

THE EFFECT OF SOURSOP LEAF EXTRACT ON PANCREATIC BETA CELL COUNT AND FASTING BLOOD GLUCOSE IN MALE WISTAR RATS EXPOSED TO A HIGH-FAT DIET AND STREPTOZOTOCIN

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ABSTRAK

Berdasarkan beberapa penelitian diketahui bahwa ekstrak daun sirsak dapat memperbaiki cedera sel beta pankreas. Tujuan penelitian ini adalah menganalisis adanya pengaruh pemberian ekstrak daun sirsak terhadap glukosa darah puasa (FBG) dan jumlah sel beta pankreas pada tikus Wistar jantan yang dipaparkan diet tinggi lemak dan streptozotocin. Rancangan penelitian ini adalah the randomized posttest only control group design. Total besar sampel adalah 50 ekor tikus Wistar jantan. Variabel bebas yaitu diet tinggi lemak, injeksi STZ, dan sonde ekstrak daun sirsak; variabel tergantung yaitu jumlah sel beta pankreas dan kadar FBG3. Data diuji normalitas dengan Kolmogorov Smirnov ($\alpha=0,05$) dan uji homogenitas dengan uji Levene ($\alpha=0,05$). Uji komparasi antar kelompok dengan uji Kruskal-Wallis ($\alpha=0,05$) yang dilanjutkan dengan uji Mann Whitney. Uji korelasi antara dosis ekstrak daun sirsak dan FBG3, serta antara dosis ekstrak daun sirsak dan jumlah sel beta pankreas dengan uji Pearson ($\alpha=0,05$). Penelitian ini mendapatkan pengaruh pemberian ekstrak daun sirsak dosis 100mg/kg BB dan 150 mg/kg BB terhadap kadar glukosa darah puasa dan jumlah sel beta pankreas; 2) Terdapat korelasi negatif yang bermakna antara sonde ekstrak daun sirsak dengan FBG3 ($r=-0,647; p<0,001$), semakin tinggi dosis ekstrak daun sirsak, semakin menurun kadar glukosa darah puasa; 3) Terdapat korelasi positif yang bermakna antara sonde ekstrak daun sirsak dengan jumlah sel beta pankreas ($r=0,759; p<0,001$), semakin tinggi dosis ekstrak daun sirsak, semakin meningkatkan jumlah sel beta pankreas. Sebagai simpulan, semakin tinggi dosis ekstrak daun sirsak, akan semakin menurunkan glukosa darah puasa dan semakin meningkatkan jumlah sel beta pankreas. (FMI 2017;53:12-17)

Kata kunci: diet tinggi lemak, STZ, ekstrak daun sirsak, glukosa darah puasa, sel beta pankreas

ABSTRACT

Based on some researches known that soursop leaf extract can improve beta cell injury. The aims of this study was to analyze the effect of soursop leaf extract on fasting blood glucose (FBG) and pancreatic beta cell number in male Wistar rats which were exposed to a high-fat diet and streptozotocin. This study design is the only randomized posttest control group design. The total sample size is 50 male Wistar rats. The independent variable: high-fat diet, STZ, and soursop leaf extract; the dependent variable: pancreatic beta cells number, and FBG3. Data tested for normality with Kolmogorov-Smirnov ($\alpha=0,05$) and tested of homogeneity with Levene ($\alpha=0,05$). Comparison test between groups with Kruskal-Wallis ($\alpha=0,05$), followed by Mann Whitney. Correlation test with Pearson ($\alpha=0,05$) between dose of the soursop leaf extract and FBG3, and between dose and the number of pancreatic beta cells. The results of this study showed that the soursop leaf extract at a dose of 100 mg/kg and 150 mg/kg have an effect on fasting blood glucose levels and pancreatic beta cells number; 2) There is a significant negative correlation between the orogastric lavage of soursop leaf extract with FBG3 ($r=-0,647; p<0,001$), the increasing doses of soursop leaf extract, further lowering fasting blood glucose levels; 3) There is a significant positive correlation between the orogastric lavage of soursop leaf extract with the number of pancreatic beta cells ($r=0,759; p<0,001$), the increasing doses of soursop leaf extract, further increasing pancreatic beta cells number. In conclusion, increasing doses of soursop leaf extract, further lowering fasting blood glucose and increasing the number of pancreatic beta cells. (FMI 2017;53:12-17)

Keywords: high-fat diet, STZ, soursop leaf extract, FBG, pancreatic beta cells.

