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Snakehead (*Channa striata*) Extracts Treatment towards Hyperglycemic Mice (*Mus musculus*) Blood Glucose Levelsand Pancreatic Histology Structure

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ABSTRACT

Hyperglycemic condition induced by islets of Langerhans (island of Langerhans) tissue damage of the pancreas. Albumin treatment was expected to generate islets of Langerhans tissue. Snakehead (*Channa striata*) extracts contained roughly a million albumin so that, it was inquired to generate its pancreatic tissue of mice (*Mus musculus*) hyperglycemic. Alloxan at 190 mg/kg of weight doses with intraperitoneal (IP) injection used to encourage mice hyperglycemic. Various doses during 14 days were carried out under it treatment. Blood glucose levels had measured by Accu-Check Roche® glucose meter (glucometer). Histological preparation for mice pancreas obtained through Haematoxylin and Eosin (H&E) Staining for paraffin sections, where it observed under Olympus® BX-41microscope image. As much of 0.14846 mL/day had generated 68.78% islets ofLangerhans tissue of the pancreas and reduced 34.42% blood glucose levels as long 14 days were obtained as the result of snakehead albumin treatment.

KEYWORDS: *Channa striata*, diabetes,blood glucose, pancreas

INTRODUCTION

Diabetes mellitus was a metabolic disease which characterized by hyperglycemia symptoms as a result of insulin secretion disorders or increasing of the cells resistance to insulin. Diabetes was distinguished into 2 types.Type I called IDDM (*Insulin Dependent Diabetes Mellitus*), it occurs due to tampering of the pancreas β cells that may lead depopulating for the number of insulin hormone secretion, so it neither able to keeping glucose from blood circulation and controllingblood glucose levels. Type IIcalledNIDDM (*Non Insulin Dependent Diabetes Mellitus*) occurs due to insulin resistance.In this case indeed insulin number was enough in amount. But it was lack of energy on a cell because it changed not more sensitive andglucose utilization worked optimally [1].

Now days, antioxidant derived from a vegetable or animal extract, was frequently used for metabolic disease healing process include diabetes mellitus. Snakehead (*Channa striata*), a freshwater fish, it has already widely known by Indonesian people. It was easily found for consumption or medicine, by these reasons its price became more expensive. Its exogenous antioxidant was expected to improve pancreatic damaged thatinduced by free radicals from alloxan, so to prove the effectiveness of its extract this research was aimed.

MATERIALS AND METHODS

This research was conducted since August to November 2013 at Zoologicallaboratory Department of Biology, Institut Teknologi Sepuluh Nopember Surabaya (ITS).Drinking and eating mice equipment, glucometer, IP injector, syringe, latex gloves, microscopes, pipette drops, micro pipette, tissue, cover glass, objects glass, dissecting set, cotton, and microtome used as tools. The ingredients that used in such as, 25 mice (*Mus musculus*) bull (two-three month), drink mouse, feed, snakehead extract, alloxan, aquades pro-injection, chloroform, alcohol 70 %; 80 %; 90 % and 95 %, absolute ethanol, xylol, formalin 10 %, paraffin, dye hematoxylin with eosin and aquades

Maintenance for mice(*Mus musculus*)

20 Bull mice were grouped around 5 groups(4 mice/group),acclimated as long as 2 weeks. They were fed up and given to drink daily. 18 hours (day 15) before blood glucose levels check, fed up was stop. Glucometer used as checking tool.

Treatment for hyperglycemic mice (*Mus musculus*)

Alloxan solution

Each mice weighed beforehand to know a weight which was associated with how many alloxan will be induced. A dose of 190 mg/kg weight, used as reference doses, it has converted into mouse weight in grams. Storage till injecting process should be

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carried on a cool temperature, to avoid alloxan damage.Alloxan who has weighed (corresponding to each conversion of weight each of mouse) then is dissolved by pro-injection water/aquades as many as 5 cc for each mouse.

Hyperglycemic mice (*Mus musculus*) induced by alloxan

After checking (blood glucose: at the 15th) its induction conducted in group B, C, D and E on the 16th day. Amount 0.5 cc of alloxan induced to mice by using a syringe 1 cc mLwith intraperitoneal injection method (on the abdominal cavity).Beforehand the mice intraperitoneal sterilized used cotton moistened with 70% alcohol.Then syringe had contained alloxan solution, injected in that area.Mice in group 1 only injected with a mere aquades pro-injection.

