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The Influences of Fibroblast Growth Factor (FGF) And Protein About to Histopathology of Rats Pancreatic β Cell.

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ABSTRACT

The research about the influence of giving egg whites chicken embryo and maryn protein to the histopathology of pancreatic β cell from diabetic rats induced Alloxan with dose 150 mg/kg BW. This research was done experimentally using rats were divided into 3 group each group consist of 5 rats, which are negative control, positive control and group of treatment with doses 30 mg/200g BW rat to white fertile chicken eggs that was incubated for 9 days and 1g/200g BW rat to animal protein from snack fish powder. This preparation were given for 28 days and measurement of blood glucose levels done on day 7th , 14th, 21st, 28th after administration of the test preparation. On the 29th day the rats were sacrificed and then dissected and pancreatic tissue is taken to look at the histopathology of the pancreas with Hematoksilin Eosin (HE). The result statistically showed there was significant effect of $p<0,05$. The description of histopathology observations that there is any change better than the positive control.

Keywords: FGF, protein, stem cell, histopathology

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INTRODUCTION

As the development of health and the pharmaceutical industry research, has been developed based on stem cell research using egg whites, chicken Avian fertile eggs were incubated until reaching the pre-embryo, for 9 days. Egg whites are separated and extracted by the method of freeze dried [1].

The avian chicken eggs is a source of nutrients that contain protein, lipid, vitamin, mineral and growth factors that are important for embryonic development, as basic nutrients for the biological function of a chicken and give defense factors to protect the embryo from bacterial and viral infections. Fibroblast growth factor (FGF) is one of the growth factors that are from the early stages of embryonic development of chicken and responsible for the progress of the initial cell stimulatory signals such as setting the pattern, proliferation, differentiation and migration to form a tissue[2].

In Indonesia, the eggs are used as ingredients in traditional beverage known as tea eggs, stamina that is processed half cooked and consumed without processing to help increase muscle mass, because the eggs contain protein. In addition to eggs, fish can also be used as an alternative source of animal protein, because protein content is high enough. So that the fish is also a food that it is proposed to give a positive effect on health.

Definitions are the cells that became the beginning of the growth of other cells that make up the whole body of the organism, including humans. Stem cells will differentiate specifically after obtaining specific stimulatory signals to generate different cell types. Stem cells can differentiate into specialized cells that have functions and special forms such as the liver cells, blood cells, endothelial cells, nerve cells, heart cells and others[3].

One of the diseases that many common metabolic disorder is diabetes mellitus. Diabetes mellitus is a degenerative disease that has characteristics of chronic hyperglycemia, which is caused by insulin deficiency or insulin resistance[3]. In this study expected the nutrients contained in the egg white powder and fish powder can improve pancreatic β cells and normalize blood sugar levels of the rats.

METHOD

Time and Place Research

The study have been conducted from May to July 2015 in the Laboratory of Pharmacology, The college of pharmacy Indonesia (STIFI) Padang and Kopertis Laboratory Region X and the Laboratory of Veterinary Bukittinggi for histopathology examination of the pancreas.

Materials

The apparatus were used is analytical balance, spatula, egg incubator, manual egg beater, aluminum pan, oven, mortar and pestle, vials, syringes ip, sonde needles, glass tools (Pyrex®), cage rat, weighing animals , digital blood glucose measuring devices (Gluco Dr®), cotton, alcohol swabs, surgical tools (tweezers and surgical scissors), glass objects, microscope Trinokuler electric, and hot plate.

The materials were used is fertile chicken eggs, aluminum foil, suspending agent (Na CMC), aquadest, flour fish snack (*Dahfa Snack ikan*®), a solution of 1% egg white powder, fish snack starch solution 1%, HNO_3, NaOH 10% , NaOH 4N, $CuSO_4$, Millon reagent (HgNO3 dan $NaNO_2$), food for rodents, the solution ether, 10% formalin, a solution of NaCl physiological, alcohol solutions of various concentrations (50%, 70%, 80%, 90%, 96%, and absolute), xylol solution, solid paraffin, Mayer's albumin (egg white-glycerin = 1: 1), water, distilled water, hematoxylin-Erlich reagents, reagent eosin-alcohol 1%.

