



The Effects of Amino Acids that contained in Fermented Soybeans Against Blood Glucose and Histopathologic Overview Pancreatic β Cells of Mice White

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ABSTRACT

A study about to what extent of the effect of giving fitoestrogen contained in soybean flour obtained from soybeans fermented bean to the improvement of pancreatic β cells. Before the treatment, mice's pancreas induced by alloxan compound. After the sample selection, mice that have been given treatment by providing tempeh flour with 3 variations doses are 40 mg; 55; 75; 105; 145 and 200 mg / 20 g BW. The mice were divided into 8 groups, group 1 is a group of negative control without being offered anything, group 2 is positive control, group of diabetic mice without being offered treatment, group 3, 4, 5, 6, 7 and 8 are given group tempeh flour with 6 dose variation. As the parameters of this study was to measure the blood glucose levels of mice on day 7th of observation; 14th and 21st. The methods used enzymatic method by using "Gluco DR". Group of mice that given tempeh flour, observed dose that can provide a decrease in blood glucose levels optimally. Significant at $p < 0.05$. Selected animal groups do dislocation of the neck, pancreatic tissue taken and then preserved using formalin. Histopathologic examination and pancreatic tissue was observed qualitatively. The results showed dose administration of 200 mg / 20g BW can lower blood glucose levels compared with the positive and negative control group and showed clear improvement tissue in the group with a dose of 200 mg / 20 g BW.

Keywords: amino acid, fermented soy beans, pancreatic β cells

INTRODUCTION

Diabetes mellitus is a chronic disease, which appears when the pancreas is unable to produce insulin in sufficient amounts, or when the body is unable to use the insulin that is produced effectively. These conditions will lead to high levels of glucose in the blood. Diabetes mellitus increases the risk of cardiovascular disease and stroke. Diabetes mellitus also cause neuropathy, nephropathy, retinopathy, cardiomyopathy, ulcers on the legs, fatty liver and impotence [1],[2]

Diabetes mellitus is a major threat to human life, and judging by the prevalence is an estimated 1.71 million people worldwide suffer from diabetes mellitus and are predicted to increase to 366 million by 2030 [3]. In 2012, an estimated 1.5 million people died due to the disease Diabetes mellitus. WHO estimates that diabetes mellitus will be ranked seventh top cause of death in 2030 (WHO, 2014, Abdulzeez, 2014). Indonesia is expected in 2030 will have raised diabetes mellitus as many as 21.3 million [4].

Although diabetes can now be controlled clinically using insulin injections, but this treatment does not lead to healing and provide an uncomfortable feeling upon use and also in the long term can lead to a number of clinical complications. Treatment with insulin injections do not the same as for the control of blood glucose levels by cell β pancreas and can not prevent the adverse consequences as the result of diabetes [5], [6], [7] Treatment with repairing the damage that occurs in β cells, remains be a big expectation, so that healing occurs is permanent [8]

Lately, there isa lot of research done about the effects of fitoesterogen that is widely available from fermented soybeans[9]. Results obtained from these studies is the provision of tempe fermented in diabetic rats can improve blood glucose levels and distribution of β cells in histopathology. Soy contains three types of isoflavones; daidzein, glisitein, and genistein.

MATERIALS AND METHODS

Times and Places

This study was conducted from July to September 2016 in the laboratory of Pharmacology, University of Andalas Padang and histopathology laboratory Veterinary Institute Baso.

Tools and Materials

The tools used were analytical scales, vials, syringes, needles oral, glass tools, mice cages, animal scales, digital blood glucose measuring devices (Gluco DR), surgical scissors, glass objects, electrical Trinokuler microscope. The materials used were soybean soybeans, alloxan, white mice and mice food.

Making Animals tested Diabetic

Animals tested were made diabetic by the administration of alloxandiabetogenic agent at a dose of 150 mg / kg BW in the IP, except the negative control group was not given anything. Before inducing, mice were fasted for 10 hours. On day 7th after the administration of alloxan, do the examination of blood glucose levels, then do the election of a random sample.

Manufacture of Tempeflour

Tempe ready-made dried by freeze dry until all the water evaporates. The dried tempe then crushed in a mortar until smooth.

Dose Calculations

Soybean flour dose given was 40, 55, 75, 105, 145 and 200 mg / 20 g BW, made suspension, administered orally. Alloxan as inducers, given a dose of 150 mg / kg BW.

Work Procedures

1. Acclimatization of animal experiments for 1 week

2. Animals classified into 8 groups consisting of:

Group 1: negative control group, the test animals are given only the standard of food and drink during the trial period. Group 2: positive control group, the test animals are given standard food, beverages and alloxan 150 mg / kg BW. Group 3, 4, 5, 6, 7 and 8 is the group given standard food, beverages and tempeh flour (40, 55, 75, 105, 145 and 200 mg / 20g BW. Flour tempe given every day and then observed the blood glucose levels of mice on day 7th.