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INTRODUCTION

Methods of induction of hyperglycemia in Wistar rats that resembled the pathophysiology of type 2 diabetes in humans either by giving exposure to the combination of

a high-fat diet (29 % energy from lard) and STZ injection of a single low dose (27.5 mg/kg). In this study, experimental animals are given a combination of exposure to high-fat diet and STZ, so it is expected to occur hyperglycemia that resembles the pathophysio-

logy of type 2 diabetes in humans (Kumar et al. 2010; Day & Bailey 2011; Srinivasan et al. 2005; Rimbun 2015).

Indonesia ranked seventh in the world with diabetes of 3.2 % of the total population in 2010 (Soewondo et al. 2013). Ninety percent of all people with diabetes in 2010, is type 2 diabetes mellitus (Chen et al. 2012). Type 2 diabetes mellitus (T2DM) is a multifactorial metabolic disease, influenced by lifestyle factors and is associated with obesity. The disease is characterized by the existence of a state of high glucose in the blood (hyperglycemia). There are two defects metabolic occurs in diabetes mellitus type 2, namely a decrease in the response of peripheral tissues to insulin (insulin resistance) and dysfunction and decreased beta (β) cells mass of the pancreas in the form of insulin secretion is inadequate as a result of the insulin resistance (Kumar et al. 2010).

Various pharmacological therapy in type 2 diabetes until recently based on some way related to the mechanism of insulin resistance and elevated insulin production. Some studies show that, often therapy in patients with diabetes mellitus have failed due to reduced function of pancreatic beta cells mass. Patients with pancreatic beta cells function decline associated with poor glycemic control in patients after receiving treatment for 4 years with these drugs (Saisho, 2014). Accordingly, the therapy in type 2 diabetes mellitus should be based on both the metabolic defect mechanisms, not only insulin resistance. Until now there has been no type 2 diabetes drugs that works based on the improvement of dysfunction associated with a reduction in pancreatic beta cell mass.

Soursop plant in ethnomedicine in some countries, such as Amazonia, Brazil, Malaysia, Peru, Togo, and the West Indies widely used as a diuretic, treatment of disorders of the liver, antiparasitic, to lower blood pressure, antidiarrheal, antihyperglykemia, and antidiabetic. Fruit, seeds, leaves, bark and roots of this soursop used in extract form (extracted with hot water), stew, powders, and fruit eaten directly (Kedari & Khan 2014).

Associated with dysfunction and a decrease in the number of cell beta pancreas, it is known that an extract of the leaves of the soursop can repair the dysfunction and injury of the pancreatic beta cells, which decrease oxidative stress in pancreatic beta cells, thereby improving the integrity of the beta cells and causing the regeneration of beta cells in the islets of Langerhans of the pancreas (Malviya et al. 2010; Adeyemi et al. 2010). Treatment with this extract also showed significant increase in antioxidant enzyme activity, and this extract has a high content of nonenzymatic antioxidants. Both of

these plays an important role in the state of oxidative stress that occurs in pancreatic beta cells (Adewole & Ojewole 2009; Muthu & Durairam 2015).

A study found that soursop leaf extract has enzymatic and non-enzymatic antioxidant activities. Enzymatic antioxidant activity such as catalase, superoxide dismutase and glutathione reductase provides a protective effect on tissue from injury by reactive oxygen species (ROS). Non-enzymatic antioxidants such as flavonoids, ascorbic acid, and reduced glutathione has a scavenging effect against ROS as well as a protective effect. In addition, these extracts can inhibit the formation of ROS (Muthu & Durairam 2015).

The general objective of this study was to analyze the influence of soursop leaf extract to pancreatic beta cells count and fasting blood glucose 3 (FBG3) in male Wistar rats which were exposed to a high-fat diet and STZ. The specific objective of this study was to analyze the influence of soursop leaf extract with the increasing dose of 50 mg/kg, 100 mg/kg and 150 mg/kg body weight to fasting blood glucose and the number of beta cells of the pancreas, as well as to prove that increasing doses of the soursop leaf extract will increase the number of pancreatic beta cells and lower fasting blood glucose in male Wistar rats which were exposed to a high-fat diet and STZ. The results of this study can be used to develop therapy of type 2 diabetes by increase in the number of beta cells that produce insulin.

MATERIALS AND METHODS

The design of the study is a laboratory experimental research design and implemented with the randomized posttest only control group design.