Procedure

Preparation of the test preparation

a) White flour eggs, eggs used are 8 pieces fertile chicken eggs, do not crack, and taken carefully, there should be no excessive shocks, then incubated at a temperature of 38-39° C for 9 days and the egg is rotated as much as 2 times a day. Then do the separation of egg white and made into flour eggs with thin-layer method / pan drying.
b) Snack fish powder used is have nutritional standards

Testing the egg white flour and flours snack fish

- *Rendemen*
- Organoleptic: form, colour, odor and taste of the preparation.
- Identification of amino acids and proteins: Biuret test, Xantoprotein test and Millon test.

Planning of the dose

The dose of Alloxan is 150 mg/kg of BW; a dose of egg white powder is 1,680 mg for 70 kgBW/day; and dose fish meal snack taken based on human protein needs 60 g /day.

Treatment of experimental animals

Acclimatization: the experimental animals used were 15 male rats (*Rattus novergicus*), healthy, which aged about 2-3 months, weighing about 150-200g, and never had received drug treatment. Before use of the experimental animals, acclimatized for 1 week with increased or decreased weight tolerance of 10%. During maintenance, the experimental animals were given food and drink sufficiently.

The animal trials were made hyperglycemia by administration of substances diabetogenic: alloxan dose of 150 mg/kgBW by intra peritoneal, before inducing, rats were fasted for 16 hours and checked for glucose levels early or before inducing. On the 14^{th} day after inducing, fasting blood glucose levels of mice are checked as glucose hyperglycemia or after inducing. Checking blood glucose levels by using GlucoDr®. Diabetic rat if when fasting blood glucose levels before and after inducing significantly different ($p<0.05$) were statistically by paired T test.

Group of the treatments

Animals were divided into 3 groups each 5 rats:

- Group 1: control group (-) is the experimental animals were given food, drink and suspending agent Na CMC standard of 1 ml of 0.5% during the trial period (28 days).
- Group 2: positive control group is the experimental animals induced alloxan, dose of alloxan is 150 mg/kgBW by intraperitoneally and given food, drink and suspending Na CMC standard 1 ml 0.5% during the trial period.
- Group 3: The group of experimental animals induced alloxan dose of 150 mg/kgBW by intraperitoneally and given suspensions flour egg whites with a dose of 30 mg/200gBW of rats and suspension snack fish powder with a dose of 1g/200gBW rat for 28 days and given food and drink standards.

Fasting blood glucose levels of rats of all groups are checked every 7 days for 28 days of suspension dosage administration. Experimental animals were sacrificed on day 29 by way of anesthetized using ether and cotton, animal dissected with surgical scissors and pancreatic organs taken for histopathology examination. Then the pancreas organ were made preparations for microscopic examination with an electric microscope magnification of 400x.

Data processing

The observation of changes in blood glucose levels in animal experiments are recorded, tabulated and analyzed statistically using One-Way ANOVA, for differences in blood glucose levels mean the control group (-) for 28 days and Two - Way ANOVA for differences in blood glucose levels mean the three groups treatment for

28 days. Then proceed with advanced test Duncan. Results of histopathology cells of pancreatic islets of Langerhans are used as supporting data and analyzed qualitative mean and standard deviation for each group was calculated from the data obtained. The value obtained from research data will be presented in the form of mean ± standard deviation.

RESULT AND DISCUSSION

a **b**

Figure 1. a: the egg white flour; b: the powder of fish snack

The egg whites that have been taken from chicken embryos which aged about 9 days, and then dried by the method of drying pan in the oven at 40-45^{O}C till not damage the proteins and growth factor which is also a protein. The Fish snacks was made in powder. Organoleptic of the egg white flour is grain powder, yellowish white, typical smell of eggs and egg flavor; organoleptic fish meal snack is a fine powder, yellow-brown, the typical smell of fish and the flavor of the fish. Then do the identification of proteins and amino acids with the color reaction use is biuret test, millon test and test of xantoprotein.

In the biuret test egg white flour and powder of snacks fish, the color of the solution changes to blue-violet after added a solution of $CuSO_4$. This indicates a positive reaction, where the purple color due to the formation of complex compounds of Cu^{2+} and molecules N of peptide bonds under alkaline conditions. The number of amino acids bound to the peptide bond will affect the color intensity of the solution formed[4]. Millon reaction involves adding Hg compounds into the protein solution so that the addition of these metals produces a white precipitate of mercury compounds. For protein-containing tyrosine or tryptophan, Millon reagent additions and heating will produce a red color. However, this reagent is not specific as it gives a positive test with their red phenol compound, so it is used specifically to test for phenol group on proteins such as tyrosine. From the identification results showed that egg white flour and powder of snack of fish results is negative.

