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Research Article

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

Yeny Sulistyowati[1*], Sri Kadarsih Soedjono[2], Mustofa[2], Budi Mulyono[2]
[1]Departement of Science Nutrition, Faculty of Health Sciencies, Respati University of Yogyakarta, 55281, Indonesia
[2]Faculty 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 Streptrozotocin-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 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 Smirnof 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 antiaterosklerotik [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 seskuiterpenoid [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.

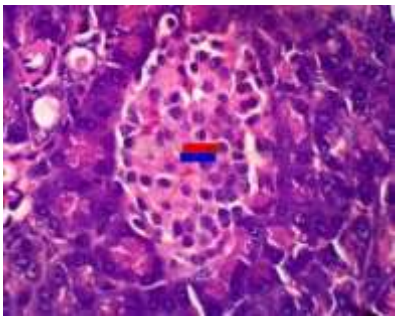
METHODOLOGY

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.

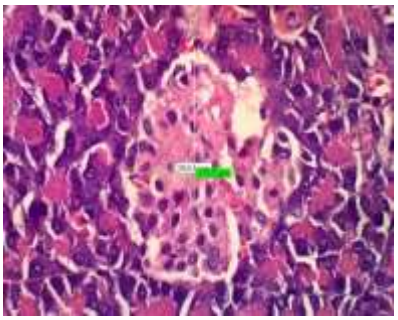
The independent variable in this study was giving physalin standardized from Physalis angulata L, a dose of 20 mg / kg BW and the dependent variable was histology of the pancreas. Uncontrolled confounding variables were the subject of research, Ciplukan herb and maintenance. Subjects of the study include 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 histofisiology. 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



DM group without Ciplukan herb (Control group)



DM group with Ciplukan herb 20 mg / kg BW (Treatment Group)

Fig. 1: Microscopic histology of the islets of Langerhans picture with 40x magnification

Table 1: The mean diameter of the islets of Langerhans between the control and treatment group

Groups	Dose of standardized physalin from Physalis angulata L	The mean diameter of islets of Langerhans (µm)	p (Anova)
Control	Without extract	265,88 ± 27,09	0,001*
Treatment	20 mg/kg BW	354,89 ± 42,16	

* p< 0.05 = significant

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 of 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 acount 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 Lengerhans 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 oxygene 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 dismutase (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 dismutase (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.

CONCLUSSION

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

1. World Health Organization; Definition, diagnosis and classification of diabetes mellitus and its complications. Geneva, WHO/NCD/NCS/99.2, 1999.
2. Cubbon RM, Adil R, Stephen BW; The impact of insulin resistance on endothelial function, progenitor cells and repair. Diab Vasc Dis Res., 2007; 4(2): 103-111.
3. Indonesian Society of Endocrinology; Consensus Management and Prevention of Type 2 Diabetes Mellitus in Indonesia, 2011, PERKENI.
4. Heydari I, Vida R, Sara R, Afsaneh A; Chronic complications of diabetes mellitus in newly diagnosed patients. Int J Diabetes Mellit., 2010; 2(1): 61–63.
5. Buchanan TA, Xiang AH, Peters RK, Kjos SL, Marroquin A, Goico J *et al*.; Preservation of pancreatic β-cell function and prevention of type 2 diabetes by pharmacological treatment of insulin resistance in high-risk hispanic women. Diabetes, 2002; 51(9): 2796-2803.
6. Jung M, Moonsoo P, Hyun CL, Yoon-Hoo K, Eun SK, Sang KK; Antidiabetic agents from medicinal plants. Curr Med Chem. 2006;13(10):1203-1218.
7. Widowati L, Dzulkarnain B, dan Sa'roni; Tanaman obat untuk diabetes mellitus (Medicinal Plants for Diabetes Mellitus). Cermin Dunia Kedokteran, 1997; 116: 53-60.
8. Anggraini M, Haryono K, Habibie; Potensi antiaterosklerotik herba ciplukan (*Physalis angulata* L) dengan uji sintesis proinflamatori dan aktivitas penghambatan oksidasi LDL (Potency antiatherosklerotik ciplukan herbs (*Physalis angulata* L) with proinflammatory synthesis test and inhibition activity of LDL Oxidation). Jurusan Farmasi: Universitas Hasanudin, 2009.
9. Guimarães ET, Lima MS, Santos LA, Ribeiro IM, Tomassini TB, Ribeiro dos Santos R*et al*.; Activity of physalins purified from *Physalis angulata* in in vitro and in vivo models of cutaneous leishmaniasis. J Antimicrob Chemoth., 2009; 64(1): 84-87.
10. Fauzi IA, Fikri A, Nurma S, Adam H, Muthi I, dan Edy M; Aktivitas antiproliferasi ekstrak etanolik herba ciplukan (*Physalis angulata* L.) terhadap sel hepar tikus betina galur sprague dawley terinduksi 7,12-dimetilbenzena. (Activities antiproliferasi ethanolic herb extract (Physalis angulata L.) against rat liver cells female sprague-dawley strain-induced dimetilbenzen). Majalah Kesehatan Pharma-Medika, 2011; 3(1): 194-199.
11. Rathore CKR, Dutt Shobharam S, Lokesh D; Antiasthmatic activity of the methanolic extract of *Physalis angulata* Linn. J Med Plants Res., 2011; 5(22): 5351-5355.

