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by Saikhu Akhmad Husen1

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Antioxidant Potency of Okra (*Abelmoschus esculentus* Moench) Pods Extract Preserve Langerhans Islet Structure and Insulin Sensitivity in Streptozotocin-induced Diabetic Mice

SAIKHU AKHMAD HUSEN[1], MUHAMAD FRENDY SETYAWAN, ARIF NUR MUHAMMAD ANSORI[2], SUHAILAH HAYAZA, RADEN JOKO KUNCORONINGRAT SUSILO, MOCHAMMAD AMIN ALAMSJAH[3], ZULFA NAILUL ILMI[4], PUGAR ARGA CRISTINA WULANDARI[4], PRATIWI PUDJIASTUTI[4], KHALIJAH AWANG[5], DWI WINARNI AND WIN DARMANTO*

Department of Biology, Faculty of Science and Technology, Universitas Airlangga, 60115, Surabaya, Indonesia
**(e-mail : windarmanto@fst.unair.ac.id; Mobile : +62-85648283644)*

ABSTRACT

This study aimed at determining the effect of various fractions of okra pods extract (VOPE) on fasting blood glucose, the diameter of the islets of Langerhans, number of β-cells of the islets of Langerhans and homeostatic model assessment for insulin resistance (HOMA-IR) in diabetic mice. This study used 35 male Balb/c strain mice. The samples were divided into seven groups, KN (normal control), KD (diabetic control), KA (acarbose control; 100 mg/kg body weight), EK (crude extract; 100 mg/kg body weight) EP (non-polar; 20.04 mg/kg body weight), ES (semi-polar; 27.39 mg/kg body weight) and EP (polar; 54.11 mg/kg body weight). VOPE and acarbose administered orally for 14 days in streptozotocin-induced diabetic mice. The diabetes was induced by intraperitoneal injection of multiple low-dose streptozotocin (30 mg/kg body weight) daily for five consecutive days. On day 15th, mice were sacrified. Interestingly, administration of VOPE decreased fasting blood glucose level, increasing the diameter of the islets of Langerhans, number of β-cells of the islets of Langerhans, and homeostatic model assessment for insulin resistance (HOMA-IR) in streptozotocin-induced diabetic mice. In sum, VOPE was a promising antioxidant agent due to amelioration of the islets of Langerhans.

Key words : *Abelmoschus esculentus* Moench, diabetic mice, Langerhnas islet diameter, fasting blood glucose, HOMA-IR

INTRODUCTION

Diabetes mellitus (DM) is a metabolic multisystem disorder that affects about 6% of the world population. The disease is characterized by hyperglycemic conditions, due to the reduction of both insulin secretion and insulin sensitivity (American Diabetes Association, 2011). Resistance to insulin is one of the causes of DM which means that the insulin fails to work properly. Therefore, it could lead to insulin insensitivity in the body (Husen *et al.*, 2017). Insulin resistance causes a disruption in glucose transporter-4 (GLUT-4) translocation to the membrane surface of the muscle cells and fatty cells. This condition damages the glucose uptake process and causes high glucose level in blood (Mukherjee *et al.*, 2013).

The prolonged hyperglycemic condition can activate polyol pathways. The excessive activation of the polyol pathway in the insulin insensitive tissues causes a lot of glucose being converted into sorbitol which can be retained in the cell. These changes force mitochondria in the cell to produce superoxide anions which

[1]Animal Histology Laboratory, Faculty of Science and Technology, Universitas Airlangga, 60115, Surabaya, Indonesia.
[2]Doctoral Program in Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, 60115, Surabaya, Indonesia.
[3]Department of Marine, Faculty of Fisheries and Marine Universitas Airlangga, Kampus C Universitas Airlangga, 60115, Surabaya, Indonesia.
[4]Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, 60115, Surabaya, Indonesia.
[5]Department of Chemistry, Faculty of Science, University of Malaya, 50603, Kuala Lumpur, Malaysia.

