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Fibroblast growth factor (FGF) identification of a fertilized chicken egg whites and their effects on stem cells regeneration in the pancreas of hyperglycemia mice

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ABSTRACT

This research aims to identify Fibroblast Growth Factor (FGF) in fertilized chicken egg whites and to see the effects of the FGF on the regeneration of stem cells in the pancreas of hyperglycemiamice. FGF identification in fertilized chicken egg whites utilizes Enzyme-Linked Immunosorbent Assay (ELISA)method. The examination of FGF effectson the regeneration of stem cells in the pancreas ofhyperglycemia miceis carried out in vivo by giving out the prepared eggwhites containing FGF indosage of 4 mg/20 g for 28 days. Then the writer observed the pancreatic histopathology using Hematoxylin-Eosin (HE) reagents and ImmunoHistoChemistry (IHC) reagents. FGF identification yielded by ELISA was positive with levels of 219 ng/L. Histopathological observations showed that the induction of FGF derived from fertilized chicken egg whites repaired cell morphologyof pancreatic endocrine and increased insulin secretion in pancreatic β cells. FGF derived from fertilized chicken egg whites helps the regeneration of stem cells in the pancreas of mice hyperglycemia.

Keywords: Fibroblast Growth Factor, Stem Cells, Hyperglycemia, Pancreas

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INTRODUCTION

Nowadays, stem cells have become a major topic of discussion of many scientists, medical experts, and even lay people all over the world. According to the words that compose it, stem cells are the cells that become the beginning of other cells growth whichbuild the whole body of the organism, including humans [1]. Stem cells have a remarkable ability to replace itself and generate a specific cell required by body. The division of stem cells will produce two cells, which may become new stem cells or differentiate into specialized cells with specific functions as well [2].

Many diseases or physical disorders that occur in connection with cell damage in organ systems that cause the organ cannot function in accordance with the needs of the body, such as Parkinson's, diabetes mellitus, or any autoimmune disorders and other diseases. Potential therapies to cure this condition is a cell-based therapy that promises the improvement of the system /organ with the primary objective to regenerate cells and restore the normal function of those organs [3].

Diabetes is a chronic disease that occurrs when the pancreas is unable to produce insulin in sufficient amount, or when human body is unable to use the insulin effectively. This situation may cause the increase concentration of glucose in blood (hyperglycemia). Diabetes can increase the risk of heart disease and stroke. 50% of people with diabetes die of cardiovascular disease (primarily heart disease and stroke). Diabetes also causes neuropathy (nerve damage), increases the possibility of ulcers on the feet, infection, and even amputation [4].

Diabetes mellitus is a major threat to human life, as seen from the prevalence of diabetes mellitus which is estimated at 2.8% worldwide (171 million people suffer from diabetes mellitus) and are predicted to increase to 4.4% (336 million suffer from diabetes mellitus) in 2030 [5]. In 2012, it is estimated that 1.5 million people died due to diabetes. WHO estimates that diabetes will become the 7thtop causes of death by 2030 [4]. It is estimated that there will be 21.3 million people in Indonesiasuffered from diabetes in 2030 [6].

Chicken eggis a source of nutrients that contain protein, lipids, vitamins, minerals and growth factors that are important for embryonic development, such as nutritional building blocks of biological functions of a chicken and builds a defensivepower to protect the embryo dealing with bacterial and viral infection[7]. FGF is responsible for the signal stimulation of early process of cell development (such as establishing a pattern, proliferation, differentiation, and migration) form a network [8].

Based on those data, the researchers want to identify FGF in fertilized chicken egg whites and examine the effectiveness of FGF induction to regenerate the pancreas stem cel of hyperglycemiamice [9],[10]
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MATERIALS AND METHODS

Materials

Fertilized chicken eggs bred alone, alloxan, chicken FGF Elisa Kit from Bioassay Technology Laboratory, the standard mice food, HE reagent, and imnuhistokimia reagent.

Methods

Preparation of Egg WhitesFlour

The egg whites of the fertilized chicken egg is taken. Gather it in a petri dish, dry it with freeze dryer until it becomes completely dry. Then, make it into powdery smooth flour.

Identification of FGF in Fertilized Chicken Egg White Flour

Identification is done by using ELISA method and Chicken bFGF Elisa Kit. The identification is done according to the practical instructions in the operational manual of Elisa Kit.

Preparation of Experimental Animal

Mice are acclimatized for 1 week. They eat standard food and drink. 40 mice are divided into 4 groups of 10 mice.

