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Sesamum Indicum and Linum Usitatissimum Extract on FGF and Pancreatic Histopatology White Male Mice

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Abstract: Research on the effect of lignan compounds from *Linumusitatissimium* L. (flaxseed) and *Sesamum indicum*L. (sesame) extracts had been done, combined with Fibroblast Growth Factor (FGF) that found in egg white powder derived from fertilized egg white. Before the research was carried out, all of the test animals' pancreases were damaged by alloxan given at a dose of 150 mg/kg for the positive control and test compound preparation, except for the negative controls. Test animals were divided into 5 treatment groups named; negative control group, positive control group, and 3 test preparation groups. The seeds' extracts were given at a dose of 800 mg/kg and sesame seed extract at a dose of 15 g/kg with FGF in egg white powder with dosages variations of 200, 400 and 800 mg/kg. Tests were carried out for 21 days, blood glucose levels were checked at 7th; 14th; and 21st day. Pancreatic preparations were done by fixation, dehydration, dipping and cutting. The results showed that in all test groups there was a decrease in blood glucose levels compared to the positive controls. The extracts combination with a dose of 800 mg/kg BB FGF was the optimum dose because the blood glucose levels was decreased from 7th, 14th and 21st day ($p < 0.05$). The results of histopathologic examination showed that there was a fairly clear improvement of pancreatic β cells.

Keywords: FGF, Lignan, Glucose, Pancreas.

INTRODUCTION

Until now, there are still no drugs that can cure diabetes mellitus permanently. We can only control the blood glucose levels both pharmacologically and non-pharmacologically. Insulin resistance, less secreted-insulin, and less sensitive target cells in the body might increase the blood glucose levels. In addition to contributing to the metabolic process of carbohydrates, insulin also plays a role in fat hydrolysis and protein uptake into cells (Dipiro, et al., 2008).

According to the International Diabetes Federation (IDF) in 2017, the prevalence of diabetes mellitus in the world (aged 20-79 years) reaches 8.8% of the total adult population of 425 million people, and is expected to increase in 2045 to 9.9%. Approximately 79% of the population lives in countries with low and middle income. In countries with high income estimated 87-91% suffer from type II diabetes, 7-12% suffer from type 1 diabetes and 1-3% suffer from other types of diabetes mellitus.

The use of insulin injection is a follow-up after oral diabetic drug administration, but insulin use does not provide a comfortable feeling in the patient. Moreover, it does not provide the same degree of control as the control function of blood glucose levels by pancreatic beta cells. The administration of antidiabetic drugs both oral and parental are still provide undesirable side effects such as hypoglycemia, gastric bloating, obesity and symptoms of hematologic symptoms and edema.

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Efforts need to be made so that the pancreatic β cells are able to differentiate and regenerate normally by stimulating the reactivation of the tyrosinkinase and PPAR enzyme enzymes and finding appropriate and suitable amino acids so that the signals return to play a role in producing diffraction and regeneration of pancreatic β cells. One method that leads there is to modify the methods of steam cells.

Chicken eggs are a source of nutrients that contain protein, lipids, vitamins, minerals and growth factors. These compounds are very important for the development and embryo defense system in the face of bacterial and viral infections (Kovacs, 2005). Fibroblast Growth Factor (FGF) is a polypeptide growth factor that regulates developmental processes including proliferation, differentiation, migration, pattern determination and cell morphogenesis (Teven, et al., 2014). FGF found in egg white flour can reduce blood glucose levels in hyperglycemic mice and help regenerate pancreatic cells (Dharma, et al., 2016).

In addition to eggs, phytoestrogen compounds also have anti-diabetic properties (Bhathena and Velasquez, 2002). Phytoestrogens are a group of plants, both grains, nuts, vegetables and fruits. All of these types have the properties of estrogen-like hormones. The most widely studied phytoestrogen groups are lignans and isoflavones.

Some plants that contain phytoestrogens among them are flaxseeds (*Linumusitatissimum* L.) and sesame (*Sesamum indicum* L.). This plant is rich in lignin and isoflavonoids. Flaxseed is the highest source of lignans, containing secoisolariciresinoldiglucoside (SDG) as the main lignan (Milder, et al., 2005; Kurzer and Xu, 1997), and sesame seeds are rich in lignans such as sesamin, sesamolin (Kumar, 2014).

