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Effect Of Giving White Egg Chicken Embryo And Green Beans *(Phaseolus Radiates)* To The Histopathology Of Pancreatic β Cell From Diabetic Rats *(Rattus Novergicus).*

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ABSTRACT

A research about the effect of giving white egg chicken embryo and green beans *(Phaseolus radiates)* to the histopathology of pancreatic β cell from diabetic rats *(Rattus Novergicus)* have been done. By giving these two active substances we hoped it can regulate the proliferation of pancreatic stem cell turn into β cell. The research was used completely randomized design with 3 treatments and 5 repetitions to find the effect of blood glucose levels with withe egg at a dose of 30 mg /200 g rat and green beans at a dose of 1 g/200 g rat and also aims to know the morphological improvement of β cell. There was a decrease of blood glucose levels in amount of 10.5 % and an the improvement histopatology of pancreatic β cell after 28 days administration. Before and after 28 days administration of blood glucose levels analyzed statistically two way analysis of varians repeated measures with $p < \alpha$ (0.000 < 0.05). The conclusions showed there was an effect of administration of white egg chicken embryo and green beans to decrease blood glucose levels and the improvement of pancreatic β cell diabetic rat.

Keywords: Chicken Embryo, *Stem Cell*, Diabetic Rats, Aloksan

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INTRODUCTION

With the development of medical research especially about metabolism disorders, a method with stem cell base had found using fertilized Avian white egg incubated for 9 days. This white egg separated and extracted with frezee dried method. A clinical test had done forthis preparation such as anti-stressed, helped mood disorder problems, energy increased and muscle shaped with increased testosteron production and also increased sexual desire, decreased LDL, triglyserida and blood pressure (Eskeland, 2006; Schult, 2009; Andujar, 2014).

The avian egg contains a multitude of the proteins, lipids, vitamins, minerals, and growth factors. There are also additional defense factors contained to protect against bacterial and viral infection, and biologically active components, making it more than just a source of nutrients. Fibroblast growth factor is one of the factor that get in chicken embryo development and has a responsible for initial cell development signal stimulation like patern confirmation, proliferation, diferentiation and migration to form a tissue (Kovacs, 2005; Seed, 1988; Fallon, 1994; Schofer, 2001; Dathe, 2005; Thisse and Thisse, 2005).

In PhilipPINES, incubated chicken eggs for 13-14 days and incubated duck eggs for 15-25 days were made as favourite snacks that called balut. The preparation for Balut was simple just boiled or made into omelet, stuffing pastry, satay and stew. While in Indonesia, the eggs was used as main ingredient for traditional medicine through certain reliance and in traditional drink knowed teh telur, serta as stamina increased which was made a half cook, even there was also consumpt without process for help increased muscle mass because this egg was contain a high protein. Beside eggs, green bean (Phaseolus radiatus) also was made as alternative nabati protein source because it has an enough high protein contents so often they was made as daily food such as green bean porridge, traditional cake, green bean drinking, and vegetable from green bean sprout. The definiton of stem cell according to the words that compose is cells that became the beginning of the growth of other cells that arranged all of organism body, including a human. In indonesia, the stem cell term is define as sel punca. In The Pinguin Dictionary of Biology" *stem cell* is undifferent cells that can dupplicate itself for produce another stem cell. Then stem cell will have a specific differentation after they had stimulated by certain signal for produce a different cell. Stem cell can different into a special cell with function and special shaped such as heart cell, blood cell,endothelial cell, nerve cell,liver cell and other cells (Atmosukarto, 2005; Halim, 2010).

One of the common metabolism disorder disease in society is diabetes mellitus. Diabetes is one of the most killer disease at this time. Diabetes is one of clinical syndrom is marked by poliuri, polidipsi, polifagi and an increase in time blood glucose levels or hiperglycemia over 200 mg/dl. Etiology found that diabetes differented into DM type 1, there is a interruption of insulin produce due to auto-imun disease or idiopatic and also by pancreatomy or induction of diabetogenic substances like aloksan and streptozotocin; DM type 2, due to insulin resistance or disruption of insulin secreation (Suherman, 2013; Nugroho, 2006).

From these data, this experiment use white egg from kampung chicken embryo aged 9 days and nabaty protein from green bean for help the decrease of blood glucose levels and pancreatic β cell improves because of the diabetogenic substance, aloksan, and is hoped the adult stem cell in pancreas tissue can different to replace the β cell.