3. Among the dose treatment group, take the dose of tempeh flour that has the optimal effect,that is 200 mg / kg BW.

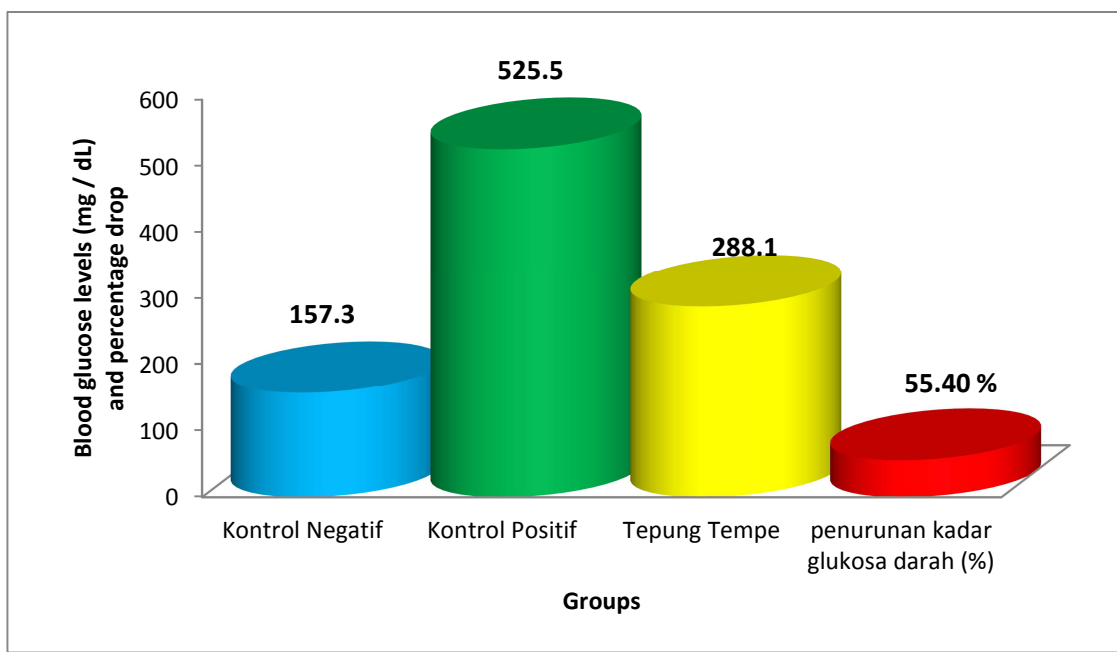
4. Do histopathological examination regarding pancreas β cell damage in the observation group at day 7th.

RESULTS AND DISCUSSION

From the observations obtained after administration of tempeh flour of different doses (40, 55, 75, 105, 145, and 200 mg / 20 g BW, then the provision of tempeh flour that can lower blood glucose levels significantly compared to the positive control is 200 mg / kg BW. The provision of tempeh flour carried out for 7 days and then was observed by measuring blood glucose levels, then obtained the following results:

Group Treatment	Negative Control (-)	Positive Control + Alloxan	Tempehflour + Alloxan	N = 9 Decreasing in blood glucose levels (%)
1. Examination of blood glucose levels (mg/dL)	157.33±6.85	525.5 ± 11.16	288.1±12.10	55,40±11.65
2. Decreasing in blood glucose levels (%)				

Research data tables and diagram cylindrical examination average glucose levels and calculation of the percentage decrease in blood glucose levels in the observation day 7 against the (-)control group and (+)control and tempeh flour dose 200 mg / 20gBW



From the the above description alloxan administration at dose of 150 mg / kg BW, seen these compounds can damage the pancreatic β cells significantly when we compare it with negative control.

On the negative control looks average blood glucose 157 mg / dl were seen lower than the positive control that has very high blood glucose levels 525 mg / dl. Both have the differences with significant value at $p < 0.05$

From the 9 samples that have been tested with the provision of tempeh flour suspected to contain fitoestrogen, dosing of 200 mg / 20 g of BW which is the best dosage to lower blood glucose levels in an optimal when compared to the others. Tempeh flour is capable of lowering blood glucose levels of 525.5 mg / dl to 288.1 mg / dl., means that the administration of tempe at dose of 200 mg / 20 g BW is the maximum dose is able to repair and differentiatecan secrete insulin back. If the decreasing of blood glucose levels are calculated as a percentage compared to the positive controls were only given alloxan. The results demonstrate that fitoestrogen allegedly found in many soy flour is capable of lowering blood glucose levels 55.40%. According to the theory, the body's cells to differentiate require amino acids to bind to phosphate tirosinkinase which will give some signal to activate the differentiation and regeneration of cells is often called stem cells (Stem Cell). Fitoestrogen which many found in soybean as genistein, daidzein and glisitein. Great as an anti-cancer properties, allegedly capable of acting as the amino acids needed in the cell for differentiation and regeneration process that will bring hope to cure diabetes mellitus permanent [10-12].