Treatment for hyperglycemic mice (*Mus musculus*)

Mice groups (C, D and E), had ≥200 mg/dL blood glucose, ready for snakehead treatment in day 20 orally. Doses formulated under Faculty of Veterinary Universitas Airlangga Surabaya, accurately. As details treatment as bellow:

- Group (A): negative control mice (normal).
- Group (B): positive control mice(hyperglycemic).
- Group (C): hyperglycemic mice fed up by snakehead treatmentwith 0.07423 mL/day doses.
- Group (D): diabetes mice fed up by snakehead treatment with 0.1248 mL/day doses.
- Group (E): diabetes mice fed up by snakehead treatment with 0.14846 mL/day.

These treatments conducted as long as 14 days until day 33.

Measurement of Blood glucose levels

Mice got fast during 18 hours before blood glucose checking used glucometer.Tail tip of mice were wiped by alcohol 70% with wet cotton. It sliced in a centimeter. Strip inserted to glucometer. Mice blood from its tail drops to glucometer box sensory when drop direction has occurs. Observe on glucometer screen for digital result (mg/dL) as its shown blood glucose levels. A strip using was only for once. Blood glucose test conducted at day 15 (after acclimated), day 19 (after hyperglycemic induction), day 27 (after snakehead treatment in day 7) and day 34 (final treatments). It may help compared between normal mice (A), hyperglycemic (B) and hyperglycemic after its treatment with distinguished snakehead doses (C, D, and E). Each blood glucose levels test conducted in an hour as long as 2 hours totally, to obtain that it was really associated to blood glucose of diabetes condition, due to glucose was easily dissolved around 2 - 3 hours even it took in sterile way. It has known as glycolysis [2]

Charts and tables were served as the results when snakehead therapy or treatment have already finished to gain lower blood glucose levels for hyperglycemic mice, it used ANOVA test based on blood glucose levels average calculation. The percentage of a decrease in blood glucose levels of mice written as follows;

$$\frac{K2 - K1}{K2} = P\,\%$$

Descriptions:
K2 = decline average rate of blood glucose
K1 = average rate of blood glucose before got decline
P = percentage of decline

Pancreatic histology slides

A sample of the pancreas that has been passed fixation in a Bio Analitika® pro-analysis 10% formalin buffer as long as 24 hours, then it followed by Haematoxylin and Eosin (H&E) Staining for paraffin sections,as its detail bellows;

1. Tissue preparations(materials, samples and tools) Anesthesia, surgery and tissues isolation of mice
2. Embalming (fixation) of tissues useda Bio Analitika® pro-analysis 10% formalin buffer as long as 24 hours for minimum
3. Execution, that made up of :
   a. Dehydration, it used alcohol 70% (20 minutes), alcohol 80% (20 minutes), alcohol 96% (2 x @30 minutes).
   b. Clearing, it used xylol (2 x @30 minutes) until reach a slightly tissue/transparant.
   c. Infiltrating/impregnating/emmbedding), it used paraffin/liquid paraplast (3 x @1 hours).
   d. Blocking/casting). Paraffin tissue/paraplast III inserted to emmbedding cassette which has already poured by parafin/ liquid paraplast then let it frozen.
4. Tissue's sectioning made up of:
   a. Apreparation, knife laid on with the microtome to specified angles. The top block paraffin cut beforehand so that equal to form the bottom. Then holder and block paraffin laid on its place in the microtome. Waterbath filled with aquades and it ignited, temperature and aquades in waterbath are set to reach liquid paraffin temperature point is about 55°C.
   b. Slicing with rotary microtome, set its thickness pieces on the microtome ±5 µm. It was regulated has a shortest distance possibly, the rotor is moved in rhythmic and clockwise so it block touch knives and slicing block paraffin accurately. Paraffin bands in early which are not contained tissue are disposed. Then after a wedge closely reach it tissue, block paraffin sliced cautiously. Paraffin bands inside were tissue desirable, then transferred carefully with a brush small upward surface water/aquades in a waterbath that has organized in 55°Ctemperature. A tissue that has become separated from the paraffin

ribbon affixed to object glass cautiously. Glass object that was containedit, laid over hot plate with temperature 40-45°C until dries so that the tissue may stick perfectly.