MEHTOD OF RESEARCH

The research time and place

The research was happaned in Mei-July 2015 at Pharmacolocy Laboratorium of STIFI Perintis Padang, Laboratorium of Kopertis Wilayah X and Laboratorium of Balai Veteriner Bukittinggi.

The Tools and Materials

The tools are digital scales, spatel, egg incubator, egg manual mixer, aluminium tray, oven, mortar and stamfer, vial, syringe, glass material (Pyrex), rat's cage, a digital gluco-check (Gluco Dr®), cotton, alcohol swabs, surgical instrument, electric trinoculer microscope and hot plate.

The ingredients kampung chicken egg , aluminium foil, NaCMC suspent agent, aqua destilata, green bean flour (Mung Bean), white egg solution 1 %, green bean solution 1%, HNO3 concentrate, NaOH 10 %, NaOH 4N, CuSO4 liquid solution, Millon reagent (HgNO3 dan NaNO2), rat's food, ether, formalin 10 %, NaCl fisiologis, alkohol solution (50 %, 70 %, 80 %, 90 %, 96 %, dan absolut), xylol, compact parafin, Mayer's albumin, water, aquadestilata, Hematoksilin-Erlich reagent, Eosin-alkohol 1 % reagent.

Methods

1. Making sample
    a. White egg flour: the eggs were used was fertilized kampung chicken eggs amount 8 eggs, wasn't cracked, and took it slowly and then incubated in temperatur 38-39 °C for 9 days and the eggs were turned around twice a day. Then, separated the white and made into egg flour with pan drying method.
    b. Green bean flour were used was green bean flour at the market and had a nutritional standard.
2. Testing sample
    a. Rendemen $= \frac{\text{bobot sampel kering}}{\text{bobot sampel basah}} \times 100\ \%$
    b. Organoleptis were shape, colour, smell and taste sample.
    c. Amino acid and protein identification used Biuret Test, Millon Test and Xantoprotein Test.
3. Dosis planning
    The dose of aloksan was 150 mg/kg BW; the dose of white egg flour was1680 mg/day and the dose of green bean flour was took from human protein needed 60 g/day.
4. Treatment of experimental animals
    a. Animals acclimatization: the animals were used was healthy male white rats (Rattus novergicus), aged 2-3 month and never got any treatment before as much as 15 rats. Before these animals were used, they acclimatized for 1 week with increased tolerance about 10 % weight. They feed enough during maintenance.
    b. Experimental animals were made hyperglicemic with diabetogenic substance: aloksan at a dose 150 mg/kg BW in intraperitonial which fasted for 16 hours before and checked they early blood glucose levels. At the 14th days after aloksan induction, their blood glucose levels will be checked again as hyperglicemic blood glucose levels. The blood glucose levels was checked with Gluco Dr. The rats was said diabetic when before and after induction blood glucose levels were different statically base on paired samples T-Test.
    c. Division of the group and treatment of experimental animals.
    The experimental animals was divided into 3 group with 5 rats for each group;
    Group 1: control (-) group was experimental animals with standard food, drink and 1 ml of NaCMC 0,5% suspension during 28 days of treatment.
    Group 2: control (+) group was experimental animals which was got aloksan induction at a dose 150 mg/kg BW with standard food, drink and 1 ml of NaCMC 0,5% suspension during 28 days of treatment.
    Group 3: sample group was experimetal animals which was got aloksan induction at a dose 150 mg/kg BW with standard food, drink and white egg flour suspension at a dose 30 mg/200 g rat and green bean flour suspensioan at a doses 1 g/200 g rat and standard food and drink during 28 days of treatment.
    d. The rats blood glucose levels was checked every 7 days during 28 days treatment.
    e. The experimental animal was killed on 29th days after28 days of treatment by drugged before it with eter. Hence, the animal will be operated to got them pancreatic for the histophatology.
    f. The pancreatic was made into a histopatology preparation to check it into an electric trinoculer microscop with 400x magnification.
5. Statisticaly analysis
    The observation result of blood glucose levels in experimental animals was recorded, tabulated and analyzed statically using one way ANOVA for different mean of blood glucose levels on control (-) group during 28 days treatment and repeated measure two way ANOVA for different mean of blood glucose levels on control (-), control (+) and sample groups during 28 days treatment. The overview of the pancreatic β cell histopatology pictures will be used as supporting data and will be

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analyzed qualitatyvely. The mean and standard deviation for each groups was calculated from data obtained.