Manufacture of soursop leaf extract

Simplicia soursop leaves obtained from Balai Materia Medica, Batu, East Java and made into extracts in the Laboratory of Phytochemistry and Pharmacognosy Faculty of Pharmacy, Universitas Airlangga. The simplicia subsequently crushed into a powder. Soursop leaf powder soaked in ethanol for 24 hours, then the liquid is separated with the powder and the liquid is evaporated. The soaking process must repeated until extraction clear. The extract was dissolved in Na-CMC (Carboxy Methyl Cellulose) before being fed to rats.

Experimental animal

Animals in this study is a rat (*Rattus norvegicus*) Wistar strain, male, age 2-3 months, weight 100-170 grams, and healthy which is characterized by smooth and shiny

fur, and normal activities. Wistar rats selected as a sample, because it is a mammal and is often used as experimental animals in experimental research.

Exposure to High-Fat Diet and Streptozotocin (Induction type 2 diabetes) and Giving Soursop Leaf Extract. K1: Negative control group (not induced hyperglycemia), K2: The positive control group (induced hyperglycemia), P1: Treatment group 1 (induced hyperglycemia and given soursop leaf extract 50 mg/kg), P2: Treatment group 2 (induced hyperglycemia and given soursop leaf extract 100 mg/kg), and P3: Treatment group 3 (induced hyperglycemia and given soursop leaf extract 150 mg/kg)

The total sample size of five groups of rats were 50 rats. Rats adapted for 7 days and placed into each group randomly, ie 2 control groups (negative control, K1; and a positive control, K2) and the 3 treatment groups (P1, P2, and P3). Before the exposure of high-fat diet and STZ, measurements of fasting blood glucose levels 1 (FBG1) was performed in all groups of rats. The K1 group was given a standard diet (4% of energy from fat) from the 1st day until the end of the study, while the K2, P1, P2, and P3 given a high-fat diet (29 % energy from lard) from the 1st day to the 36th day and continue given the standard diet until the end of the study.

In Group K2, P1, P2, and P3 given STZ injection of 27.5 mg/kg intraperitoneally on the 29th day. After exposure to high-fat diet and STZ, measurements were taken at FBG2 levels in all groups. P1, P2, and P3 groups given soursop leaf extract on the 37th-58th day. The P1 group was given a dose of soursop leaf extract 50 mg/kg, P2 group is given a dose of 100 mg/kg, and P3 group was given a dose of 150 mg/kg body weight.

Research data collection

Before sacrificed on the 59th day, FBG3 level measurements carried out in all groups (after fasting for 8 hours). The experimental animals are anesthetized using ketamine HCL 60 mg/kg body weight intramuscularly. The experimental animals sacrificed by decapitation method. Next, an incision made in the abdominal cavity, as well as the cutting of the peritoneal cavity to take the pancreas. FBG3 levels measured with a glucometer, while the pancreas processed for staining with monoclonal antiinsulin antibodies using immunohistochemical methods. Beta cells in the islets of Langerhans seen through the light microscope with 400x magnification. Pancreatic beta cells that produce insulin in the islets of Langerhans calculated and compared to the total number of cells in the islets of Langerhans of the pancreas.

Data analysis

All data were tested for normality with the Kolmogorov-Smirnov ($\alpha=0.05$) and tested of homogeneity with Levene test ($\alpha=0.05$). Statistical analysis by one way ANOVA test ($\alpha=0.05$) or Kruskal-Wallis test ($\alpha=0.05$), followed by LSD (Least Significant Difference) test or Mann Whitney test. Correlation test performed with Spearman's test ($\alpha=0.05$) or Pearson test ($\alpha=0.05$).

After normality and homogeneity test of the FBG1, FBG2, FBG3 levels and the number of pancreatic beta cells; if the data were normally distributed, one way ANOVA test done and continued to LSD (Least Significant Difference) test. If the data distribution is not normal or variant data is not homogeneous, Kruskal-Wallis test was conducted and followed by Mann Whitney test.

Normality test performed on the soursop leaf extract orogastric lavage, FBG3 and the number of pancreatic beta cells in the K2, P1, P2, and P3 groups, if the data were normally distributed, the Pearson correlation test was performed. If the data distribution is not normal, the correlation Spearman test was performed.

RESULTS

Normality test results on FBG3 levels and the number of beta cells of the pancreas showed that FBG3 have no normal distribution, while the number of pancreatic beta cells have a normal distribution. Homogeneity test showed that, FBG3 has a homogeneous variance, while the number of pancreatic beta cells is not homogenous. The FBG3 levels and the number of pancreatic beta cells tested with Kruskal Wallis test, which showed significant differences between groups ($p<0.05$).