increase ROS. Thus, the oxidative stress of the cell also rises (Hayaza *et al.*, 2019a). This increase in oxidative stress will trigger lipid peroxidation in the cell membrane. MDA is a lipid peroxidation product that has been recognized as one of the biological markers for reliable oxidative stress. MDA concentrations in serum, blood plasma and tissue as a way to determine the antioxidant activity which also acts as a major indicator of potential oxidative stress in the diabetic patient. The increased ROS production which exceeds the cell antioxidant capacity leads to a rise in the oxidative stress accompanied by the dysfunction and β-cells damage in the pancreatic tissue (Ansori *et al.*, 2019; Husen *et al.*, 2019a). As a result, it decreases insulin secretion. The prolonged hyperglycemic condition can also cause the levels of RNS to increase. ROS and RNS can directly oxidize and damage DNA, proteins and lipids (Fadholly *et al.*, 2019; Tacharina *et al.*, 2020).

Free radicals are commonly known to have many harmful effects that can be prevented by the intake of antioxidants due to its ability in providing electrons to avoid undesirable damage to lipids, cell membranes, blood vessels and DNA. It also prevents other damages caused by reactive compounds, such as ROS and RNS (Ansori *et al.*, 2019; Husen *et al.*, 2019b). The amount of ROS produced by body cells can cause an imbalance between endogenous antioxidants and ROS levels in the cells. The occurring imbalance will decrease the endogenous antioxidant levels (Hayaza *et al.*, 2019b).

Indonesia has a high number of biodiversity which contains various natural potentials that can be utilized for the treatment of various diseases (Ansori *et al.*, 2018, 2020; Tacharina *et al.*, 2020). Okra (*Abelmoschus esculentus* Moench) is one of Indonesian plants that has huge possibility to be natural medication, such as diarrhea, cancer, hepatitis and contains great amount of antioxidant (Husen *et al.*, 2019a). Quercetin, which is a flavonoid compound of okra, has a great potential to overcome free radicals (Wahyuningsih *et al.*, 2018). This study expects the antioxidants to possess the ability in reducing oxidative stress, especially in numerous affected cells due to prolonged hyperglycemic conditions. Hence, this study was aimed at exploring the antioxidant potential of okra (*Abelmoschus esculentus* Moench) pods to overcome the high free radicals in DM patients so that it can be used to improve tissue sensitivity to insulin and ameliorate the damages of the islets of Langerhans in diabetic mice.

MATERIALS AND METHODS

Taxonomic identification of okra (*Abelmoschus esculentus* Moench) fruit was carried out by the Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia. This study made use of several drugs such as acarbose 100 mg (Dexa Medika, Indonesia), streptozotocin (Sigma-Aldrich, USA), and other chemicals such as lard to provide the hyperlipidemic conditions in mice, ethanol (Sigma-Aldrich, USA) for crude extraction and polar fraction of the okra pods extract, n-Hexan (Sigma-Aldrich, USA) for non-polar extraction of the okra pods, ethylacetate (Sigma-Aldrich, USA) for semi-polar extract of the okra pods, ketaminehydrochloride/xylazinehydrochloride solution (Sigma-Aldrich, USA) for animal anesthesia when taking the intra-cardiac blood and organs from experimental animals, Sodium carboxymethyl cellulose (Sigma-Aldrich, USA), 10% formalin buffer, citrate buffer (Sigma-Aldrich, USA), phosphate buffered saline (Sigma-Aldrich, USA) and Gomori's chrome alumhematoxylin-phloxinestain (Sigma-Aldrich, USA). All chemicals and reagents used were of the analytical grade.

There were more or less 20 kg of okra pods which were gained from one of the local markets in Surabaya, Indonesia. The extraction and fractions were based on Husen *et al.* (2019b). This study used adult mice, *Mus musculus* which were strain BALB/C, 3-4 months old, and having the body weight ranging from 30 to 40 g. They were obtained from the Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia. All treatment procedures were tested through Ethical Clearance at the Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia (Approval Reference Number : 2.KE.069.04.2018).

