The Difference Between Physalin Standardized Extract from *Physalis Angulata* L. and Control on Pancreatic Function of Sprague Dawley Rat Induced by *Streptozotocin-Nicotinamide*

Yeny Sulistyowati¹, Sri Kadarsh Soedjono², Mustofa³, Budi Mulyono²

¹Departement of Science Nutrition, Faculty of Health Sciences, Respati University of Yogyakarta, 55281, Indonesia
²Faculty of Medicine, Gadjah Mada University, 55281, Indonesia

*Corresponding author*
Yeny Sulistyowati
Email: yeny.sulistyowati6@gmail.com

Abstract: Diabetes mellitus (DM) is a metabolic disorder of carbohydrate, fat, and protein characterized by chronic hyperglycemia conditions. Oxidative stress due to hyperglycemia in patients with diabetes may fasten the process of pancreatic tissue damage. Physalis angulata L or Ciplukan herb is one of Indonesian medicinal plants that has been used traditionally and empirically proven as an antidiabetic. Objective the study was determine whether there is a difference between physalin standardized extract from Physalis angulata L and control on pancreatic function of Sprague Dawley rat induced by Streptozotocin and Nicotinamide. The study was true experimental research design with a post-test only control group design. The subjects of this study were 32 male Sprague Dawley rat induced by Streptozotocin and Nicotinamide. The extract was given a dose of 20 mg / kg BW for 21 days. At the end of the study, pancreas organs were taken for histological preparations. There were continued by observed of the diameter of Langerhans islets. Normality test data Kolmogorov Smirnoff was to determine the differences analysed by t-test. There was a significant difference between the diameter of the islets of Langerhans treatment and control group (p = 0.001). The provision of physalin standardized extract from *Physalis angulata* L at a dose of 20 mg / kg BW can repair the size of the islets of Langerhans. The treatment can prevent oxidative stress and are able to maintain cell function of pancreas.

Keywords: Diabetes Mellitus, *Physalis angulata* L (Ciplukan herb), pancreatic function.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of carbohydrate, fat, and protein caused by decreased insulin secretion, decreased tissue sensitivity to insulin, or both [1, 2]. Prevalence of the world's population suffered from diabetes in 2000 is estimated about 150 million people. For Indonesia, the World Health Organization (WHO) predicts the increase in the number of people from 8.4 million in 2000 to 21.3 million in 2030 [3]. Meanwhile, the International Diabetes Federation (IDF) predicts that the number of people with diabetes in Indonesia from 7 million in 2009 increase to 12 million in 2030[3].

Conditions of hyperglycemia in diabetes will cause long term damage and failure of various organ functions. Frequent complication of diabetes mellitus is divided into two major subtypes, namely diabetes-specific microvascular complications and macrovascular complications [4]. Oxidative stress and hyperglycemia due to insulin resistance in patients with diabetes may fasten the process of β-pancreatic cell damage [5].

The high cost of therapy makes the herbs as the alternative for people with diabetes. In addition, many natural products and herbal medicines are recommended as the treatment of diabetes mellitus [6]. Indonesian medicinal plants that have been used traditionally and empirically proven as an antidiabetic, among others: gardenia, bitter, noni, vinca, ciplukan, etc. [7].

Ciplukan herb (*Physalis angulata* L) has been used as antiatherosclerotic [8], antibacterial [9], anticancer [10], asthma medication [11] and antidiabetic [12-14]. Antidiabetic effects of Ciplukan herb occurs because the chemical constituents of this plant, such as unsaturated fatty acid, alkaloids, flavonoids, saponins, polyphenols, steroids and triterpenoids, monoterpenoid, and sesquiterpenoid [12, 13, 15].

Effects of Ciplukan herb (*Physalis angulata* L.) as a potential antioxidant against pancreatic function in diabetic people has not been known. Based on the results of existing studies, it is necessary to investigate whether there is a difference between...
physalin standardized from Physalis angulata L and the control on pancreatic function of Sprague Dawley rat induced by Streptozotocin-Nicotinamide.

**METHODOLOGY**

This study was true experiment with post-test only control group design using control and treatment groups. The subject of the study was a male rats strain Sprague Dawley. This research was conducted in the Laboratory of Pharmacology and Toxicology Faculty of Medicine, Gadjah Mada University as the manufacture of herbal extracts Ciplukan. Preparation of pancreas histofisiologi was conducted at Anatomical Pathology of the Faculty of Medicine, Gadjah Mada University. Maintenance of animals was conducted in LPPT 4 Gadjah Mada University. The observations of the pancreas histofisiologi was conducted in Histology and Cell Biology Faculty of Medicine, Gadjah Mada University.