Table 1: Flax scientific classification

Kingdom	Plantae
Order	Malphighiales
Family	Linaceae
Genus	Linum
Species	*Linumusitatissimum*

Table 2: Sesamum scientific classification

Kingdom	Plantae
Order	Lamiales
Family	Pedaliaceae
Genus	Sesamum
Species	*Sesamum indicum*

The purpose of this study is to observe the effect of the combination of fibroblast growth factor derived from fertilized chicken egg white by combining it with lignan compounds derived from flaxseed extract and sesame.

MATERIAL AND METHOD

Materials

The materials used were fertilized eggs (*Gallus domesticus*), flaxseeds (*Linumusitatissimum* L.), sesame seeds (*Sesamum indicum* L.), male white mice (*Mus musculus*), Sigma-Aldrich® alloxan monohydrate.

Extract Preparations

Flaxseeds were grounded to powder and its fat removed with hexane. It was then macerated with 70% ethanol at room temperature for 24 - 48 hours. The solvent was evaporated with a rotary evaporator at 50 ° C at a speed of 90 rpm until a bright yellow viscous solution was formed. The thick extract was hydrolyzed with 1M NaOH at room temperature for 12 hours, then acidified with 0.5 M HCl to pH 6. Then cooled at 15 ° C for 48 hours and the precipitate formed was filtered (Zhang, et al., 2007).

Sesame seeds are dried by oven at 60 ° C for 4 hours, then cooled at room temperature and ground. Sesame seeds were macerated with hexane for 48 hours, then the solvent is evaporated with a rotary evaporator. After the solvent was evaporated, an extract in the form of sesame oil was obtained (Kumar, 2014).

Preparation of FGF in Egg White Powder

The eggs used are native chicken eggs (*Gallus domesticus*), fertile, not cracked, taken carefully, and there should be no excessive shocks. The eggs were putted into an incubator which has a temperature of 38-39˚C for 9 days and the eggs were rotated 2 timesevery day. The egg was broke and the white part was separated from the yellow. Egg white was made into powder with freeze dry method.

Egg whites were homogenized with a manual egg beater and poured onto a petri dish., then it was dried with a freeze dryer.

Test Animals Preparation

Animals used were 45 male mice (*Mus musculus*) aged 2-3 months with an average body weight of 20-30 grams. Test animals were divided into 5 groups in which each group consisted of 9 mice. Animals used were healthy and given enough food and drink. Test animals were acclimatized for seven days.

Dosage Planning

In this study, 800 mg/kg flaxseed extract and at 15 g/kg sesame seed extract were used. Egg white powder (FGF) consisted of three dosage variations which were 200, 400, and 800 mg/kg. As an induction for diabetes, alloxan were also given to the mice at a dose of 150 mg/kg.

Treatment against Experimental Animals

- The administration of alloxan solution in the positive control group and the test preparation group (groups 1-3) where previously mice were fasted for 16 hours, carried out intraperitonially after 7 days of mice were acclimatized.
- Provision of test items in groups 1-3 for 21 days on an oral basis with a predetermined dose. Each test preparation was given at different times. Blood glucose levels were examined on the 7th, 14th and 21st days after the test administered before the mice were fasted for 6-8 hours. Blood glucose level was examined with a GlukoDr® digital device.
- The immolation of test animals is done after the examination of blood glucose levels is completed on the 21st day. Animal sacrifice is done by dislocating the neck and animals dissected with a surgical instrument so that the pancreas can be taken for histopathological examination.

Histopathological Examination

The pancreatic tissue was taken then being made as preparations through a fixation process which was soaked in 10% neutral formalin buffer solution, then dehydrated using multilevel alcohol and cleaned with xylol. The dehydrated preparations were planted in a mold that had been filled with liquid paraffin and then was cooled to cold plate. The hardened molds were removed from the mold and the blocks obtained could be stored in the refrigerator until they were ready to be cut with microtomes. Preparations in paraffin blocks were cut using microtomes with a thickness of 5 μm to form a-ribbon-like and placed on the surface of warm water to prevent folds on the ribbon. The preparation was then placed on an object glass and dried at room temperature. The preparations were then stained with Hematoxylin-Eosin (HE) and observed with an electric microscope.