Group 1: Negative control group. The experimental animals are only given standard food and drink during the probation.

Group 2: Positive control group. The experimental animals are induced with alloxan of 150 mg/kg BB dosage. They also eat standard food and drink during the probation.

Group 3: Comparative Group. The experimental animalsare induced with alloxan of 150 mg /kg BB dosage. They are given comparative drug solvent of 4 mg/20g BB dosage. The mice are put in probation for 28 daysand only eat standard food and drink.

Group 4: Test Stock Group. The experimental animals are induced with alloxan of 150 mg / kg BB dosage. They are givenFGF solventin form of egg whites extract of 4 mg/20g BB dosage. The mice are put in probation for 28 daysand only eat standard food and drink.

Pancreatic Histopathological Observation of Mice

After giving egg whites flour containing FGF, the pancreas organ is taken for histopathological observation which is done with HE reagent and immunohistochemical reagent.

RESULTS AND DISCUSSION

FGF Identification of Egg Whites Flour of Fertilized Chicken

The result of FGF test on the egg whites flour of fertilized chicken is as follows:

Table 1: FGF levels in fertilized chicken egg white powder

Samples	Concentration (ng/L)	The Average Concentration (ng/L)
Sample 1	222.134	219.006
Sample 2	215.878	

From the identification and assay of FGF in the egg whites flour of fertilized chicken using ELISA method, it found FGF level of 219 ng/ L (Table 1). ELISA method separated some components of the mixture in the sample, by absorbing certain component in stationary phase in the form of solid (well). The sample solvent is added to the stationary phase which has tied particular compound and then followed by the addition of various reagents sequently, then incubated and washed until the color changes in the final solvent which is yielded from the product enzymatic reaction. This color intensity is measured as the quantitative of a sample.