On histopathologic examination it appears that in the (-)control group the description of β cells pancreas appear normal β cell seen in large quantities, the condition of the cell wall all are in normal condition, not seen cells undergoing lysis and core are colored black, all in situation is clear and bright. Below is the image looks normal cells with a magnification of 400 X

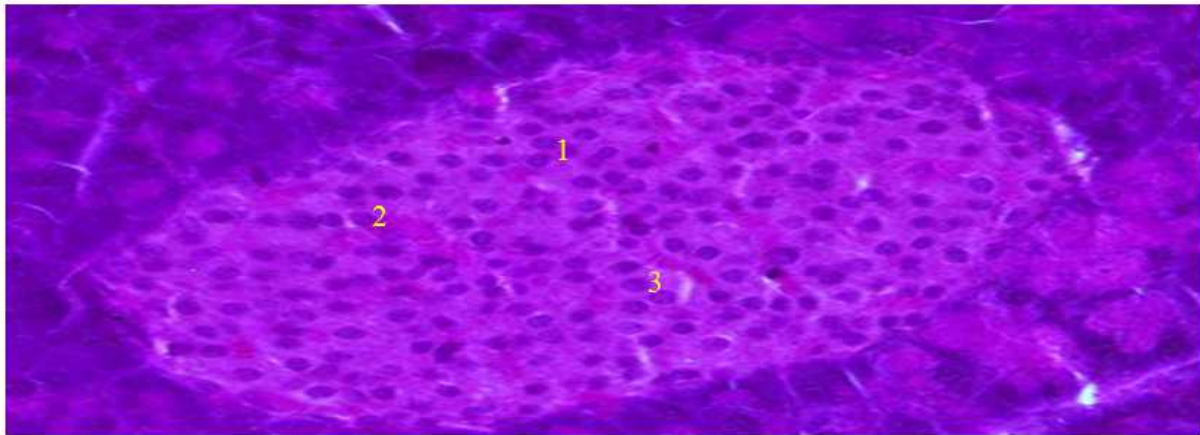


Figure 1. The pancreas gland in normal conditions
 Description: 1. β cells 2. α cells 3. Acini cells

Impression: β cells, α cells and acini cells are in normal condition, no abnormalities cell edge, cell meetings nothing is broken

On the (+)control that is induced by alloxan group aims to damage the pancreatic β cells. Alloxan is a strong oxidizing agent that is looking for a pair electrons into the cell, the cell becomes damaged, uneven edge of the cell, the cell nucleus is not seen colors are vivid, arising lysis and finally apoptosis. Permanent damage to these cells, the cells can not differentiated and regenerate back because until now still not known exactly. Some theories hold that the situation is due to the absence of an amino acid present in β cell so that the differentiation process does not occur. Several attempts have been sought but until now still not clearly revealed. In the picture below looks morphological changes in the pancreas of mice that apoptosis that is not clearly seen.