RESULT AND DISCUSSION

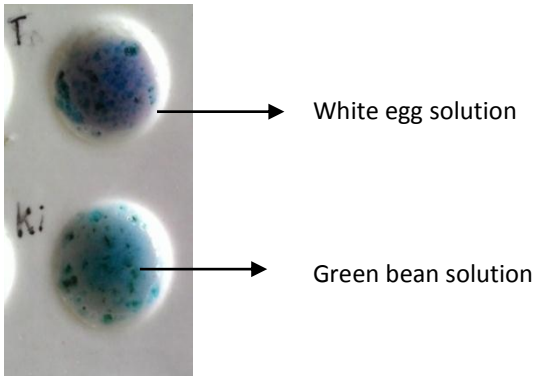
The samples of white egg flour and green bean flour

The withe egg flour was took from kampung chicken embryo aged 9 days and was dried into oven 40 – 50 °C with pan driying method so that it didn't crush the protein and growth factor contain that was also a protein too. While green bean was used home industry product which have a flour form. The yield of white egg flour is 31.98%. The organoleptis of white egg is powder, cream colour, egg specific odor and has an egg flavor; and the organoleptis of green bean flour is fine powder,snuff-coloured, green bean specific odor and has a green bean flavor.

Pic 1. White egg flour

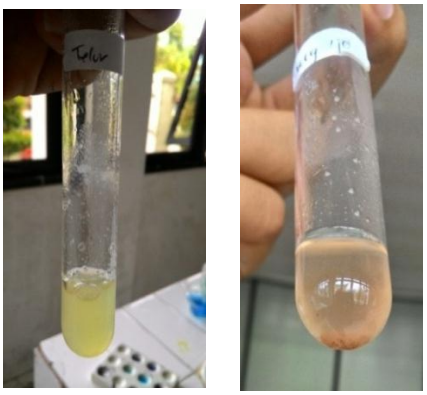
Pic 2. Green bean flour

Then the identification of protein an amino acid was did with colour reaction using Biuret test, Millon test and Xantoprotein test. The result of the third test to white egg flour and green bean flour are show in this picture bellow:



Pic 3. Biuret test

(a) (b)



Pic 4. Millon Test

(a) (b)

Pic 5. Xantoprotein test

In the biuret test of white egg flour and green bean flour, the solution colours change into blue-violet after the addition of CuSO4 dilitus solution. This is show a positive reaction, where this colour cause of complex compounds formed between Cu2+ and N from peptide bond in alkali condition. The amount of amino acid bound in peptide bound will influence the colour intens had form (Sumardjo, 2009). The Millon reaction involve a mercury in protein solution so that in logam addition will make a white sediment from mercury compound. For the protein that contains tyrosin or tryptofan, the addition of Millon reagent and heating will produce red colour. However, this reagent is not too specific because it will give a red colour too if there was a fenol group**,** so that this test was specific for fenol group in protein like tyrosin. From the identification of these two flour, we get the result are the green bean has a tyrosin amino acid but not with the white egg flour (Sumardjo, 2009).

The xantoprotein is a protein qulaitative identification with benzen core like fenilalanin, tyrosin and tryptofan with nitric acid and heating so the lysis of protein chains will happen into benzen group. The positive reaction showed while the yellow colour appeared because of the nitration process in benzen core and will be changed into orange if we add some base solution (Sumardjo, 2009).