Statistical Analysis

Data analysis that used in this study was a two-way Analysis of Variance (ANOVA) statistical test. Two-way ANOVA was used to compare differences in average blood glucose levels due to group factors and duration of administration. 95% confidence level and the results were said to be meaningful if $p < 0.05$. The further analysis used was the Duncan test (Duncan's Multiple Range Test).

RESULTS

Test Preparation

Egg white powder was obtained by freeze drying method to not cause damage on proteins and growth factors which were also a composition of amino acids. The preparations of egg white powder were in form of granules and yellowish white. Flaxseed extract was obtained in the form of sediment and brownish yellow, while sesame seed extract obtained was in the form of oil and yellow.

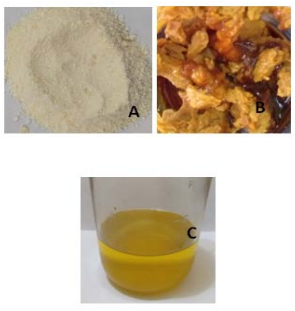

Figure 1: (A) Egg white powder contains FGF. (B) Flaxseed extract. (C) Sesame seed extract

Measurement of Blood Glucose Levels

This study used alloxan as achemical compound toinduced a damage to pancreatic β cells so that the blood glucose levels increasement were occurred. The function of alloxan was to increase the blood glucose levels because alloxan is selectively toxic to pancreatic beta cells that produce insulin, so, alloxan might accumulates specifically through glucose transporter, GLUT 2 (Lenzen, 2008).

Measurement of blood glucose levels was carried out at 7th, 14th, and 21st day. The parameters observed were blood glucose levels that were influenced by the type of treatment and time. The type of the treatment or group that observed was the test group which had the effect of decreasing blood glucose levels. Whereas for the time seen with the duration of administration, it was expected that the effect in decreasing blood glucose levels was better.

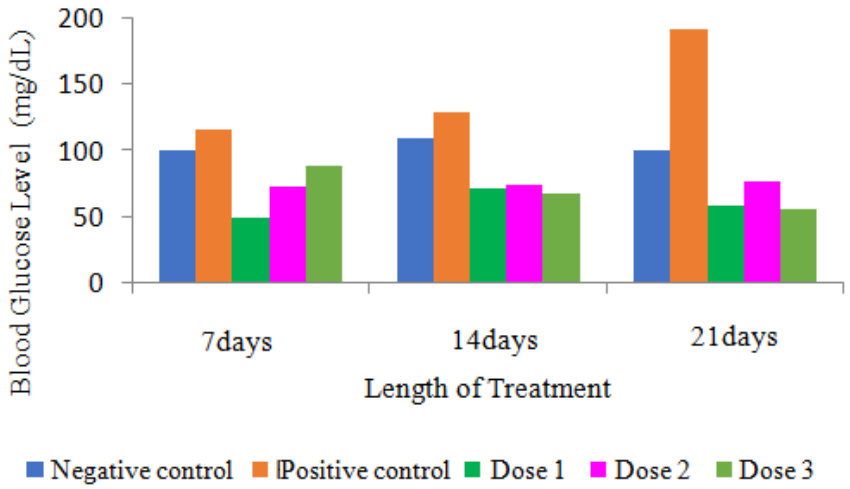

Figure 2: Comparative bar diagram of the average blood glucose levels of mice during observations at 7th, 14th and 21st day.

Table 1: The average decrease in blood glucose levels (mg/dl) and the percentage decreasein mice's blood glucose levels were compared with the positive control at the same observation time.

Group	The average of blood glucose levels (mg / dl) ± SD and the percentage reduction in blood glucose levels (%) n = 9					
	7th day		14th day		21st day	
	Concentrations (mg/dl)	%	Concentration (mg/dl)	%	Concentration (mg/dL)	%
Negative Control	99,67 ± 10,83	13,75	108,78 ± 8,09	15,16	99,56 ± 15,29	47,78
Positive Control	115,56 ± 15,02	-	128,22 ± 7,03	-	190,67 ± 14,87	-
Dosage 1	49,00 ± 8,26	57,59	71,22 ± 10,52	44,45	58,33 ± 8,66	69,40
Dosage 2	72,22 ± 7,59	37,50	73,78 ± 9,93	42,45	76,78 ± 9,85	59,73
Dosage 3	88,33 ± 13,45	23,56	67,22 ± 10,52	47,57	56,11 ± 9,86	70,57