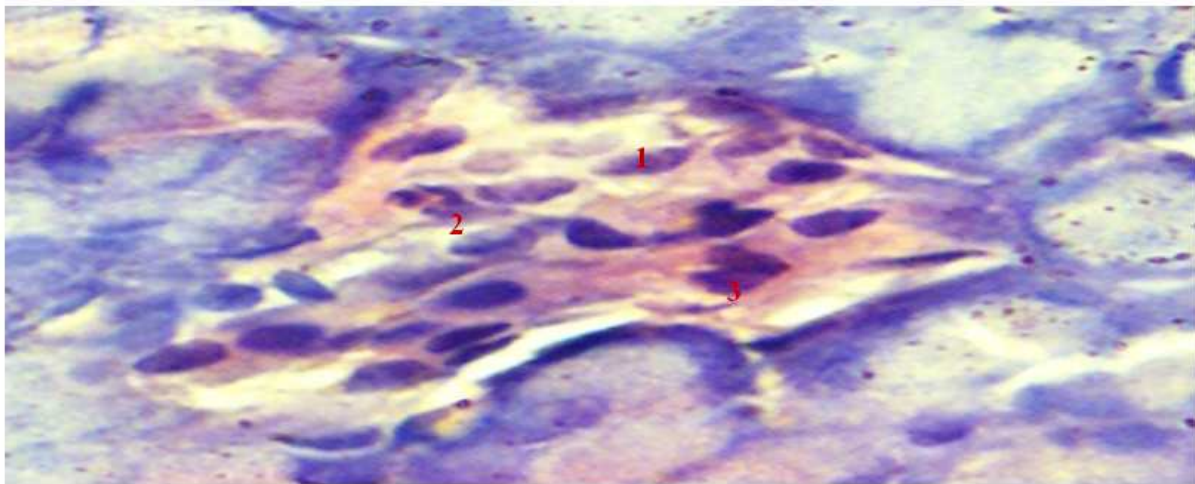


Figure 2. The pancreas gland in alloxan administration of 150 mg / kg BW (Control +)
 Description: 1. β Cells 2. α Cells 3. Acini Cells

Impression: The cell walls and cell nuclei are not clear, reduced cell number, look apoptosis, cells were also damaged acini strong.

In the group administration soybean flour, which is expected to be able to improve and differentiate as well as regeneration of β cells. To regenerate and differentiate require amino acids that are expected amino acids from this fitoesterogen group. Soybean flour is rich with amino acids such as genesteinfitoesterogen group, daidzein and glisitein. This amino acid content should be able to bind to phosphate tirosinkinase which is the active enzyme in the

cell so that the bond will be signals which will enable the regeneration and differentiation β cells in the pancreas. In this condition the cells had been seen improvement when compared to the control (+)group. Cells are not so many more damaged, the cell nucleus had started to clear and somewhat light, the cell walls are a little start slippery. This condition is seen not resembled to the (-)control group. Below this is an overview loaded histopathological tissue after administration tempeh flour dose of 200 mg / 20 gBW.

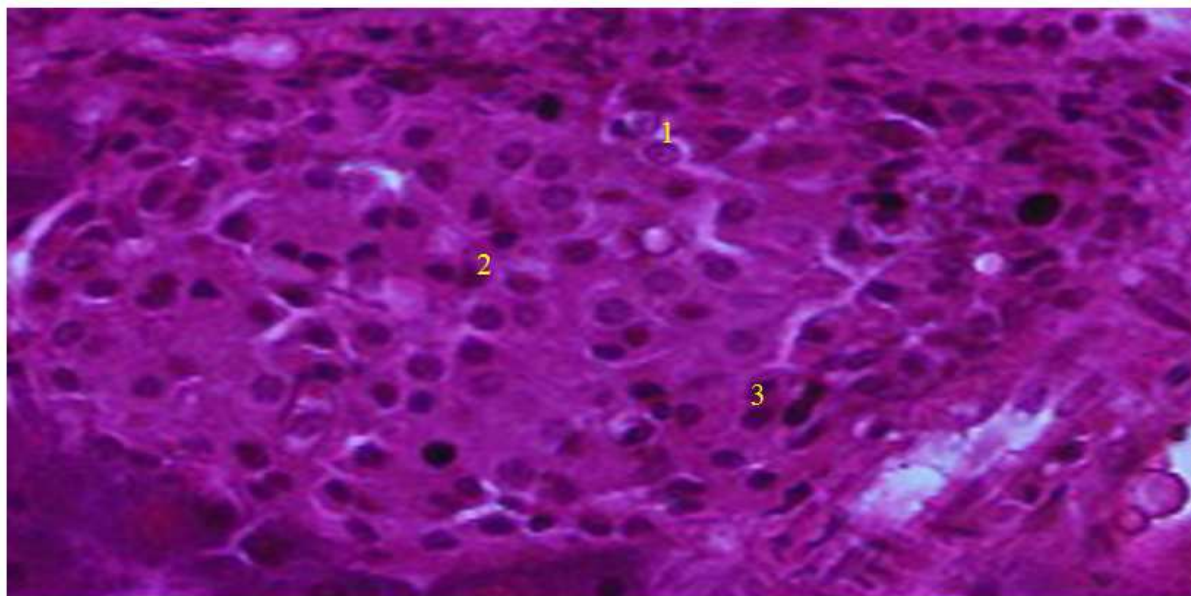


Figure 3. Pancreas gland after administration tempeh flour at dose of 200 mg / 20 g of BW with a magnification of 400 X

Description: 1. β Cells 2. α Cells 3. Acini Cells

Impression: Pancreatic β cells are already somewhat improved, is still spreading and has not compactus

CONCLUSION

From the research we concluded that administration of amino acids contained in soybean flour has the ability to lower blood glucose levels significantly at $p < 0.05$ compared to (+) and (-)control and histopathologic observation, administration of amino acids contained in flour, pancreatic β cells capable of repairing the damaged but still not resembling the (-)control

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