5. Tissue's staining consists of:
   a. Deparaffinated, a tissue soaked in xylol (3 x @5 minutes).
   b. Hydrated, a tissue soaked in alcohol 96% (2 x @2 minutes), alcohol 80% (2 x @2 minutes) and alcohol 70% (2 minutes) then aquades (10 minutes).
   c. A tissue incubated within Haematoxylin solution around 10 minutes.
   d. It diluted with water flows around 10 minutes.
   e. It has counterstaining with Eosin around 2 minutes.
   f. Dehydration, it tissue soaked in alcohol 70% (2 minutes), alcohol 80% (2 minutes) and alcohol 90% (2 x @2 minutes).
   g. It has clearing with xylol (2 x @5 minutes).
   h. It has mounting with object glass
   i. Labelling, put some notes about it treatment and how many replication duringthe research.

Pancreatic histology microscopic observation

Observation of histological pancreatic microscopically performed by looking at the diameter of the island of Langerhans without cell β scoring system using a compound microscope under roughly 40-1000 magnification. Then it followed by made a comparison between the diameter average of the pancreatic Langerhans islets mice at all groups, as well as conducted a comparison to blood glucose levels with its diameter.

Snakehead albumin therapy for β cell repairing of mice hyperglycemic pancreas served with an observation of Langerhans islands, its size in diameter and the results of the ANOVA test diameter average for each group of treatment. The percentage of its repair obtained from the formula as follows;

$$\frac{D2 - D1}{D2} = P\,\%$$

Descriptions:
D2 = islets ofLangerhansdiameter average after repairing.
D1 = islets of Langerhans diameter average before repairing.
P = islets of Langerhans percentage repairing value.

Data analysis

Draft research composed using randomized complete design (RAL). Quantitative Data (average blood glucose levels and the diameter of the islands of Langerhans) obtained from mice, analyzed using one-way ANOVA statistically. Four replications were used in each group. Tukey confidence test with interval 95% used to see a significant difference,

RESULTS AND DISCUSSION

Snakehead (*Channa striata*)albumin therapy towards islets ofLangerhansregeneration

Snakehead albumin used as the therapy aims to determine its effects on the regeneration of pancreatic Langerhans islets in hyperglycemic mice (*Mus musculus*). The pancreas was used as a parameter because it was an important instrumental in glucose setting for the blood system immediately. The β cells of pancreas wereacted for secreting insulin. Insulin activation signaled by the presence of an excess of glucose levels in the blood. It was carried by the insulin to metabolized in tissues that are in the body [3].

Haematoxylin-eosin staining to the islands of Langerhans visible contrast used as the preparations of pancreas organ. The islands of Langerhans in the pancreas was composed of 4primary cell types such as β cells (produced insulin), which form a 60-80% of the cells mass. α cells, secreting glucagon nearly 25% and delta cells produced somatostatin as much as 2 to 8% [2]. Therefore its diameter size can be represented as an indicator of pancreas organ damage or repair on hyperglycemic. Figure 1 below was thepancreatichistological microscopic slides observation result that represents its condition in each treatment group. ANOVA test determined therapy influence on the histology of the pancreas which presented in Figure 1 below;

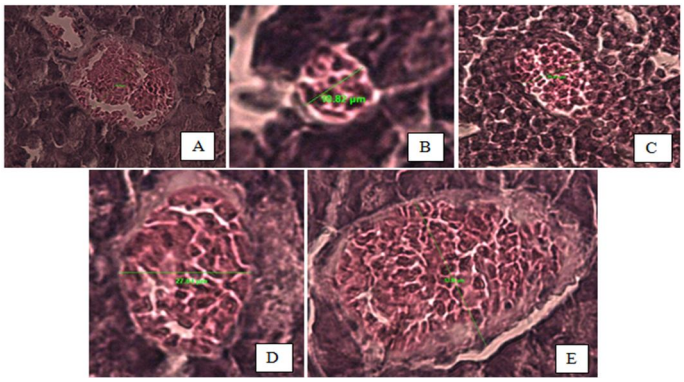

Figure 1. Islets of Langerhansof pancreas microscopic images (1000 magnification).

Descriptions:

A. Negative control. Diameter 66.14 µm.
B. Positive control. Diameter 10.82 µm.
C. Snakehead albumin bottom doses (0.07423 mL). Diameter 25.05 µm.
D. Snakehead albumin mid doses (0.1248 mL). Diameter 27.64 µm.
E. Snakehead albumin top doses (0.14846 mL). Diameter 53,96 µm.