DISCUSSION

The parameters that used in this study were blood glucose levels and histopathological examination of mice's pancreatic tissue represented by each group. Specifically, for the tissue's histopathological examination was that at 21st day observation, ranging from the negative control, positive control, 800 mg/kg flaxseed extract and 15000 mg/kg sesame extract. These dose were taken based on the highest dose which had optimal ability to reduce blood pressure. Both types of phytoesterogens were combined with FGF using three dosage variations (200, 400 and 800) mg/kg. Observations were made based on the dosage differences with 21 days observation. Observations were made on the 7th; 14th and 21st day. Data were processed statistically using two-way Analysis of Variance (ANOVA) between doses and time of observation.

The results of data analysis showed that there was an interaction between the treatment group and the treatment time of blood glucose levels ($p < 0.05$). The treatment group had a significant effect on blood glucose levels ($p < 0.05$). Likewise, the treatment time showed a significant effect on blood glucose levels ($p < 0.05$)

If it was calculated based on the percentage value of the decrease in blood glucose levels of mice, it was found that in all test groups (groups 1-3) the extracts could reduce blood glucose levels of mice for 21 days compared against the positive control groups. However, the group 3 of dose is the only one which showed a linear decrease from the 7th, 14th and 21st day examinations.

From the data analysis, there was an effect of flaxseed extract, sesame seed extract and egg white flour given to decrease the blood glucose levels. All data are shown in table 2 and figure 1.

Histopathology of mice's pancreas was done by observing the picture of Langerhans island endocrine cells because the beta cells that produce insulin were found on the Langerhans cells. Histopathology was performed using Hematoxylin-Eosin (HE) staining, consists of two colours components named hematoxylin and eosin. Hematoxilin is a blue alkaline substance so that it can give colour to acidic cell nuclei. Meanwhile, eosin is a red substance which is acidic so it can give colour the alkaline cytoplasm (Rathet all., 2017).

Histopathological observation of mouse pancreas was seen from the form of cells undergoing the stages of necrosis, named cell death. Necrosis is caused by anoxia, lack of energy supply and the effects of harmful substances that damage cell function. Signs of necrosis in cells can be seen as the occurrence of karioreksis (tearing of the core membrane and core fragmentation), kariolisis (chromatin lysis) and piknosis (clumping of the contents of the cell nucleus) (Djamnov, 2000).

Regular arrangement that started in endocrine cells was indicated as an improvement of endocrine cells, and one of it was beta cells.

Beta cell refinement caused insulin to re-produced and worked to enter blood glucose into cells to be used as an energy source.

Beta cell refinement and proliferation were caused by the activated stem cells in pancreatic tissue which were stimulated by growth factors. One of the growth factors was Fibroblast Growth Factor (FGF) which was found in fertilized chicken egg white.

In addition to FGF, the content of lignans in flaxseed extract and sesame seed extract also had an effect in reducing blood glucose levels and cell repairs. Lignan compounds have a high antioxidant effect which could provide a protective effect on pancreatic beta cells by reducing oxidative stress factors that could damage cells(Akhila and Bevvy, 2015). In addition, lignans work synergistically with FGF by influencing gene expression (Morris, 2007). Gene expression can be found in the RAS/mitogen-activating protein (MAP) kinase pathways that regulate cell proliferation and differentiation (Teven, et al., 2014).

CONCLUSION

1. The results presented in this study indicated that the administration of egg white powder containing FGF in a combination with flaxseed extract and sesame seed extract could significantly reduce blood glucose levels ($p < 0.05$)
2. The highest percentage of decrease in blood glucose levels that could be seen in the 3 treatment groups was 70, 57%, at the FGF dose of 400 mg/kg in combination flaxseed (800 mg/kg) and sesame 15,000 mg/kg.
3. From the results of histopathological observations there was also an improvement in Langerhans island cells approaching normal which especially seen at dose 3 at the 21st day of observation compared to negative, positive and 3 dose variations of FGF (100, 200 and 400) mg/kg.

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AUTHOR CONTRIBUTIONS

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There were no problems during the writing of this manuscript.

CONFLICT OF INTEREST

There is no conflict of interest.

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