All body weight and blood glucose levels were recorded before and after the administration of lard and streptozotocin. Mice were divided into seven groups : KN (normal control), KD (diabetic control), KA (acarbose control; 100

mg/kg body weight), EK (crude extract; 100 mg/kg body weight), NP (non-polar; 20.04 mg/kg body weight), SP (semi-polar; 27.39 mg/kg body weight) and EP (polar; 54.11 mg/kg body weight). All groups were in control of environmental conditions (25±5°C, humidity of 50±10% and 12 light/dark cycle). Mice were fed with standard pellet and drink (*ad libitum*). For induction of diabetic mice, this study used streptozotocin. Fasting blood glucose levels were measured before and after streptozotocin induction on 7th and 14th day. Only mice with fasting blood glucose levels of more than 130 mg/dL were used as a diabetic group. Blood collection of mice was carried out on 15th day intracardially. Furthermore, the islet diameters were measured using calibrated micrometer. Data with normal distribution and homogenous variation were analyzed using one-way variance analysis continued with Duncan test. All statistical test was conducted at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The data of mice's two hours post-prandial blood glucose level, after VOPE treatments, are presented in Fig. 1. The Langerhans islet diameter data are presented in Fig. 2. The data of β-cells number are presented in Fig. 3. While the data of the HOMA-IR index are presented in Fig. 4. In addition, histology of pancreatic β-cells of islets of Langerhans is presented in Fig. 5.

The administration of streptozotocin was able to cause diabetic conditions in experimental animals. Free radicals were formed due to the presence of oxidants that enter the body of living things and antioxidants possessed the

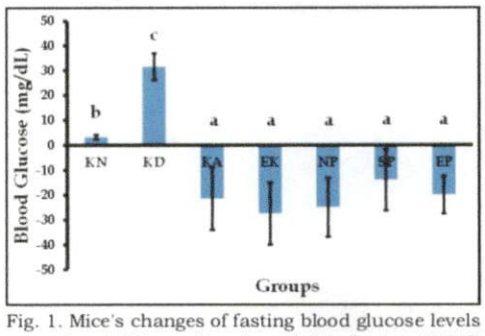

Fig. 1. Mice's changes of fasting blood glucose levels (mg/dL) on day 1 and 14 during VOPE administration. The different letters indicate a significant difference (α=0.05).

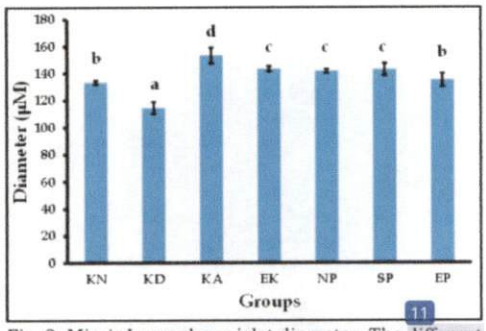

Fig. 2. Mice's Langerhans islet diameter. The different letters indicate a significant difference (α=0.05).

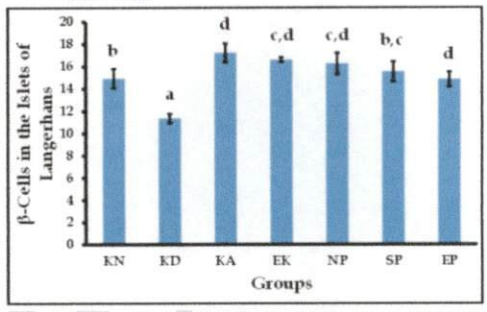

Fig. 3. The mean of β-cells number in the islets of Langerhans on day 15 during VOPE administration. The different letters indicate a significant difference (α=0.05).