The white rat's blood glucose levels

Kelompok	Kadar Glukosa Darah Puasa Tikus (mg/dl)					
	Sebelum induksi	Sesudah induksi/ Awal Diabetes	Setelah pemberian sediaan			
			hari ke-7	hari ke-14	hari ke-21	hari ke-28
Kontrol (-)	78 72 83 93 79	89 66 75 96 81	72 57 93 96 81	79 67 92 88 85	65 72 83 78 81	84 75 99 67 88
Rerata ± Standar Deviasi	81 ± 7,778	81,4 ± 11,718	79,8 ± 15,959	82,2 ± 9,731	75,8 ± 7,328	82,6 ± 12,260
Kontrol (+)	99 71 80 61 88	172 177 165 137 192	162 202 154 146 143	132 108 121 118 126	121 146 113 129 139	119 98 105 111 121
Rerata ± Standar Deviasi	79,8 ± 14,721	168,6 ± 20,256	161,4 ± 23,870	121 ± 9,000	129,6 ± 13,297	110,8 ± 9,602
Sediaan uji (tepung putih telur dan tepung kacang hijau)	96 71 83 78 67	171 154 163 181 147	139 141 121 137 133	125 121 112 137 120	110 105 149 123 101	117 89 94 104 92
Rerata ± Standar Deviasi	79 ± 11,336	163,2 ± 13,461	134,2 ± 7,950	123 ± 9,138	117,6 ± 19,411	99,2 ± 11,432

The diabetic contidion can cause by an induction of toxic compounds and can cause the destruction of pancreatic β cell. Alloxan induction can cause male white rats have a hiperglycemic because it will have an oxidation-reduction metabolism that produce free radicals and alloxan radicals. The effect of β cell destruction, the insulin could not be produce in β cell of Langerhans (Szkudelski, 2001; Nugroho, 2006).

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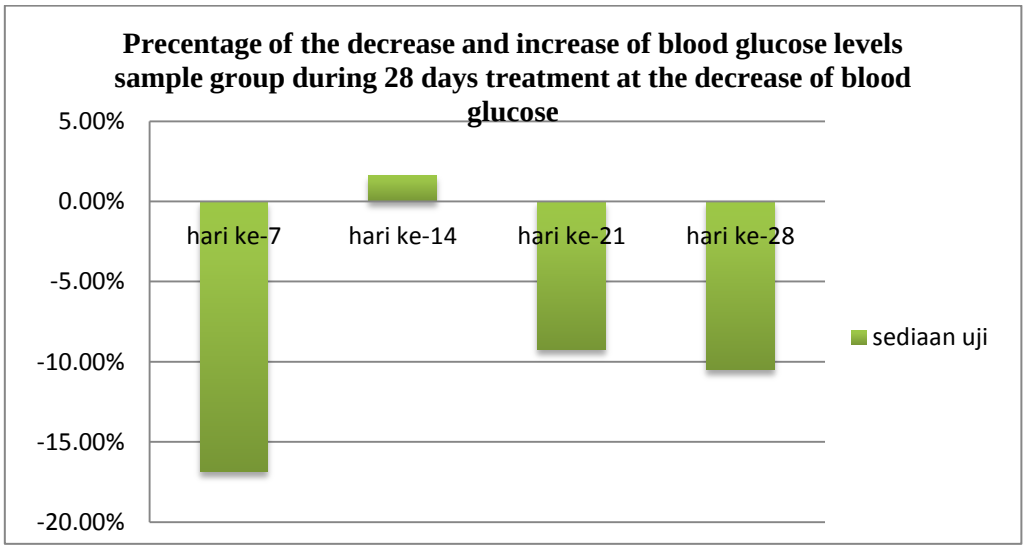
In before and after induction of alloxan at control (-) group test use paired samples T-Test of blood glucose levels, it get p value < 0.05 so that mean the blood glucose levels between the early and diabetic is not different. While in control (+) and sample group get p value < 0.05 so the blood glucose levels between the early and diabetic are different. And this experiment can be followed to the next step is the treatment of sample group.

The green bean had the second protein contain beside their carbohidrat and they are one of Leguminoceae family which be a second of the biggest food source for human being in the world next to cereal and it is important in diet for developing country. This family is the main consumed in the world because it had many varietas and have high nutritional quality; they are an excellent sources of starch and protein and are fairly good sources of dietary fiber, minerals, vitamins, and polyunsaturated fatty acid (Du, 2014).

Green bean had carbohidrat component is about 62.9 g/100 g of green bean. This starch is composed of two main components, amylose and amylopectin. According to the enzyme digestion rate, starch could be classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). The most desirable feature of common beans is their starches cotaining significant amounts of SDS and RS, which result in low glycemic index (GI) response. Consumption og low GI foods could prevent diabetes, heart disease, cardiovasculardisease, obesity and even certain cancers (Rukamana, 1997; Du, 2014).

