(Effect of Pleurotus ostreatus on Pancreatic Beta Cells of Diabetes Mellitus Mice Model)

(Efek Pleurotus Ostreatus terhadap Sel Beta Pankreas pada Mencit Model Diabetes Mellitus)

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Abstract: Over the last 30 years, the number of people suffering from diabetes mellitus has doubled globally. Adipose tissue dysfunction plays an important role in insulin resistance. Mushroom has been traditionally used to prevent diabetes. This research aims to study the anti-oxidative effect of Pleurotus ostreatus on pancreatic beta cells. This study is an experimental post test only control group design. The subjects were 24 male wistar mice, divided into six groups. Group P0 was given distilled water and citrate buffer. Group P1 was given high fat diet (HFD) and low dose streptozotocin (STZ). Group P2 and P3 were given HFD and low dose STZ along with Pleurotus ostreatus ethanol extract. Group P4 and P5 were given HFD and low dose STZ, and then given Pleurotus ostreatus ethanol extract. Blood glucose levels and pancreatic beta cells area count were done after treatment. Data obtained was analyzed using one-way ANOVA test. One-way ANOVA test showed significant difference in all the groups (p<0.05). Post Hoc test results showed difference in blood glucose levels and pancreatic beta cells area count. Pleurotus ostreatus ethanol extract can prevent cellular damage to murine pancreatic beta cells but unable to reverse the damage to the beta cells.

Keywords: diabetes mellitus type 2, pancreatic beta cells, pleurotus ostreatus.


Kata kunci: diabetes melitus tipe 2, sel beta pankreas, pleurotus ostreatus.

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INTRODUCTION

DIABETES mellitus (DM) is a clinically and genetically heterogeneous group of disorders characterized by abnormally high levels of glucose in the blood\(^1\). Over the last 30 years, the number of people suffering from diabetes mellitus has doubled globally and becomes a challenge in several countries\(^2\).

DM type 2 is the most common type of diabetes\(^3\), amounting to 90% of the total diabetes case in 2010\(^2\). The DM type 2 prevalence has reached epidemic proportion worldwide and has become a world health threat\(^1,4\). DM type 2 is more prevalent in developing countries compared to developed countries with 80% of the people with DM type 2 in developing countries. Among 10 countries predicted to have the highest incidence of DM type 2 in 2030, 5 is in Asia with China, India, Pakistan, Indonesia, and Bangladesh making the list\(^3\).

Among the factors contributing to the incidence of DM type 2 are obesity, genetic factors, smoking and alcohol consumption\(^2,4\), sedentary lifestyle\(^3\), sleeping disorders and depression\(^3\). Involvement of high fat diet in DM type 2 has been reported by decades\(^5\).

Recent studies showed that obesity may cause DM type 2 through involvement of pro-inflammatory cytokines (TNF & IL-6), insulin resistance, impaired fatty acid metabolism, and impaired cellular process such as mitochondria dysfunction\(^6\). The type of fat in high fat diet affects the membrane fatty acid composition and may affect cellular membrane function, including membrane instability, ion permeability, and insulin receptor binding affinity\(^3\).

Adipose tissue dysfunction plays an important role in insulin resistance\(^7\). Adipose tissue secretes TNFα along with monocytes and lymphocytes, where it is involved in inflammatory response and is associated with insulin resistance, obesity, and diabetes\(^8\). Insulin resistance contributed by inflammation on skeletal muscle and lipid metabolism, is the main metabolic abnormality in most of DM type 2 patients\(^8\), where intracellular lipid accumulation in the skeletal muscle and liver is observed in both man and murine. The lipid accumulation causes disruption in GLUT4, which reduces the amount of glucose into the cell and lowers glucose oxidation and glycogen synthesis\(^9\).

STZ administration induce increase of food and water intake, static mass gain, and increase in blood glucose level\(^10\). Microscopic observation of STZ induced mice showed histomorphologic abnormality of the pancreas. Langerhans Island cells were misshaped, reduced in size, and reduction in pancreatic endocrine cells\(^11\). Polysaccharide (beta glucan) in mushrooms can recover pancreatic tissue function by increasing insulin secretion from functional β-cells, which lowers the blood glucose levels and repair peripheral cells sensitivity to circulating insulin\(^12,13\).

Pleurotus species is a member of phylum Basidiomycota. Pleurotus ostreatus increase the antioxidative enzyme which reduces oxidative damage in human. Pleuratus ostreatus contains polyphenols known to hamper mutagenic and carcinogenic effect\(^14\). Ikrimah in 2012, showed that oyster mushroom extract was proven to regenerate Langerhans Island cells shown with increase of pancreatic mass and lower blood glucose levels\(^15\). Based on the observations above, it is interesting to see the effect of Pleurotus ostreatus on DM type 2 induced mice pancreatic β-cells with high fat diet and low-dose streptozotocin.

MATERIALS AND METHODS

METHODS. Reaserch Design. This study is an experimental posttest only control group design. The study was conducted from November 2013 until January 2014.