The independent variable in this study was giving physalin standardized from Physalis angulata L, a dose of 20 mg / kg BW and the dependent variable was histology of the pancreas. Uncontrolled confounding variables were the subject of research, Ciplukan herb and maintenance. Subjects of the study include 32 male rats strain Sprague Dawley weighing range 160-200 g aged 3 months. Animal made STZ-induced diabetes by means of a dose of 45 mg / kg BW in intraperitonial. Diabetes Mellitus rats expressed when blood sugar levels > 100 mg / dL pp [16-18]. Giving Nicotinamide was much as 110 mg / kg BW and it was given 15 minutes before administration of STZ. Ciplukan herb treatment was given for 21 days then on day 22, the animals euthanized for pancreas histofisiologi. Histological observation of the pancreas used histofisiologi test with hematoxylin and eosin staining technique and observations of the diameter of the islets of Langerhans [19] with 40x magnification. Diameter of the islets of Langerhans was statistically analysed. Analysis of the data in the form of test data normality was Kolmogorov Smirnov. Different test between the control and treatment groups was analysed by independent t test trials.

**RESULTS AND DISCUSSION**

**Microscopic Histology of the Islets of Langerhans**

Based on Figure 1, it can be seen that the microscopic picture of the histology of the pancreas (islets of Langerhans) control group showed abnormalities which are round and elongated autolysis. Microscopic picture of group I, showing the form that is normal islets of Langerhans are oval and endocrine cells appear to degenerate density compared to the control group and the other treatment.

**The Difference between the Diameter of the Islets of Langerhans Treatment and Control Group**

Histological observation of the pancreas uses histofisiologi test with hematoxylin and eosin staining techniques. The mean diameter of the islets of Langerhans can be seen in table 1.

Based on Table 1 it can be seen that the average diameter of the islets of Langerhans control group is 265.88 µm (SD ± 27.09), the mean diameter of the islets of Langerhans group with herbal treatment Ciplukan 20 mg/kg body weight is 354.89 µm (SD ± 42.16). The normality test performs on the mean diameter of the islets of Langerhans data using Kolmogorov Smirnov statistical test with a significance > 0.05.
Statistical analysis of data showed the average diameter of the islets of Langerhans were normally distributed (p = 0.74). Different test between the control and treatment groups by t-test statistical test showed significant difference (p = 0.001).

The pancreas is a gland that produces hormones (insulin and glucagon), digestive enzymes, and bicarbonate are secreted into the duodenum to aid digestion [20]. Patients with type 2 diabetes mellitus, chronic decline in the function of pancreatic β cells in the form of a decrease in the number and diameter of the islets of Langerhans resulting in decreased insulin secretion. Decreased function of the pancreas due to an imbalance of antioxidants and free radical or oxidative stress due to the state of chronic hyperglycemia [21].

In this study, the condition of hyperglycemia in experimental animals given injections done by Streptozotocin (STZ) at a dose of 45 mg / kg BW. Streptozotocin a diabetogenic substances that are cytotoxic to pancreatic β cells [16, 22]. After STZ induced, animal adapted for 3 days and were treated for 21 days. Day 22 after treatment of experimental animals euthanized for histological preparations.

Histological preparations are conducted using hematoxylin and eosin staining techniques for observation of the shape and diameter of Langerhans. The results of measurements of the diameter of the islets of Langerhans is being an indicator of the extent of damage to the pancreatic endocrine cells of experimental animals after STZ induced and after being given Ciplukan herb extract for 21 days treatment. Islets of Langerhans have properties dependent plasticity of pancreatic β cell mass in them. There for the diameter of the islets of Langerhans is directly proportional to the amount and mass of β - cells of the pancreas [23].

Statistical analysis of the diameter of the islets of Langerhans, shows that there are significant differences between the control and treatment groups (p = 0.001). Based on the microscopic appearance histology pancreas, islets of Langerhans control group show abnormalities which are elongated shape and smaller than the treatment group. While the treatment group Ciplukan herb dose 20 mg/kg BW show Langerhans islets in the shape and the diameter is larger than the normal control.

Changes in the shape and diameter of the islets of Langerhans control group occurred due to an increase in free radical production after STZ -induced pancreatic tissue damage resulting.

The increase in free radical production after induction of STZ describes the pathogenesis of the condition of hyperglycemia in chronic type 2 diabetic patients. The increase in free radical production is being offset by an increase in the concentration of antioxidants leads to oxidative stress. Oxidative stress of the patients with diabetes can lead to various complications and pancreatic tissue damage.

The imbalance between free radicals and antioxidants can lead to oxidative stress. In patients with chronic type 2 diabetes mellitus, oxidative stress can lead to microvascular and macrovascular complications [4]. Complications occurred mainly in the form of impaired cardiovascular function of endothelial dysfunction and atherosclerosis [24, 25].