The Result of Histopathological Observation Using HE Reagent

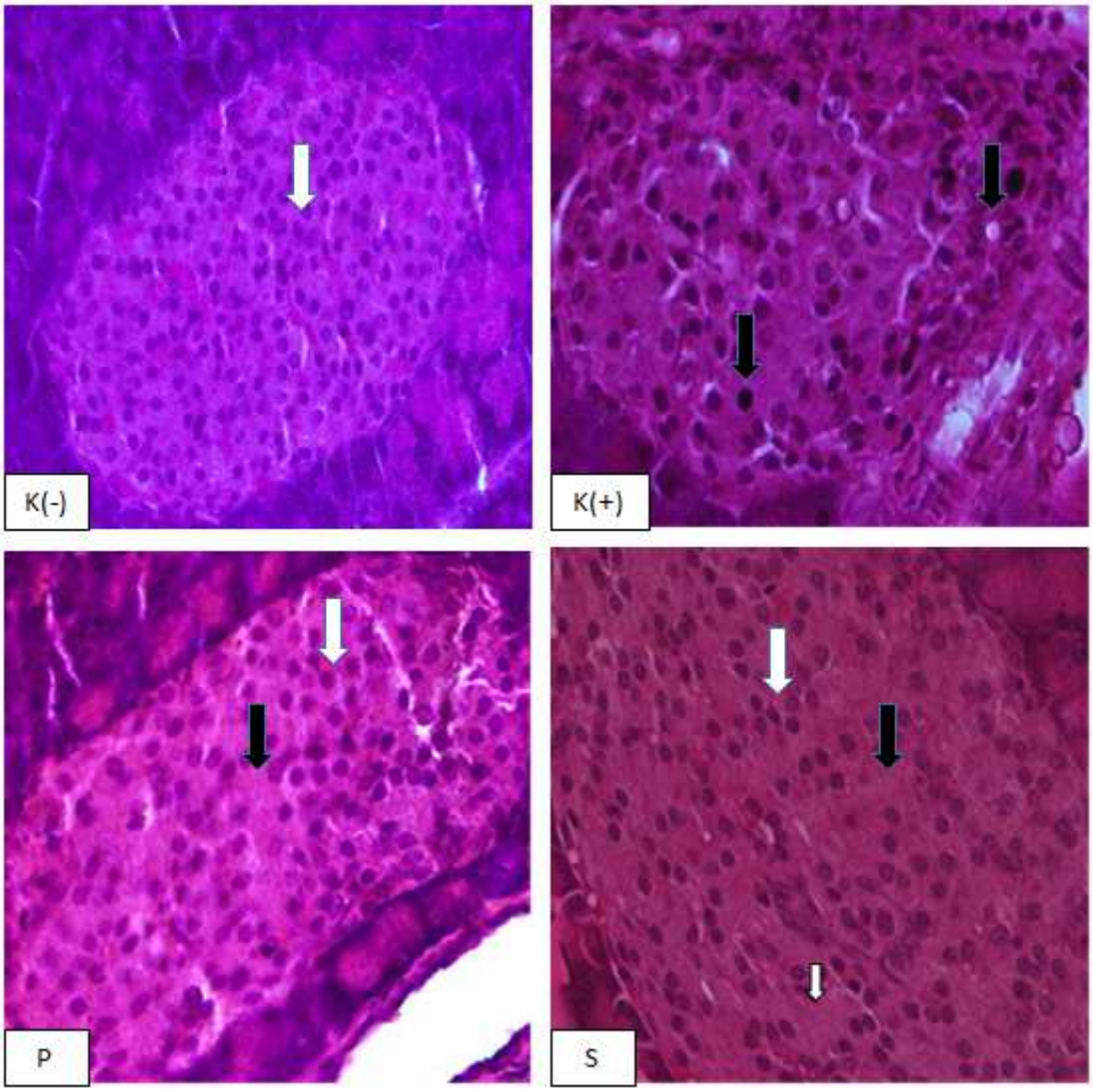

Fig 1. Picture of Pancreatic Histopathologic Using HE Reagent

Notes: K(-): Control (-), K(+): Control (+), P: Comparator, S: Stock, signmeans normal endocrine cells, sign means endocrine cell that encounters degeneration. At K(-), we see the presence of regular structure of endocrine cells in islets of Langerhans in quite uniform cells and the size of cytoplasm seems proportional towards the size of the essence and it does not change(still normal). In K(+) we see the process degeneration to necrosis cell with the occurenceof pyknosis, karyorrhexis, karyolysis and even cell vacuolle.In P we find that endocrine cell almost becomes a normal form with the cellstructured in good order although some cells are still experiencing pyknosis.In S, we see that the endocrine cell has began to regenerate, but there is cellencounters karyorrhexis and karyorlysisis.

The result of Hematoxylin-eosin (HE) colouration on the pieces of pancreatic system in all groups is observed towards the morphology of pancreatic system cell. In the colouration of HE in the parts of endocrine (islets of Langerhans) has lighter colour (light-red) than exocrine part, and blood arteries stream was discovered [11]. The pancreatic histopathological observationwas done by monitoring the morphology structure of mice's pancreatic system that was coloured by Hematoxylin-eosin (HE) colouration.

From the illustration of Histopathologywith HE reagent, the conclusion is that the induction of FGF from egg whites of fertilized chicken is able to help the process of pancreatic stem cell regeneration of hyperglycemia micebecause there seems the rehabilitation of endocrine cell morphology in the islets of Langerhans on stock group.