Table 1. Langerhans islands diameter of mice(*Mus musculus*) panceratic based on ANOVA test.

n	Diameter of LangerhansIsland Pancreas In Each Groups (µm)				
	Control		Doses		
	(-)	(+)	Bottom	Mid	Top
1	66.14	14.63	21.87	27.64	52.62
2	62.74	10.82	20.75	26.18	40.14
3	86.62	18.27	25.05	30.94	53.68
4	90.39	18.85	25.28	33.87	53.96
Mean	76.47^{A}	15.64^{C}	23.24^{C}	29.66^{C}	50.10^{B}

Based on the table 1 above, the diameter of the island of Langerhans in the top therapeutic dose was larger (50.10 µm) than its bottom (23.24 µm)and (29.66 µm). The percentage of increase in the diameter average of its island between the control (+) with top doses over by 68.78 %.Meanwhile the percentage of diameter increase from control (+) to bottom and mid doses of successive was 23.68 % and 47.25 %.On ANOVA test results above with (P) value < 0.05, its normal diameter larger than the top dosage. A snakehead albumin extract played as exogenous antioxidant which containing 70% protein (major composer of a cell) and 21% albumin (played as regenerator cells damaged) [4]. Langerhans Island regeneration process at the top dose treatment group hadn't approached normal Langerhans island conditions yet like on the negative control, group because at 14 days therapy need a longer healing time for getting closer to its normal. Albumin acts as an exogenous antioxidants which expected to stabilize free radicals (molecules that have a group of atoms with electrons are not paired). If free radicals were not attenuated, its reaktivitymay destroy all types of cellular macromolecules, include carbohydrates, proteins, lipids and nucleic acids[5]. Its free radicals were created from a hyperglycemic induced diabetogenik substance. Albumin was known as powerful antioxidants in the plasma. It was a substance needed to neutralize free radicals and prevent damage caused by free radicals against normal cells, proteins and fats [6]. It neutralized free radicals which complement the electron deficient owned by free radicals and inhibits the occurrence of a chain reaction of free radicals that may cause oxidative stress by removing potential toxical oxygen (pro-oxidants), pressing its formation or against pro-oxidants that work [7].

In addition, high in albumin of snakehead extract was instrumental in helping the regeneration of damaged cells, defense system (immunity) also does its own body. The body has many mechanisms for defending against free radicals. The varied defenses are complementary with each other because it worked on a different oxidant or in different parts of the cell. An important line of Defense was a protective enzyme system of free radicals. Among these was initiation (the beginning of the formation of free radicals), propagation (a series of reactions that develop over the onset of free radical-transfer or addition of atoms) and termination (inactivation of endogenous free radical by antioxidants or superoxide dismutase enzymes and exogenous) [7].

Therapy (snakehead albumin) effect towards hyperglycemic mice blood glucose

This therapy aimed to lower the blood glucose levels of hyperglycemic mice (levels of glucose in the blood ≥ 2265 200 mg/dL). Measurements conducted on the 15^{th} as a representation of blood glucose levels after acclimated, the 19^{th} after induction hyperglycemic, the 27^{th} day was 7 days after snakehead albumin therapy goes and day 34 at the end of reserach (completed therapy during 14 days). Each of these measurement results showed the average blood glucose levels were different mice in each

group. The increase and decrease in blood glucose levels mice are presented in Figure 2, while the results data using ANOVA about the influence of its extract as lowering blood glucose levels in hyperglycemic mice are presented in Figure 2.

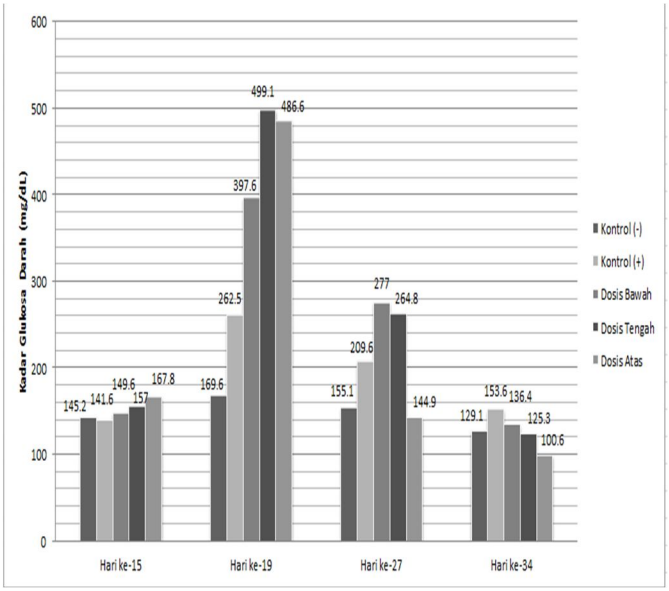

Figure 2. Blood glucose levels of mice (*Mus musculus*).