Test is a qualitative test protein Xantoprotein with benzene nucleus such as tyrosine, and tryptophan fenilalain using nitric acid and heating enable the breaking the protein chain into a cluster benzene. A positive reaction is indicated when the appearance of a yellow color reaction due process and the nitration of the benzene ring will change to orange when combined with an alkaline solution[4].

Diabetic condition can be caused by the induction of toxic compounds that can cause damage to the β-cells of the pancreas. Induction with alloxan can cause mice become hyperglycemic because alloxan in the body will be metabolized reduction oxidation produces free radicals and radical alloxan. These radicals cause damage to the β-cells of Langerhans. As a result of the destruction of β-cells, insulin can not be produced, causing diabetic is characterized by a state of hyperglycemia[5][6].

In a paired T test blood glucose levels before and after induction in the control group (-) obtained $p>0.05$. While in the control group (+) and the test preparation was obtained $p<0.05$, so that the initial blood glucose levels and diabetic were statistically significant. And this research should be expanded into treatment groups of the test preparation.

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The content of protein in a snack of fish on the market, 100g snack was obtained 28.0g of protein. Fish is a potential source of animal protein 11-27% is the largest component after water. With high protein content of fish is a food that is the recommended, Omega-3 its positive effects. Type of protein found in fish are globulin, such as myosin, actin, and trophomyosine. The total of protein is approximately 50% of the protein in the meat of fish[7].

Based on the number of protein intake is allowed for a day (recommended daily allowance or RDA), man should acquire around 10% of energy from protein. 10% protein needs of each individual can be varied so as to ensure an adequate intake for everyone, recommended 10% protein in the diet. 10% of protein in the diet is equivalent to 50-60 g of protein a day, depending on body weight and total calorie intake.

The result of the calculation of the percentage reduction in blood glucose levels and increase the average is calculated from the difference between the control group (+) with the test preparation group compared with the control group (+). The results obtained are on the 7th day decreased blood glucose levels by 14.86%; day 14th there was an increase in blood glucose levels 15.20%; day 21st decreased by 14.81% and day 28th decreased by 12.09%. The graphs can be described as follows:

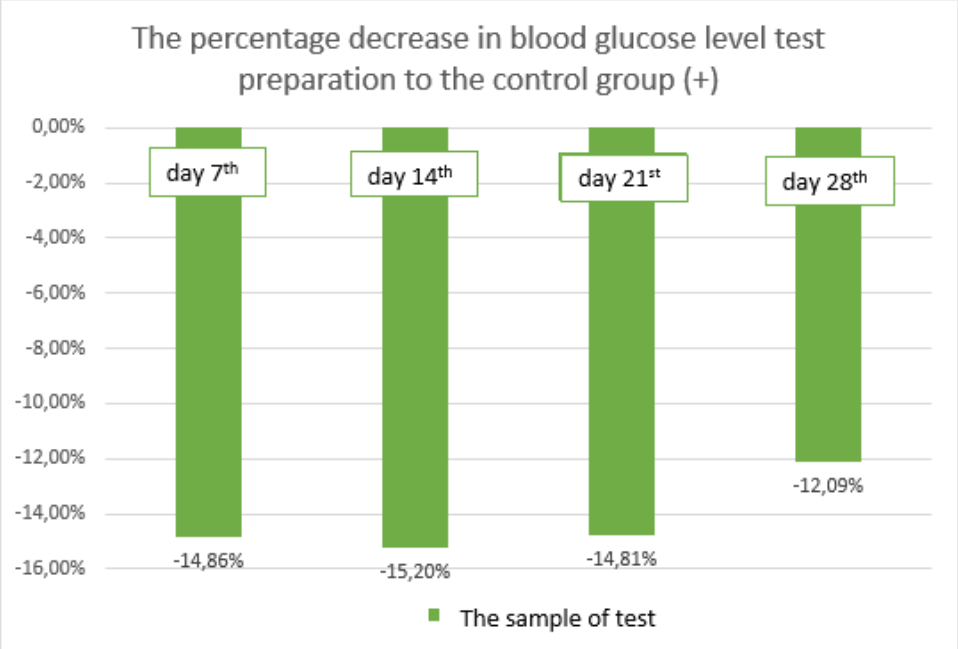

Figure 2. The percentage of decrease and increase in fasting blood glucose levels rat against a control group of test preparation (+)