The result of mean decrease and increase blood glucose levels percentage is counted from the control (+) group and sample group difference. The result is show at the 7th day it happen a decrease of blood glcose levels about 16.85%; at the 14th day it happen an increase about 1.65%; at the 21th day it happen a decrease about 9,25% and at the 28th day it happen a decrease about 10.5%. The grafik is showed in this picture bellow:



Pic 6. The decrease and increase graphic of blood gulose levels in sample group

From the result of one way ANOVA at control (-) gruop during 28 days treatment is got p value > 0.05 and it means the blood glucose levels difference during 28 days treatment is not different. While the result of repeated measured two way ANOVA between the control (-), control (+) and sample group during 28 days treatment at the decrease of blood glucose levels every 7 days of check are got p value < a (0.029 < 0.05) that is mean at the level two (the 7th) has a significant different yet. The result of level 3, 4 and 5 check are also show a significant different with p value is 0.000.

The result of mean blood glucose levels difference according to group different in 28 days show a significant different between the third group with p value are 0.000 < α, and the result of interacton between the day and the group also get a signifficant different too that happen from the 14th day to the 28th day of treatment (Pritasari, 2013).

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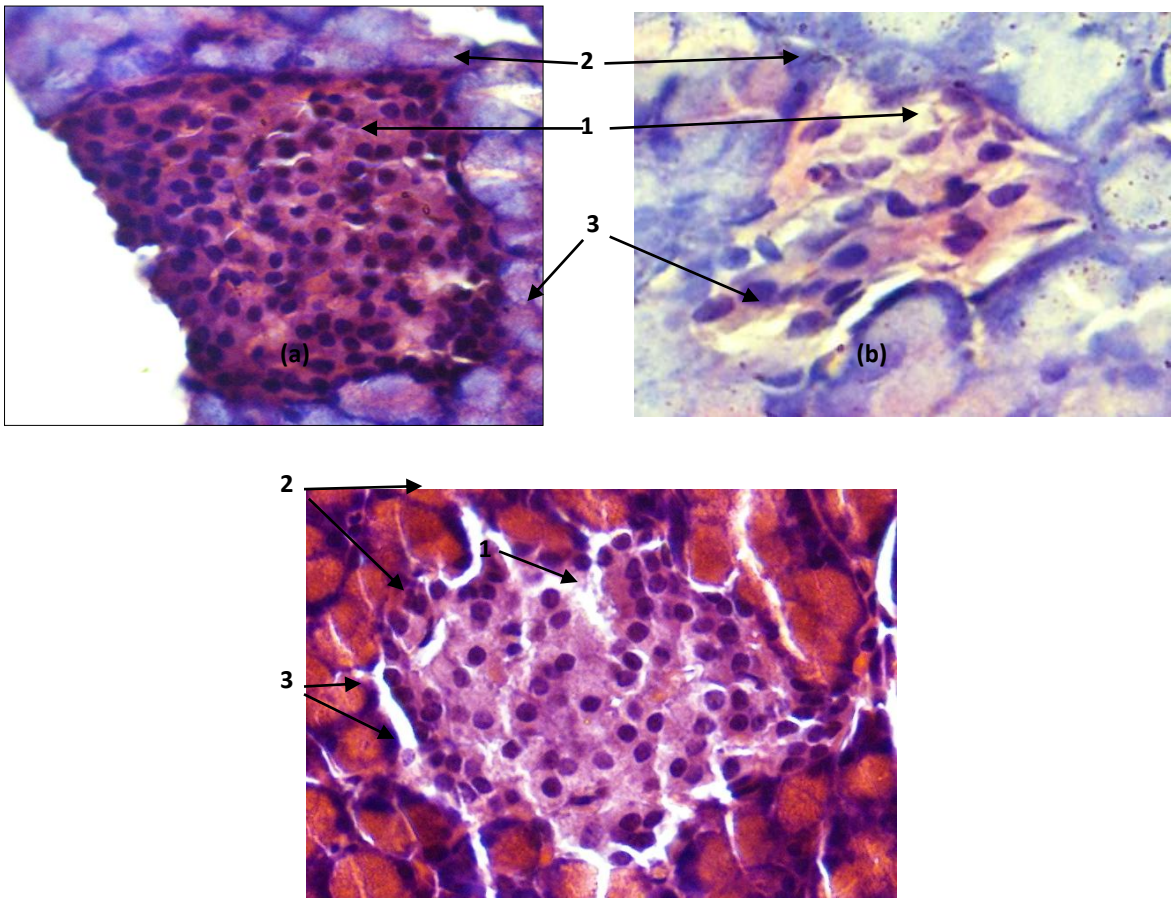
The white rat's pancreas hystopathology examination