Table 2. Snakehead albumin therapy towards blood glucose levels in hyperglycemic mice based on ANOVA test.

n	Blood Glucose Levels in Each Groups (mg/dL)				
	Control		Doses		
	(-)	(+)	Bottom	Mid	Top
1	139	147	155	148	123
2	135	159	149	101	119
3	111	163	130	112	73
4	132	147	113	141	89
Mean	129.25^{AB}	154.00^{A}	136.75^{AB}	125.50^{AB}	101.00^{B}

Based on Figure 2 above shown that among the conditions after acclimated (the 15th) and after induction of hyperglycemic (day 7), shows an average increase of blood glucose levels 129.25 mg/dL to 150 mg/dL that was as much as a 16.07%. It suggests that the induction of hyperglycemic in mice using alloxan single-dose as many as 190 mg/kg body weight (intraperitoneal injection) may cause hyperglycemic to entire mice Group B, C, D and E. It occurs because alloxan induced mitochondrial Ca^{2+} ions that resulting in impaired cell oxidation process. Discharge of Ca^{2+} ion from mitochondria affected in disruption of homeostasis as the cell death early. Alloxan also inhibited the activity of calmodulin (CaM) that played a role in Ca^{2+}transport in the cell and was a binding protein Ca^{2+} ions [8]-[10]-[10]. Alloxan cytotoxic action was mediated by the presence of reactive oxygen species (free radicals). Alloxan and it reduced products, acids in the, dialurate acid, yielding a redox cycle with the formation of superoxide radicals. Free radicals underwent a dismutase into hydrogen peroxide. After that, the highly reactive hydroxyl radicals formed from the reaction of Fenton. Reaction of reactive oxygen species with calcium within cells caused quickly damage on pancreas β cells, so that it increased the levels of sugar in the blood [10].

The therapy on hyperglycemic mice performed on day 20 to 34. Results according to Table 2, seen that decrease blood glucose levels of its conditions (day 19) most therapy group on snakehead albumin top doses (0.14846 mL/day/oral) which was as much as 34.42%, followed by groups of middle-dose therapy (0.1248 mL/day/oral) and lower doses (0.07423 mL/day/oral) the respective percentages of decrease as much as 11.20% and 18.51%. ANOVA test results also showed that the therapy using its extract effect on mice blood glucose levels decrease with p. value < 0.05. This known that the island of Langerhans also got significant improvement of its therapy. Where, setting up blood glucose levels conducted by the hormone insulin which was secreted by the β cells of the pancreas.

In the islands of Langerhans there wereα cells that secreting the glucagon, β cells that produce insulin and somatostatin which resulted by δ cells. A third type of cell layout this was anatomically adjacent so that there was polypeptide hormones secretion coordination, especially between two antagonists, glucagon and insulin. Blood glucose levels were maintained through the interaction of insulin secretion and glucagon. The secretion was inhibited by both somatostatin. Its secretion stimulated by glucagon. The primary stimuli for this interaction were the levels of glucose in the blood [11]. In the metabolism process, insulin levels played an important role which included glucose into the cell which was used as fuel. Insulin was a substance or a hormone produced by the beta cells of the pancreas, where insulin was not there then glucose may not enter the cells. Glucose

remains in the blood plasma which means that glucose levels in the blood increased [12]. Therefore, it can be assumed that as greater the Langerhans islets diameter of the pancreas as much more beta cells. So that it argued that insulin secretion was proportional by the number of cells pancreatic Langerhans islets beta cells in normal conditions.

Conclusion

Snakehead extracts may generate islets of Langerhans (island of Langerhans) within pancreas. Doses at 0.14846 mL/day named as the best doses according this final research result. It has 68.78% of hyperglycemic improvement percentage index. Blood glucose levels drove down by 0.14846 mL/day doses in mice hyperglycemic treatmentwith34.42% drop percentage of blood glucose levels.

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