The study is consistent with research conducted by Oberley [26]. The increase in free radicals after induction of STZ leads to pancreatic tissue damage within 2-4 days after induced. Increased pancreatic tissue damage will continue if there is a condition of uncontrolled hyperglycemia. Conditions of chronic hyperglycemia in diabetes may increase the production of free radicals and reactive oxygen species (ROS). Research Kaneto et al. [27], shows the same thing that chronic hyperglycemia can increase oxidative stress that can damage β-cells of the pancreas. Oxidative stress can also lower the amount of glucose transporter (GLUT) in the blood resulting in an increase in insulin resistance. In addition, the lack of insulin signaling due to oxidative stress can decrease the synthesis and secretion of insulin by β-cells of the pancreas.

Based on the results of this research conducted Sulistyowati et al. [14] show that in the control group there was an increase in blood sugar levels, from 333.62 mg / dL increased to 403.03 mg / dL. Increased blood sugar levels due to decreased insulin secretion by β-cells of the pancreas. It is proven by the diameter of the islets of Langerhans in the control group under normal.

The results of research in the treatment group show a different histological features of the pancreas with a control group based on the shape and diameter of the islets of Langerhans (p = 0.001). The statistical result of the giving physalin standardized extract from Ciplukan herb on pancreatic histology shows significant difference (p = 0.001). Water extract of Ciplukan herb in the group treated with a dose of 20 mg/kg BW could prevent the destruction of pancreatic tissue of experimental animals after STZ induced. Mechanism of action of water extract Ciplukan herb in maintaining the function of the pancreas after induction of STZ has not been known.

However, it is predicted because of the effect of the active compounds contained in Ciplukan herbs, namely alkaloids, flavonid, steroids, saponins, polyphenols, monoterpenoid, teriterpenoid and seskuiterpenoid, unsaturated fatty acids and physalin.

Physalin is a steroid derivated compound with high antioxidant activity that can prevent the
occurrence of oxidative stress. Physalin contained in Ciplukan herb can increase enzyme activity superoksidase dismutatase (SOD) and catalase [28]. Dismutase and catalase enzymes superoksidase an enzymatic antioxidants can protect cells or tissues and organ systems of the body from free radicals and ROS. Antioxidants work by providing a rapid hydrogen atom to the radical compound that turns into a more stable compound [29].

Similar results were also shown by the results of the study of El-Mehiry et al. [28], showed that the antioxidant extract of physalin can increase enzyme activity superoksidase dismutatase (SOD) and catalase. Increased activity of SOD and catalase enzyme able to reduce levels of protein oxidation and lipid peroxides. There is a balance between oxidants and antioxidants are able to prevent damage to the cells of the pancreas.

Research Kaneto et al. [27] has shown that DM treatment with antioxidants can prevent oxidative stress and are able to maintain cell function of pancreas. Oxidative stress occurs due to the condition of uncontrolled hyperglycemia can damage β-cells of the pancreas that decreased synthesis of insulin. Antioxidant treatments on DM are able to protect cells from the pancreatic β-oxidative stress and maintain β-cell function of the pancreas.

Results of research conducted Sulistyowati et al. [14] Show that the administration of the dose variation Ciplukan herb can lower blood sugar levels after giving treatment group treated for 21 days. The decrease in blood sugar levels in the treatment group shows that pancreatic function can be maintained in the group. This is proven by histology islets of Langerhans that the treatment is better than the control group.

CONCLUSION

Microscopic structure of rat pancreas in the treatment group size is larger than the control. The mean diameter of the islets of Langerhans in the control group is 26.88 + 27.09, while the treatment group is 354.89 + 42.16. There is a significant difference islets of Langerhans in the control group and the treatment (p = 0.001). The provision of physalin standardized extract from Physalis angulata L at a dose of 20 mg / kg BW can repair the size of the islets of Langerhans. The treatment can prevent oxidative stress and are able to maintain cell function of pancreas.

Based on the results, it is recommended to determine the diet that can affect energy and nutrient intake. Creating the form checklist aspects of diabetes symptoms of polyphagia, polydipsia, and polyuria will be conducted next research. It will be better to determine the effects on other biochemical parameters and organ others, such as the kidney, aorta and heart.

REFERENCES
3. Indonesian Society of Endocrinology; Consensus Management and Prevention of Type 2 Diabetes Mellitus in Indonesia, 2011, PERKENI.


23. Ridwan A, Raden T, Astrian dan Angraini B; Pengukuran efek antihiperaibitis polifenol (polyphenon 60) berdasarkan kadar glukosa darah dan histologi pankreas menci (Mus musculus L.) S.W. Jantan yang dikondisikan diabetes mellitus (Antidiabetic effect the measurement of polyphenols (polyphenon 60) based on blood glucose and pancreas histology of male mice (Mus musculus L.) SW conditioned by diabetes mellitus). Jurnal Matematika dan Sains, 2012; 17(2): 78-83.