12. Sediarso HS, dan Nurul A; Efek antidiabetik dan identifikasi senyawa dominan dalam fraksi kloroform herba ciplukan (*Physalis angulata* L.) (Antidiabetic effects and identification of dominant compounds in ciplukan herbs chloroform fraction (*Physalis angulata* L.)). Jurnal Farmasi Indonesia, 2008; 4(2): 63-69.
13. Sutjiatmo AB, Elin YS, Yulia R, Suswini K, Asri W, dan Suci N; Efek antidiabetes herba ciplukan (*Physalis angulata* LINN.) pada mencit diabetes dengan induksi aloksan (Antidiabetic effect of ciplukan herb (*Physalis angulata* LINN.) in diabetes mice with induction of aloxan). Jurnal Farmasi Indonesia, 2011; 5(4): 166-171.
14. Sulistyowati Y, Septriana Rafika M; Effect of water extract herbs ciplukan (*Physalis angulata* L) on blood sugar and lipid profile of sprague dawley male rats injected by streptozotocin and lipopholysacharide. Proceeding and Abstract book of 8th Asia Pacific Conggres on Clinical Nutrition. Tokyo, Japan, 2013: 116.
15. Choi EM, Hwang JK; Effect of some medicinal plants on plasma antioxidant system and lipid levels in rats. Phytotherapy Research, 2005; 19(5): 382-386.
16. Ahmed RG; The physiological and biochemical effects of diabetes on the balance between oxidative stress and antioxidant defense system. Medical Journal of Islamic World Academy of Sciences, 2005; 15(1): 31-42.
17. Srinivasan K, Ramarao P; Animal model in type 2 diabetes research: An overview. Indian Journal Medical Research, 2007; 125(3): 451-472.
18. Aragno M, Parola S, Brignardello E, Manti R, Betteto S, Tamagno E *et al*.; Oxidative stress and eicosanoids in the kidney of hyperglycemic rats treated with dehydroepiandrosterons. Free Radical Biol Med., 2001; 31(8): 935-942.
19. Jellies MM, dan Makiyah N; Pengaruh pemberian infusa tumbuhan sarang semut (hydnophytum formicarum) terhadap gambaran histologi pankreas pada tikus (Rattus norvegicus) diabetes terinduksi aloksan (Effect of hydnophytum formicarum against the preview of pancreatic histology of rat (Rattus norvegicus) alloxan induced diabetes). Majalah Kesehatan Pharma Medika, 2011; 3(1): 200-203.
20. Price SA, Wilson LM; Pathophysiology: clinical concepts of disease processes. 6th edition, Mosby, 2002.
21. Moussa SA; Oxidative Stress in Diabetes Mellitus. Rom J Phys., 2008; 18(3): 225-236.
22. Takasu N, Ichiro K, Takayuki A, Yoshitaka N, Takashi Y; Streptozocin- and alloxan-induced h_2o_2 generation and DNA fragmentation in pancreatic islets: H_2O_2 as mediator for DNA fragmentation. Diabetes, 1991; 40(9): 1141-45.
23. Ridwan A, Raden T, Astrian dan Anggraini B; Pengukuran efek antidiabetes polifenol (polyphenon 60) berdasarkan kadar glukosa darah dan histologi pankreas mencit (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.
24. Watskin Peter J; ABC of Diabetes. 5th edition. London: BMJ Publishing, 2003.
25. Johansen JS, Alex KH, David JR, Adviye E; Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. Cardiovasc Diabetol., 2005; 4(5): 1-11.
26. Oberley LW; Free radicals and diabetes. Free Radical Bio Med., 1988; 5(2): 113-124.
27. Kaneto H, Yoshitaka K, Jun-Ichiro M, Taka-Aki M, Yoshio F, Yutaka U *et al*.; Beneficial effects of antioxidants in diabetes; possible protection of pancreatic β-cells againts glucose toxicity. Diabetes, 1999; 48(12): 2398-2406.
28. El-Mehiry HF, Helmy HM, El-Ghany MAA; Antidiabetic and antioxydative activity of physalis powder or extract with chromium in rats. World Journal of Medical Sciences, 2012; 7(1): 27-33.
29. Percival M; Antioxidants. Clinical Nutrition Insights. 1998; 31: 1-4.