Quantitative histopathological examination of mice's pancreas

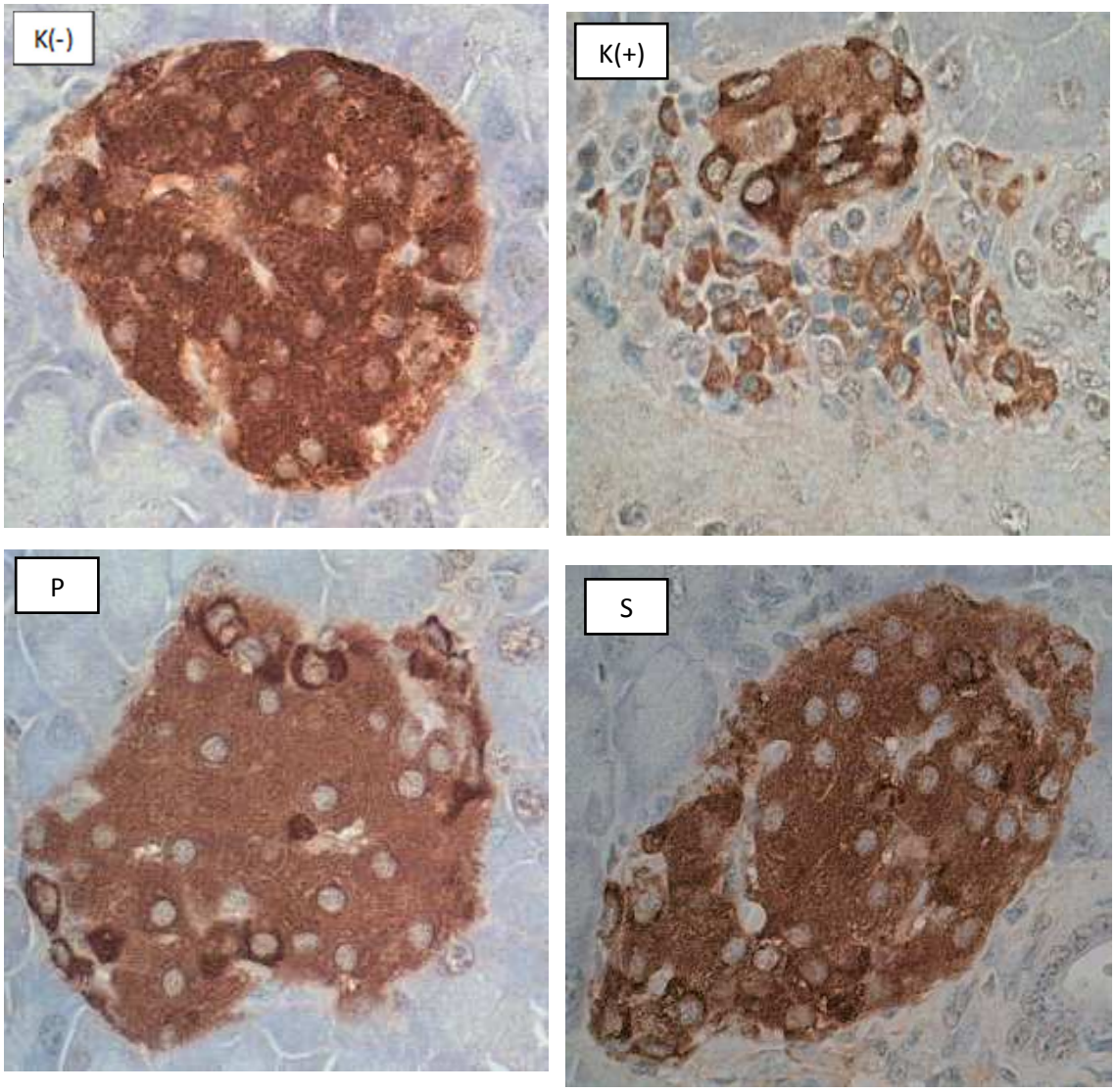

Fig 2: Histopathological illustration of Mice's Pancreas using Reagen Immunohistochemistry

Notes: K(-): the population of β cell in islets of Langerhans shows a positive antigen reaction and insulin antibody on β cell which has dark brown shape, it means that β cell secretes insulin in a large amount. K (+): the β cellpopulation that produces insulin is so limited in amount with the intensity of light brown colour. P: the β cell population that produces insulin in a fair amount with medium intensity of medium brown colour. S: the β cellpopulation in medium brown colour, it means that the β cell produces insulin in a fair amount.

The observation towards the piece of pancreatic system especially on beta cell which is coloured with imunohistochemistryis done in descriptive method by observing the population and the display of antigen reaction level and beta cell antibody that encounter change. The observation using immunohistochemical

colouration technique can present the distribution of the beta cell that produces insulin in the islets of Langerhans indicated by brown-coloured cell [12].

Based on the histopathological preparatobservation using immunohistochemical reagen, the control group(-) has β cell population that makes many insulin secretion because of the thick dark brown colour intensity that shows the result of antigen reaction and many insulin antibody. Different from control group (+),β cell population that makes only a little bit insulinsecretion, because the intensity of brown colour which is caused by antigen reaction and insulin is in small amount. It shows that there occursβ celldegeneration, so that β cell is not able to produce insulin in large amount. Comparative group produces the intensity of medium brown colour, which shows the capability of β cell in producing insulin has been almost in normal condition because the difference of brown colour is not quite far. In the same way, the test stock group that produces the intensity of medium brown colour is almost the same as incontrolgroup (-). From this quantitative examination, we can conclude that giving FGF from egg whites flour of fertilized chickencan raise the capability of β cell for insulin secretion.

FGF mediate cellular responses by binding and activating the four groups of the receptor tyrosine kinase (RTK) as indicated by the FGF receptor (FGFR1-FGFR4). FGF also binds to heparin or heparan sulfate proteoglycans (HSPG) to activate the FGFR and to induce a mixed response that can cause cellular responses induced by appropriate growth factor [13].

CONCLUSION

The result of FGF identification using ELISA method is positive with Level of 219 ng/L. Histopathological indicates that the FGF induction of egg whites of fertilized chicken can rehabilitatethe cell morphologiy of pancreatic endocrine and can also improve insulin secretion inpancreatic β cell.

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