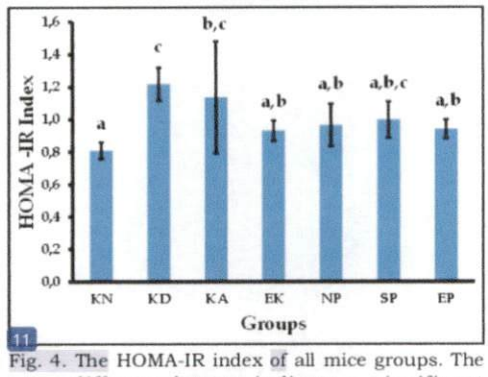

Fig. 4. The HOMA-IR index of all mice groups. The different letters indicate a significant difference (α=0.05).

ability to neutralize them (Purnamasari *et al.*, 2019). One of the oxidant compounds that often causes oxidative stress and increased blood glucose levels was streptozotocin. Streptozotocin was a bacterium that was toxic to pancreatic β-cells. Streptozotocin caused

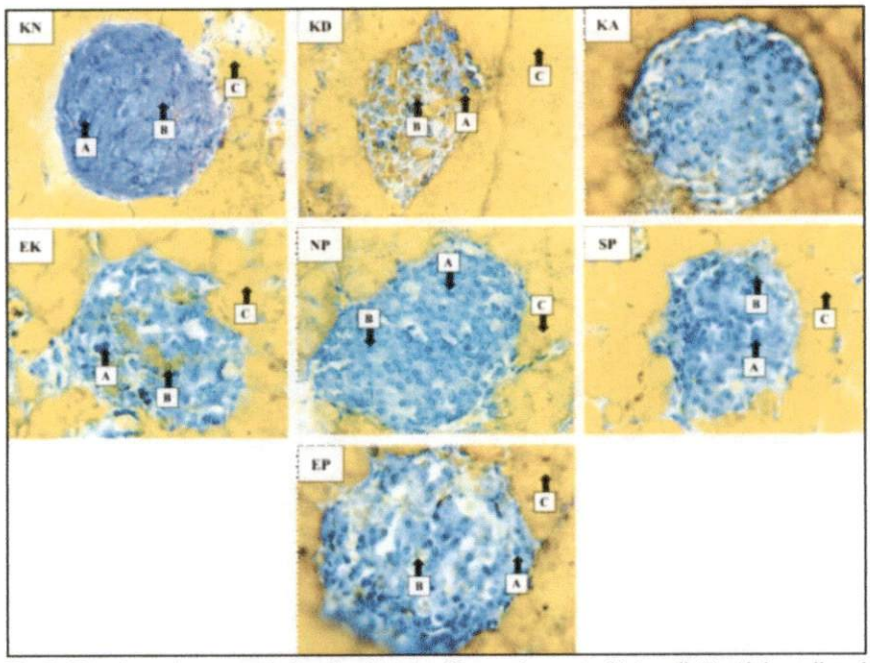

Fig. 5. Histology of pancreatic β-cells of islets of Langerhans. A : Beta cell, B : Alpha cell and C : Acinus cell. Magnification 400×.

DNA fragmentation in pancreatic β-cells so that it reduced cellular nucleotide and its components such as NAD^+ (Tacharina *et al.*, 2020). This condition led to pancreatic β-cells to necrosis (Hayaza *et al.*, 2019a). Fig. 1 shows that several doses of VOPE were able to act as antioxidants that overcame β-cells damage in the islets of Langerhans from the attack of free radicals coming from streptozotocin compounds (Husen *et al.*, 2019b).

The ability to reduce blood glucose levels performed by VOPE treatment groups was caused by the presence of antioxidant activity in okra fruits, both in the EK, NP, SP and EP groups. The increase of blood glucose levels was generally followed by an increase in the levels of free fatty acids, which resulted in superoxide production by mitochondria and increased the risk of the cell to be damaged by ROS. This increase in superoxide production resulted in an increase in nitric oxide (NO) caused by the induction of NO synthase enzymes. It interfered with the secretion and the function of insulin. This condition was characterized by the decrease of Langerhans islet diameters, so that it increased complications of DM itself (Mukherjee *et al.*, 2013).

