pm$ 12.09	98.8 $\pm$ 7.66	93.4 $\pm$ 6.91	92.8 $\pm$ 6.14	94.4 $\pm$ 5.81	0.719
35	90.2 $\pm$ 9.17	408.2 $\pm$ 12.35	287 $\pm$ 82.86	252.6 $\pm$ 61.91	431.4 $\pm$ 113.44	416.8 $\pm$ 28.04	0.000
42	103.8 $\pm$ 7.41	355 $\pm$ 47.93	184.8 $\pm$ 25.86	189.221	203.6 $\pm$ 20.98	229.4 $\pm$ 44.25	0.000
49	113 $\pm$ 8.16	370 $\pm$ 48.14	124.2 $\pm$ 28.82	133.6 $\pm$ 15.58	140.8 $\pm$ 25.07	167.8 $\pm$ 37.69	0.000

Table 3. Results of mouse pancreatic histology scoring

Rats	Pancreatic Damage Level Scoring Data					
	Normal Control	Negative Control (Na CMC)	Positive Control (Metformin)	Ethanol Extract Doses of 20g / kg BB	Simplicia steeping Doses of 20g / kg BB	Juice Doses 20g / kg BB
1	0	3	1	0	2	1
2	0	3	1	0	1	2
3	0	3	2	1	2	1
4	0	2	0	1	2	1
Average	0	2,75	1	0,5	1,5	1,25
SD±	0	0,5	0,681	0,5	0,5	0,43

Remarks: score 0 = no damage, score 1 = damage ¼ part cell, score 2 = damage ½ part cell, score 3 = damage ¾ part cell

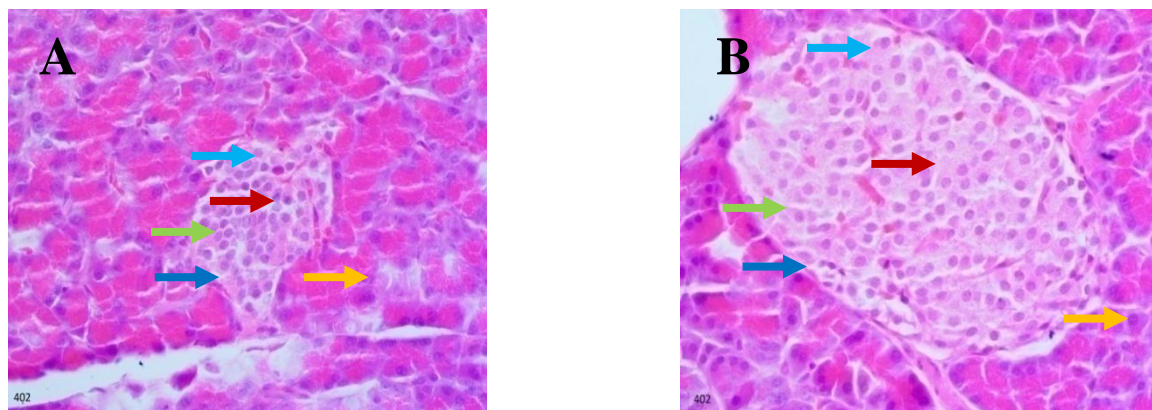


Figure 1 white mouse pancreatic tissue score 0 (Normal) staining HE 400X magnification

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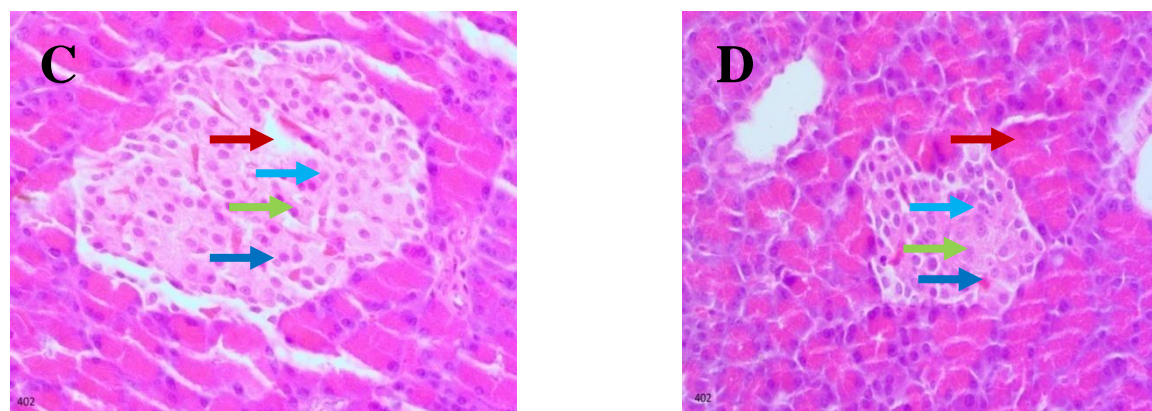
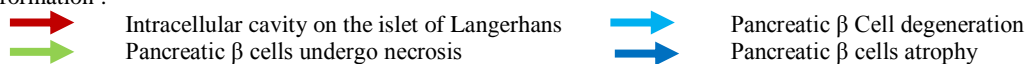


Figure 2 white rat pancreatic tissue score 1 staining HE 400X magnification damage

Information :