Furthermore, the FBG3 levels and the number of pancreatic beta cells tested with Mann Whitney test, which showed that there were significant differences ($p<0.05$) in the levels of FBG3 between the K1 and K2, K1 and P1, K2 and P2, K2 and P3, P1 and P2, and P1 and P3. Significant differences of the number of pancreatic beta cells are also found between the K1 and K2, K1 and P1, K1 and P2, K1 and P3, K2 and P2, K2 and P3, P1 and P2, and P1 and P3.

Normality test results on the soursop leaf extract orogastric lavage, FBG3 levels and the number of pancreatic beta cells indicate that the data has a normal distribution, so the Pearson correlation test was used. The results of Pearson correlation test between soursop leaf extract orogastric lavage and FBG3 levels showed

that there is a significant negative correlation between them ($r=-0.647$; $p<0.001$). This suggests that, dose of soursop leaf extract higher will cause FBG3 levels more decreases.

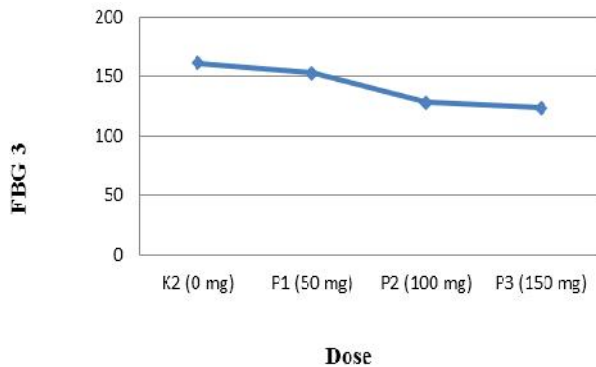


Fig. 1. The dose response relationship between FBG3 and dose of the soursop leaf extract.

The results of Pearson correlation test between soursop leaf extract orogastric lavage and the number of beta cells of the pancreas showed that there were significant positive correlation between them ($r=0,759$; $p<0,001$). This suggests that, dose of soursop leaf extract higher will cause the beta cells of the pancreas more increases.

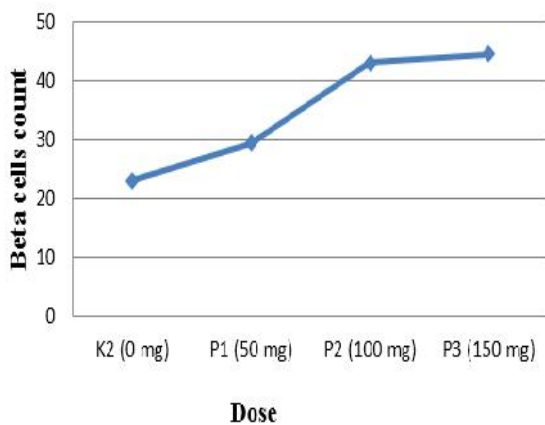


Fig. 2. The dose response relationship between beta cells count and dose of the soursop leaf extract.

DISCUSSION

Based on the test results depending on FBG3 levels showed that there were significant differences between group K1 with K2 and P1, but there is no significant difference between K1 with P2 and P3. In addition, there is a significant difference between P1 to P2 and P3. This shows that, an increase in the dose of soursop leaf extract, then further increase FBG3 levels close to normal.

Based on correlation analysis in the positive control group (K2) and the treatment group (P1, P2 and P3), indicates that there is a significant negative correlation between the gastric lavage of soursop leaf extract with FBG3 levels. This shows that the higher the dose of extract of soursop leaf extract will further lower fasting blood glucose levels, which is below the dose limit of 200 mg (dose toxicity) (Adeyemi et al. 2010).