Four keys to biochemical changes activated through oxidative stress conditions in hyperglycemia were the increased glucose metabolism through polyol pathways, the increased formation of advanced glycation end products (AGEs), the activation of protein kinase C (PKC) and the increased transport of excess glucose through hexosamine pathway. These biochemical changes further worsen the formation of free radicals which affected the occurrence of damages in the body of people with diabetes (Ceriello, 2011). During diabetic conditions, the increasing blood glucose levels contributed to the formation of AGEs and ROS production. Advanced glycation end products also produced ROS and both were associated with cell death, tissue damage, and renal dysfunction (Ansori *et al.*, 2019; Tacharina *et al.*, 2020). The hyperglycemic condition activated the polyol pathway and produced fructose from glucose. Fructose and its metabolites as well as glucose were involved in the non-enzymatic glycation of cellular proteins (Hayaza *et al.*, 2019a). In addition,

sorbitol passed through the cell membranes easily, accumulated and caused the osmotic damage to the cells (swelling) (Ansori *et al.*, 2019).

The data of the Langerhans islet diameter showed a major change among KN group and the other groups (Fig. 2). There had been a lot of damages to Langerhans β-cells in the KD group, which was characterized by a smaller diameter of the Langerhans islet. Further in the KA, EK, NP, SP and EP groups, there was a significant increase in Langerhans islet diameter. This showed that acarbose and VOPEs were able to repair β-cells Langerhans islet damaged by free radicals. VOPE containing exogenous antioxidants increased endogenous antioxidant levels so that it significantly improved the structure and function of the pancreatic gland. This was because VOPE contained flavonoid which in addition worked as an H atom donor. Flavonoids such as quercetin were able to bind to endogenous enzymes in cells so that these enzymes binded to cofactors (Zn or Cu) (Wahyuningsih *et al.*, 2018). This condition reduced ROS levels so that endogenous enzyme activity increased (Husen *et al.*, 2017).

The administration of VOPE significantly improved the β-cells of islets of Langerhans (Fig. 3). Four VOPE groups were significantly different compared to KD. Acarbose most effectively increased the number of β-cells of islets of Langerhans compared to other groups. Furthermore, Fig. 4 shows that the value of the HOMA-IR index calculation showed the comparison of insulin levels, fasting blood sugar levels, and HOMA-IR index values from each treatment group, after being given VOPE treatment, showing a significant difference between KN groups and other groups. The VOPE groups tended to experience improvement in fasting blood glucose levels, so the HOMA-IR index value differed significantly compared to KD group. Thus, it was inferred that the administration of VOPE reduced fasting blood glucose levels and insulin resistance to glucose in insulin-sensitive tissues (Husen *et al.*, 2017).

In this study, we revealed that administration of VOPE could increase the diameter and number of the β-cells in the islets of Langerhans (Fig. 5). We demonstrated that increasing the number of β-cells per mm^2 could increase the amount of serum insulin in diabetic mice so that the administration of VOPE could increase the diameter of the islets of Langerhans, the number of ?-cells of the islets of Langerhans per mm^2 and the level of serum insulin in diabetic mice (Hayaza *et al.*, 2019a, Husen *et al.*, 2019 b; Tacharina *et al.*, 2020). Our previous study showed that the administration of *Terminalia catappa* L. leaves crude extract could increase mice's tissue glucose tolerance, Langerhans islet diameter and also reduce the fasting blood glucose level (Hayaza *et al.*, 2019 a). In addition, Tacharina *et al.* (2020) stated that the non-polisaccharide fraction of the aquoeus extract of grinting grass (*Cynodon dactylon*) had high antidiabetic effect and hypoglicemic effect and increased the number of pancreatic β-cells of islets of Langerhans. Further, the aquoeus extract of grinting grass (*Cynodon dactylon*) was found to be more effective than metformin which is a standard antidiabetic drug.

CONCLUSION

In sum, it can be concluded that administration of VOPE had the ability to lower the level of blood glucose, increase the diameter and number of β-cells in the islets of Langerhans, and reduce tissue resistance to glucose in diabetic mice significantly.

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