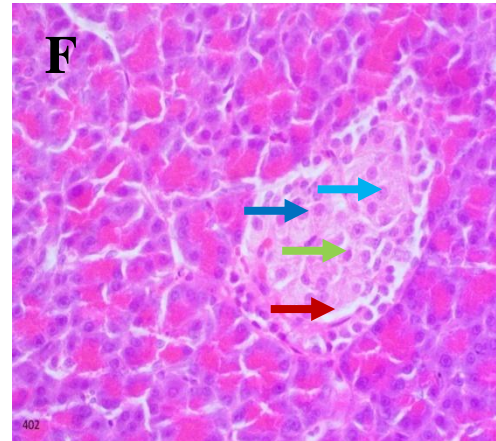
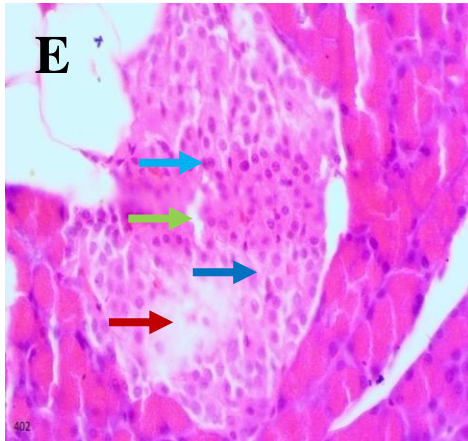


Figure 3 white rat pancreatic tissue score 2 staining HE 400X magnification damage

Information :

- Intracellular cavity on the islet of Langerhans
  - Pancreatic  $\beta$  cells undergo necrosis
- Pancreatic  $\beta$  Cell degeneration
  - Pancreatic  $\beta$  cells atrophy

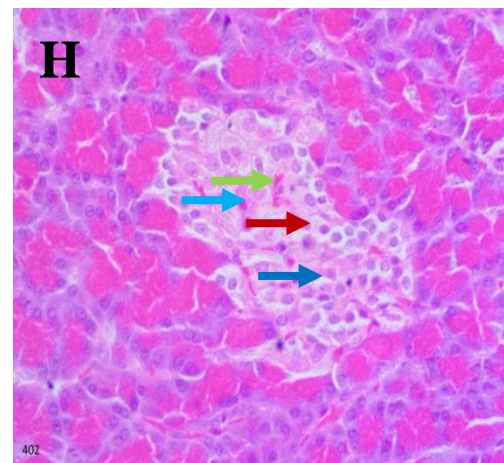
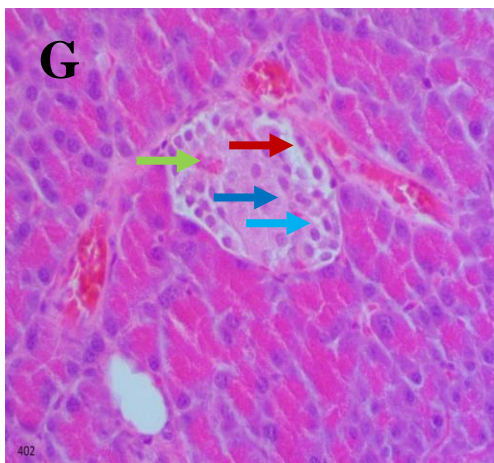


Figure 4 white rat pancreatic tissue score 3 HE staining 400X magnification damage

Information :

- Intracellular cavity on the islet of Langerhans
  - Pancreatic  $\beta$  cells undergo necrosis
- Pancreatic  $\beta$  Cell degeneration
  - Pancreatic  $\beta$  cells atrophy

A Langerhans islet score of 0 indicates normal, homogeneously distributed cells, appearing to be regular and uniform in size so that they are easily distinguished from the exocrine pancreas [12]

In pictures C and D, where the pancreatic beta cells with the lightest necrosis level are 1-25% in figures C and D where pancreatic beta cells are still homogeneously distributed, it appears that the regularity between endocrine cells with a uniform

shape of cytoplasmic cell nuclei with 10% damage occurs. degeneration, atrophy, necrosis and accompanied by the presence of intracellular cavities [12].

Figures E and F Langerhans islands with a necrosis rate of 25-50% and in Figures E and F there is 45% damage, namely degeneration, atrophy, necrosis, and accompanied by intracellular cavities. Endocrine cells appear irregular, but pancreatic beta cells are still

distributed homogeneously with different shapes and sizes in nucleus and cytoplasm [12].

Langerhans Island with a necrosis rate of 50-75%. In pictures G and H there is 50-70% damage, namely degeneration, atrophy, necrosis, accompanied by a larger intracellular cavity. Endocrine cells appear irregular between  $\alpha$  cells,  $\beta$  cells,  $\delta$  cells, F cells and  $\beta$  cells of the pancreas are irregularly arranged with different shapes and sizes, nucleus and cytoplasm due to inflammation[12].

## ■ Conclusion

Secondary metabolites are found in ethanol extract, steeping and juices of forest onion bulbs (*Eleutherine bulbosa* (Mill) Urb) namely flavonoids, tannins, saponins, alkaloids and steroids and ethanol extract of 20 g / kg can have a better effect on blood glucose levels and  $\beta$  cell regeneration of pancreas in white mice compared with simplicia steeping and forest onion tuber juice

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