Study Subjects. The subjects were 24 male wistar...
mice, divided into six groups (n=4/treatment). Group P₀ was given distilled water and citrate buffer. Group P₁ was given high fat diet and low dose streptozotocin (STZ) of 30 mg/kg body weight. Group P₂ and P₃ were given high fat diet and low dose STZ along with Pleurotus ostreatus ethanol extract (200 mg/kg BW/day and 250 mg/kg BW/day, respectively). Group P₄ and P₅ were given high fat diet and low dose STZ, and then given Pleurotus ostreatus ethanol extract (200 mg/kg BW/day and 250 mg/kg BW/day, respectively).

Preparation of Pleurotus ostreatus Extract. Pleurotus ostreatus extract was prepared as followed: all parts of the mushroom cut into small strips and then dried to be a powder. The powder was macerated in 96% ethanol for 72 hours. The ethanolic extract was then evaporated in a rotary evaporator to get concentrated Pleurotus ostreatus extract. The concentrated Pleurotus ostreatus extract was then dried using freeze dryer to evaporate the remaining solvent. The dried Pleurotus ostreatus extract then made into suspension with CMC solvent.

Treatment. All 24 mice were randomly assigned to 6 groups of 4 mice each. The groups are as follows: P₀: Aquadest+normal feed (56 days)+citrate buffer injection (day 15 & 22); P₁: high fat diet (14 days)+STZ injection (30 mg/kg BW day 15 & 22); P₂ = high fat diet & Pleurotus ostreatus ethanol extract 200 mg/kg BW (14 days)+STZ injection (30 mg/kg BW day 15 & 22)+Pleurotus ostreatus ethanol extract 200 mg/kg BW (7 days); P₃ = high fat diet & Pleurotus ostreatus ethanol extract 250mg/kg BW (14 days)+STZ injection (30mg/kg BW day 15 & 22)+Pleurotus ostreatus ethanol extract 200 mg/kg BW (7 days); P₄ = high fat diet & Pleurotus ostreatus ethanol extract 200 mg/kg BW (14 days)+STZ injection (30 mg/kg BW day 15 & 22)+Pleurotus ostreatus ethanol extract 200 mg/kg BW (28 days); P₅ = high fat diet & Pleurotus ostreatus ethanol extract 200 mg/kg BW (28 days).

Examination Procedure. The mice pancreatic β-cells were extracted and made into immunohistochemistry slide from a paraffin block. The slide is then analyzed under a light microscope with automatic camera (Matsuoka Nissei, Japan), under 400x magnification. The images were analyzed using ImageJ (National Institute of Health, USA). The blood glucose levels were analyzed using Diasys kit and spectrophotometer.

Data Analysis. The data were analyzed statistically using One Way ANOVA. If ANOVA showed p<0.05, then Post Hoc test will be performed to see differences between treatment groups. In this experiment, only α≤0.05 is considered statistically significant.

Ethical Clearance. This study has received ethical clearance from the Animal Research Ethics Committees (AREC), Faculty of Science and Mathematics, University of North Sumatera under No. 122/KEPH-FMIPA/2013.

RESULT AND DISCUSSION

The average blood glucose level for P₀ is 127.75 mg/dL, P₁ is 463 mg/dL, P₂ is 250.75 mg/dL, and P₃ is 131.25 mg/dL. There is a significant difference in blood glucose levels between P₀ with P₁ and P₂. However, no significant difference observed between P₀ and P₃. The average pancreatic β-cell area for P₀ is 74.38%, P₁ is 23.04%, P₂ is 49.16%, and P₃ is 62.10%. The graphic showed that there is a significant difference between P₀ with P₁ and P₂. However, no significant difference observed between P₀ and P₃.

The average blood glucose level for P₀ is 127.75 mg/dL, P₁ is 463 mg/dL, P₄ is 314 mg/dL, and P₅ is 331 mg/dL. There is a significant difference in blood glucose levels between P₀ with P₁, P₄, and P₅. However, no significant difference observed between P₁ and P₅.

The average pancreatic β-cell area for P₀ is 74.38%, P₁ is 23.04%, P₄ is 28.63%, and P₅ is 32.37%. The graphic showed that there is a significant difference between P₀ with P₁, P₄, and P₅. However,
also showed similar result to that of P<sub>1</sub>. STZ alkylates DNA via GLUT2 and causing DNA damage. The DNA damage leads to reduced synthesis and secretion of insulin<sup>(17)</sup>. Pancreatic β-cell analysis on groups P<sub>4</sub> and P<sub>5</sub> showed less β-cells area due to damage caused by high fat diet and low-dose STZ injection. Though white oyster mushroom extract were given as a curative treatment, it did not show significant effect when compared to the positive control group (P<sub>1</sub>). This result showed that the white oyster mushroom extract did not have significant regenerative effect on pancreatic β-cells.

**CONCLUSION**

This research aims to study the effect of white oyster mushroom extract on male wistar mice pancreatic β-cells induced with high fat diet and streptozotocin induced DM type 2. The results showed that the *Pleurotus ostreatus* ethanol extract can prevent pancreatic β-cell damage but unable to restore damaged β-cells. Further studies with various dosages and longer study time are needed.

**REFERENCES**