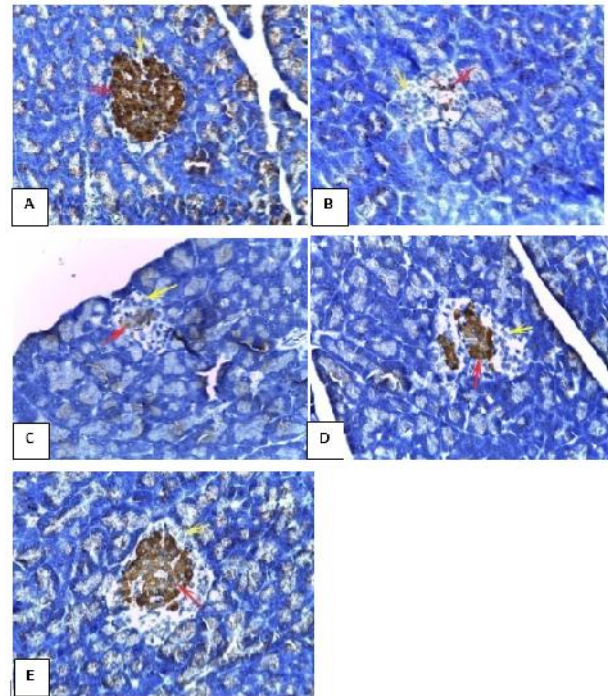


Fig. 3. Microscopic photograph on pancreatic beta cells that showed expression of antiinsulin antibodies. Immunohistochemistry staining with antiinsulin antibodies and hematoxylin counterstain. 400X magnification. A. The K1 group; B. The K2 group; C. The P1group; D. The P2 group; E. The P3 group. Red arrow: the pancreatic beta cells showed expression of antiinsulin antibodies; The yellow arrow: other types of cells in the islets of Langerhans.

Based on previous research, indicate that regeneration occurs in pancreatic beta cells after being given the leaf extract of soursop (Adeyemi et al. 2010). It can be associated with increased production of insulin by the pancreatic beta cells, resulting in a decrease in fasting blood glucose levels. Other pharmacological research also showed that the soursop leaf extract has an antihyperglycemic effect (Kedari & Khan 2014).

Examination of the number of pancreatic beta cells in the islets of Langerhans is intended to determine the effect of soursop leaf extract on beta cells of the pancreas as a producer of insulin. In a previous study, soursop leaf extract showed a significant increase in the number, diameter, and volume of beta cells in the islets of Langerhans of the pancreas. This research indicates that the regeneration of beta cells in the islets of Langerhans of the pancreas of rat given soursop leaf extract (Adeyemi et al. 2010).

The results showed significant differences between groups K2 with P2 and P3, but no significant difference between K2 with P1 on the number of pancreatic beta cells. In addition, there is a significant difference between P1 to P2 and P3. Therefore, an increase in the dose of soursop leaf extract, then further increase the number of pancreatic beta cells and close to normal.

Based on correlation analysis in the positive control group (K2) and the treatment group (P1, P2 and P3), indicates that there is a significant positive correlation between the gastric lavage of soursop leaf extract with the number of pancreatic beta cells. This shows that the higher the dose of soursop leaf extract, will further higher the number of pancreatic beta cells, which is below the dose limit of 200 mg (dose toxicity) (Adeyemi et al. 2010).

Research conducted by Adeyemi et al. showed that the soursop leaf extract can lead to regeneration of the beta cells in the islets of Langerhans of the pancreas (Adeyemi et al. 2010). Other experimental studies showed that treatment with the soursop leaf extract also showed significant increase in enzymatic antioxidant activity, and this extract contains high non-enzymatic antioxidants. Both of these play an important role in the state of oxidative stress that occurs in pancreatic beta cells (Adewole & Ojewole 2009; Muthu & Duraira 2015).

Enzymatic antioxidant content of soursop leaf extract may mediate protective action against apoptosis of pancreatic beta cells, preventing the degeneration of pancreatic beta cells, and stimulate endogenous regeneration of islets of Langerhans. Other than as a nonenzymatic antioxidant, regeneration mechanism by flavonoids is through interaction with proteins, modulate intracellular cascade, and modulate gene expression. Flavonoids can interact with insulin signaling pathway in other cell types, so that modulate beta cell function, insulin secretion and beta cell proliferation (Pinent et al. 2008).

The mechanism of regeneration of pancreatic beta cells is controversial, but it is believed that the pancreatic

beta cells can be regenerated through replication of beta cells still exist (left) or neogenesis stem cells and progenitor cells in and outside of the islets of Langerhans. Neogenesis can also be derived from other types of cells, namely alpha cells, delta cells, epithelial ductal, acinar cells, and centroacinar cells. The whole process is also dependent on extra-pancreatic hormone activator, growth hormone, and others (Hosseini et al. 2015).