From the results of one-way ANOVA test against the group (-) during the 28 days of treatment was obtained p>0.05, meaning that differences in blood glucose levels for 28 days were not significantly different. While two-way ANOVA test results of the control negative group(-), control positive(+) and the test preparation for 28 days of treatment p value in the test group and the day and the interaction between the groups and the day (0.000)<0.05, the meaning that blood glucose levels average of the three groups during the 28 day treatment significantly influenced by group differences and duration of administration[8].

Histopathology examination the pancreas of rats

Histopathology examination using hematoxylin-eosin coloring (HE), colorants consists of two color components, hematoxylin and eosin. Haematoxylin is blue dye alkaline so as to color the cell nucleus that is acidic, while eosin is a red dye that is acidic so it can stain the cytoplasm alkaline. The parameters observed from HE coloration on pancreas preparation is a common morphology island of Langerhans of the pancreas and the state of β cells contained in the middle of the island Langehans[9].

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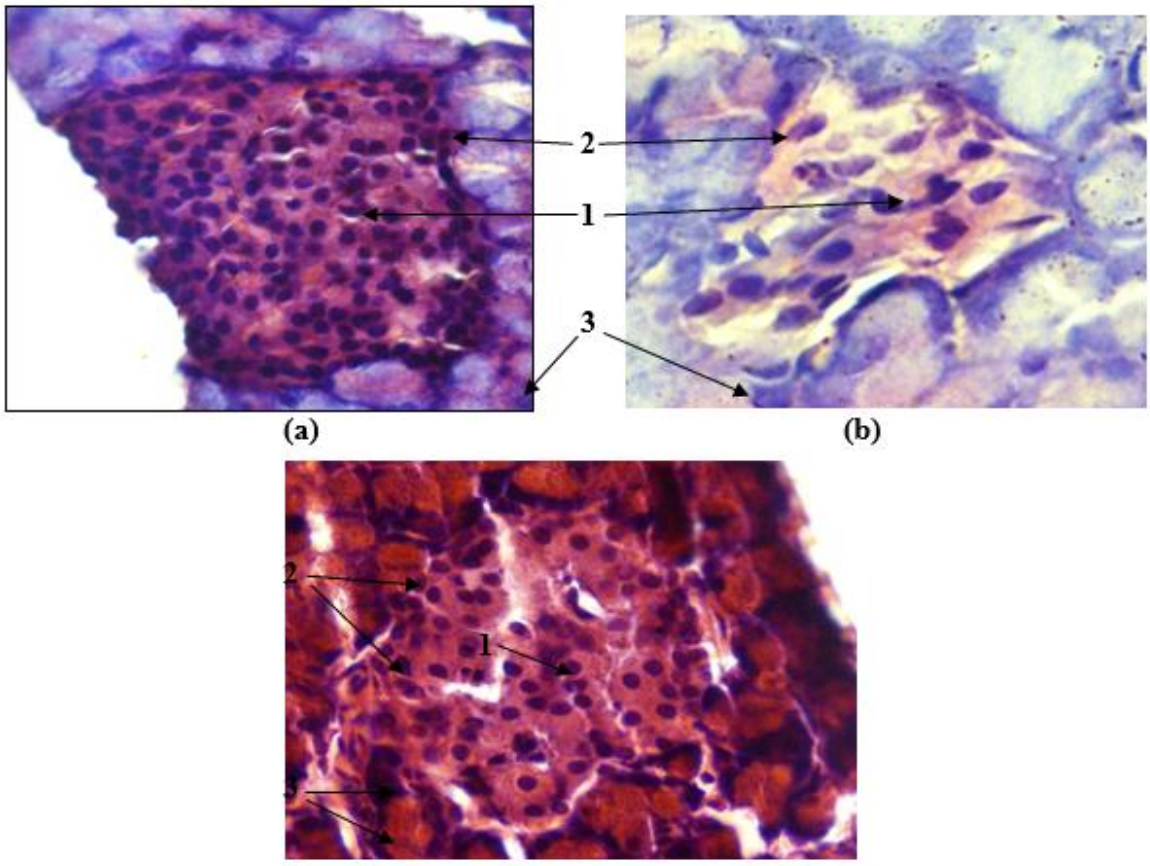