The hystopatholofy examination use a Hematoksilin-Eosin (HE) colours where is this colours compose from two component of colours, the blue has an alkalinity so it can colour the nucleolus that has acidic while the red one has an acidicity that can colour the sytoplasma. The parameter that is observed from the HE colours in hystopathology preparation is the common morfology of Langerhans Island and β cell condition at the middle of the island (Sudiana, 2005).

The hystopathology of Langerhans Island morfology is saw microscopic with 400x magnification.



(c)

Gambar 7. Pancreatic hystopathology

Description
1. b cell 2. A cell 3. Acini cell
(a) = the Langerhans Island hystopatology of control (-) group
(b) = the Langerhans Island hystopatology of control (+) group
(c) = the Langerhans Island hystopatology of control sample group

The result of pancreatic hystopatology microscopic with 400x magnification is showed the pancreatic cells in control (-) group is still healthy that show a same shape and has an orderly arrangement from β cell in the middle of Langerhans Island. The endocrin cells of control (+) group is far away from the both group. The morfology of control (+) group is showed the cells have degeneration even a necrosis that is marked with the shape and arrange of endocrin cells especially the β cell is not clear and not uniform and also the line between Langerhans Island and acini cells is not clear too in the picture. The check of b cell morfology in the sample group hystopathology picture is showed a better result from the control (+) group that is marked with the

shape and the Langerhans Island size that almost correspond to control (-) group; and also is marked with the shape and arrange of endocrin cells especially β cell that almost perfect spread like the control (-) group.

The correlation between blood glucose levels and the hystopathology of rat's β cell is showed at 28th day after sample giving. This is so clear that at 28th day, the blood glucose levels at the sample group have a decrease about 10.5% from the contol (+) froup (pic 6) and the value of mean blood glucose levels at 28th day is 99.2 mg/dl, it also approach the blood glucose of control (-) group is 82.6 mg/dl. While the of the control (+) group is about 110.8 mg/dl.

The hystopathology picture of sample group at the 28th day (pic 7c) also show repair signs in the shape of b cell in the middle of Langerhans Island is look like the endocrin cells in the middle of control (-) group Langerhans Island (pic 7a) and it isn't has a lysis like what happen in control (+) group (pic 7b). With this repair of β cell shape, it can be confirmed that insulin has already is produced and has work to make the glucose through the cell to make an energy. This repair of shape and β cell proliferation in sample group can be caused by actived the pancratic stem cell in pancreatic tissue after the growth factor induction, one of that is fibroblast growth factor (FGF) that is expected terdapat in whtie egg chicken embryo aged 9 days.

In the past experiments, the FGF was found bound by protein binding in the stage of chicken embryo development from the early stage, but the amount was not also same until the embryo ready to hatch. The FGF value in the whole tissue of embryo from the 2nd - 6th day was constan. But the value decreased while the embryo had 6th - 7th day and increased when they were at 9th - 13th day. This decreased and increased of FGF value indicated that a different in FGF structure for the certain organs development and at the same time, the receptor of FGF (FGFR) had also a desreased in embryo body and it maybe the amount of increased FGF was kept in extracellular matrix that can be loose while the trauma happaned (Seed,1988; Olwin, 1990).

Beside that, at the development of embryo stage, the amnion vessel shaped allantois vessel in the 18 th stage (embryo 2-3 days) and started had a function when the in the 20th stage (after 3 days) as respiration tools and excretion organ and also as nutrition collecting ducts from the white egg and the calsium from egg shell (Smith,1914; Hamburger-Hamilton, 1951). This is give a evidence of presumption what it say as extracellular matrix before is the white egg so that if the destruction of β cell will happen, this FGF can be a β cell proliferation induction.

CONCLUSSION

From this experiment it can be concluded that an influence of the administration of white egg kampung chicken embryo aged 9 days at a dose 30 mg/200 g rat and green beans at a dose 1 g/200 g rat to decrease blood glucose levels and the improvement of pancreatic β cell diabetic rat in the 28th day.

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