CONCLUSION

The conclusions of this research are the soursop leaf extract dose of 100 mg/kg and 150 mg/kg body weight affect the fasting blood glucose and pancreatic beta cell number in male Wistar rats which were exposed to a high-fat diet and STZ; and increasing doses of soursop leaf extract will further lowering fasting blood glucose and increasing the number of pancreatic beta cells in male Wistar rats which were exposed to a high-fat diet and STZ. The suggestions for this research are further research is necessary to observe the extension of the islets of Langerhans associated with soursop leaf extract; further research is needed for a closer look at the types of cells other than pancreatic beta cells in IHC staining, in order to obtain the percentage of pancreatic beta cells more precisely; and further research on the effect of soursop leaf extract against other organs.

REFERENCES

- Adewole, SO, Ojewole, AO 2009, 'Protective Effects Of *Annona muricata* Linn. (Annonaceae) Leaf Aqueous Extract On Serum Lipid Profiles And Oxidative Stress In Hepatocytes of Streptozotocin-Treated Diabetic Rats', *Afr. J. Trad. CAM*, vol 6, no. 1, pp. 30 – 41.
- Adeyemi, DO, Komolafe, OA, Adewole, OS, Obuotor, EM, Abiodun, AA, Adenowo, TK 2010, 'Histomorphological and morphometric studies of the pancreatic islet cells of diabetic rats treated with extract of *Annona muricata*', *Via Medica*, vol. 69, no. 2, pp. 92-100.
- Chen, L, Magliano, DJ, Zimmet, PZ 2012, 'The worldwide epidemiology of type 2 diabetes mellitus-present and future perspectives', *Endocrinol*, vol. 8, pp. 228-236.
- Day, C, Bailey, CJ 2011, 'Obesity in the pathogenesis of type 2 diabetes' *The British Journal of Diabetes & Vascular Disease*, vol. 11, no. 55, pp. 54-61.
- Hosseini, A, Shafiee-Nick, R, Ghorbani, A 2015, 'Pancreatic beta cell protection/regeneration with phytotherapy', *Brazilian Journal of Pharmaceutical Sciences*, vol.51, no. 1, pp. 1-16.

- Kedari, TS, Khan, AA 2014, 'Guyabano (*Annona Muricata*): A review of its Traditional uses Phytochemistry and Pharmacology', *American Journal of Research Communication*, vol. 2, no. 10, pp. 247-22.
- Kumar, V, Abbas, AK, Fausto, N, Aster, JC 2010, 'The Endocrine Pancreas', in Robbin and Cotran *Pathologic Basis of Disease* (8th ed), Saunders, an imprint of Elsevier Inc, Philadelphia, pp. 1130-1148.
- Malviya, N, Jain, S, Malviya S 2010, 'Antidiabetic Potential of Medicinal Plants', *Acta Poloniae Pharmaceutica-Drug Research*, vol. 67, no. 2, pp. 113-118.
- Muthu & Duraira 2015, 'Evaluation of antioxidant and free radical scavenging activity of *Annona muricata*', *European Journal of Experimental Biology*, vol. 5, no. 3, pp. 39-45.
- Pinet, M, Castell, A, Baiges, I, Montagut, G, Arola, L, Ard'evol, A 2008, 'Bioactivity of Flavonoids on Insulin-Secreting Cells', *Comprehensive Reviews in Food Science and Food Safety*, vol. 7, pp. 299-308.
- Ramachandran, A, Snehalatha, C, Shetty, AS, Nanditha, A 2012, 'Trends in prevalence of diabetes in Asian countries', *World Journal of Diabetes*, vol. 3, no. 6, pp. 110-117.
- Rimbun 2015, 'Tesis Profil Glukosa Darah Luas Pulau Langerhans dan Ekspresi Reseptor Insulin Otot pada Berbagai Model Diabetes Mellitus Tipe 2', Program Studi Kedokteran Dasar Jenjang Magister Fakultas Kedokteran Universitas Airlangga, Surabaya.
- Saisho, Y 2014, 'Importance of Beta Cell Function for the Treatment of Type 2 Diabetes', *J. Clin. Med*, vol. 3, pp. 923-943.
- Scottish Intercollegiate Guidelines Network 2010, 'Management of diabetes'.
- Shaw, JE, Sicree, RA, Zimmet, PZ 2010, 'Global estimates of the prevalence of diabetes for 2010 and 2030', *Diabetes Research and Clinical Practice*, vol. 87, pp. 4-14.
- Soewondo, P, Ferrario, A, Tahapary L 2013, 'Challenges in diabetes management in Indonesia: a literature review', *Globalization and Health*, vol. 9, no. 63, pp. 1-17.
- Srinivasan, K, Ramarao, P 2007, 'Animal models in type 2 diabetes research: An overview', *Indian J Med Res*, vol. 125, pp. 451-472.