Figure 3. Histopathology of the pancreas

1.β Cell 2. A Cell 3. Asini Cell
(A) = Histopathology Langerhans of negative control group (-)
(B) = Histopathology Langerhans of positive control group (+)
(C) = Histopathology of the islets of Langerhans group test preparation

The histopathology of the pancreas from the results of microscopic examination with a magnification of 400x to the preparations of rat pancreas, seen gland acini are arranged around the island of Langerhans with cell nucleus is located along side compactus gland acini. Their regularity of arrangement of the cells in the endocrine pancreas that spreads island of Langerhans cells with the same form. On the island of Langerhans cells are generally situated on the edge of the island α and β cells deeper part or middle part of the island, pancreatic endocrine cell types most often found is the β cells[10]. Sel-pancreatic endocrine cells, especially β cells capable of secreting insulin in large quantities , so that the body does not lack insulin and hyperglycaemia occurs.

On the positive control be seen changes in the pancreatic morphology of rat, necrosis and exocrine multifocal on the endocrine pancreas, so that the cells can not be seen clearly. In the picture also looks exocrine cells no clear borders. This happens due to the induction of alloxan. Alloxan causes hyperglycaemia in male rats[6].

Histopathology of pancreas group hyperglycaemia rat given suspension test preparation showed a better result than the positive control group (+), characterized by the cells making up the endocrine pancreas exist greater improvement but not compact because it still looks the empty space, there uniformity in the form and the size of the cells in the endocrine pancreas that spreads island of Langerhans. This shows that pancreatic condition began to improve compared to histopathology pancreas hyperglycaemia groups of rats.

The correlation between blood glucose levels and histopathology β cells rat be seen on the 14th day after the administration of a preparation. Chicken egg white flour and animal protein from fish snack gives the

effect of lowering blood glucose levels rat hyperglycaemia on day 14th, but the duration of effect of preparations 21st and 28th days does not look because blood glucose levels did not differ significantly by day 14th.

Histopathology group test preparation at day 28th also showed signs of improvement such as cells form β who are in the middle of islets of Langerhans resembles the shape of endocrine cells located in the central part of the islets of Langerhans negative control group (-) and not through lysis as which occurred in the positive control group (+). With the improvement of β cells form, it can be confirmed insulin has been re-produced and have been working to incorporate a blood glucose into cells to be used as an energy source of cells. Repair forms and β cell proliferation in the test preparation can be caused by active stem cells in pancreatic tissue after induction of growth factors, one of them is the fibroblast growth factor (FGF) which is suppose found in egg whites 9 day old chicken embryos.

In the previous study, FGF found bound to proteins in chicken embryo progress stages began to from early stage of progress, but the numbers are not always the same until the embryo is ready to hatch. FGF existing value in the whole embryo tissue from the second day until the sixth day is a constant. But the numbers are declining at the age of 6-7 days and increased when embryos aged 9-13 days. Decrease and increase in the number of FGF indicate a change in the composition of FGF to the development of specific organs and at the same time, the number of receptors FGF (FGFR) was also reduced in the body of the embryo and the possibility of FGF whose numbers are increasing are stored in the extracellular matrix that will be released during -time when there is trauma and pain.

In addition, at the stage of embryonic development, the amnion embryos formed allantois vessel to the stage-18 (at aged 2-3 days) and started to work as he entered the stage of the 20th (after 3 days) as respirators and excretory organs as well as the channel taking nutrients from egg whites and calcium from eggshell. This gives a evidence that the extracellular matrix is intended as a place to store a number of FGF is the white part of the egg so that in case of damage to the β cells, FGF is to stimulate cell proliferation β.

From the research that has been done can be concluded that the administration of egg white powder at a dose of 30 mg/200gBW rat and fish snacks 1 g/200gBW rat showed improvement in pancreatic tissue histopathology. The ability of the decrease in blood glucose levels seen on day 14th, but the duration of effect of preparations 21st and 28th days because there was no blood glucose levels are not significant to the 14th day.

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