The Effect of Alpha-mangostin in Glucose Level, Cholesterol Level and Diameter of the Islets of Langerhans of STZ-induced Diabetic Mice

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Abstract: The increasing prevalence of Diabetes Mellitus (DM) worldwide is an issue of major socio-economic concern. DM is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fat, and protein. Medicinal plants play an important role in the management of DM, especially in developing countries. The aim of this study is to investigate the antidiabetic and antilipidemic effects of alpha-mangostin in STZ-induced diabetic mice. We conducted the study using the male BALB/C mice. The mice were divided into two groups: the normal control (KN) and the STZ-induced diabetic mice. Streptozotocin (STZ) induction was performed using multiple low-doses of 30 mg/kg bw injected for five consecutive days. The diabetic mice were grouped again into three subgroups: diabetic control (KD), metformin HCL treated diabetic mice (KM), and diabetic mice which were treated using alpha-mangostin at 2 mg/kg bw (P1), 4 mg/kg bw (P2), and 8 mg/kg bw (P3). Before and after STZ injection, the blood glucose and the cholesterol levels were observed. The blood glucose and the cholesterol levels were also measured on the 1st, 7th, and 14th day of alpha-mangostin treatments. Treatment was given for 14 days. At the 15th day, the pancreases were collected and then processed into histological slides. The results of this experimental study indicated that alpha-mangostin has hypoglycemic and hypolipidemic activities which can ameliorate the damaged islets of Langerhans in STZ-induced diabetic mice. Therefore, we concluded that alpha-mangostin is a promising antidiabetic and antilipidemic agent due to its antioxidant activity.

1 INTRODUCTION

DM is a metabolic disorder that affects about 6% of the world population. DM is characterized by the prolonged hyperglycemic conditions due to the reduction of both insulin secretion and insulin sensitivity (Kang et al., 2014). DM is divided into type-1 DM (insulin dependent DM) and type-2 DM (non-insulin dependent DM). Type-1 DM is an autoimmune disease which causes the immune system to attack pancreatic cells, thus damaging a person's ability to produce insulin. Type-2 DM is a metabolic disorder that is characterized by an insulin resistance, a decrease of cell sensitivity to insulin, and a relative lack of insulin due to the damage suffered by pancreatic islet β-cells (American Diabetes Association, 2013). The decrease of cell sensitivity to insulin is a typical condition, as well as the cause of type-2 DM. Progressive decrease in insulin secretion is generally a result of decreased tissue sensitivity to insulin (McClung et al., 2004; Husen et al., 2017a; Husen et al. 2017b).

Beside the prolonged hyperglycemic conditions, one of the factors which causes DM is obesity due to the increased levels of fat in the body caused by hyperlipidemic and escalating levels of cholesterol in the blood (Husen et al., 2016; Husen et al., 2017a). The increased blood cholesterol levels can be followed by the dilated levels of free fatty acids, which generate the enlarged superoxide production by mitochondria and the higher risk of cell exposure by the reactive oxygen species (ROS). A growth in superoxide production will lead to an increase in nitric oxide (NO) caused by the induction of NO synthase enzymes. This condition leads to the

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production of reactive nitrogen species (RNS), which will oxidize the sulfhydryl groups of proteins, especially amino nitrate acids such as tyrosine, which can increase lipid peroxidation and cause a harmful DNA damage to cells (Novelli et al., 2010; Husen et al., 2017a).

The condition of hyperlipidemia in the people with obesity can boost the oxidative stress in the body, which can lead to some complications. People with obesity also experience heightened levels of cholesterol in the body (hypercholesterolemia) caused by excessive accumulation of fat in the body. One of the negative effects of obesity is the insulin resistance, which is the inability of insulin to produce the normal biological functions, which causes decreased tissue sensitivity to insulin. The cell resistance to cellular action of insulin is developed in people with obesity, which is characterized by the reduced ability of insulin to support the glucose intake in fat and muscle resulting in the hyperglycemic conditions (Husen et al., 2017a; Husen et al., 2017b). The condition of hyperglycemia leads directly to the increased levels of ROS and RNS. ROS and RNS can directly oxidize and destroy DNA, proteins, and lipids. High levels of ROS and RNS also can damage the macromolecules indirectly, which causes oxidative stress. Oxidative stress occurs when there is an imbalance between the number of highly reactive molecules (ROS and RNS) with the existing antioxidants (Novelli et al., 2010; Husen et al., 2018; Ansori et al., 2018).

Antioxidants are substances that can prevent the negative effects of free radicals by providing electrons to enable to suppress damages of lipids, cell membranes, blood vessels, DNA, and other damages caused by the reactive compounds, such as ROS and RNS. To reduce the occurrence of the free radical's effect, extra antioxidants from the outside (exogenous), such as vitamin E, vitamin C and other antioxidants obtained from consuming various types of fruits and vegetables that contain high antioxidants, are needed. One type of antioxidants which still provides a chance to overcome free radicals up until today is alpha-mangostin. The alpha-mangostin compound is a pigment of *Garcinia mangostana*, which is able to contribute hydrogen atoms and stabilize the free radicals by resonance, which is hard to participate in other radical reactions (Husen et al., 2018). In addition to neutralizing free radicals, antioxidants are expected to reduce oxidative stress, mainly in various cells affected by the prolonged hyperglycemic conditions, such as hepatocyte cells in the liver and renal tubular cells (Vallon, 2011; Ansori et al., 2018).

Indonesia has a high number of biodiversity, which contains various natural potentials that can be utilized for the treatment of various diseases (Wahyuni et al., 2017; Ansori et al., 2018). One of Indonesia's original flora which currently has great potential to be developed as a medicinal raw material is mangosteen (Ansori et al., 2018). The pericarp of the mangosteen fruit contains an active compound known as xanthone. Beside having anti-hypertensive and anti-inflammatory potential, xanthone compounds also play an important role as a powerful antioxidant compared to vitamin C and vitamin E in preventing free radicals and cell damage, as well as inhibiting cell degeneration processes (Chin et al., 2008). The xanthone compounds contained in the pericarp of the mangosteen, especially alpha-mangostin compounds, have been proven ameliorate the damaged pancreatic islet β-cells so that insulin can be produced optimally (Husen et al., 2017b; Husen et al., 2018). Based on the above background information, it has now been widely reported that the raw mangosteen pericarp extract is able to lower glucose and blood cholesterol levels. However, presently there has been no scientific explanation about the potential of alpha-mangostin to reduce blood glucose and cholesterol levels, as well as to ameliorate the pancreatic islet β-cells damages caused by the prolonged hyperglycemic conditions. Thus, this study was aimed to investigate the effects of alpha-mangostin in glucose level, cholesterol level, and diameter of the islets of Langerhans of STZ-induced diabetic mice.

2 MATERIALS AND METHODS

This study was conducted at the Animal Laboratory, Animal Histology Laboratory, and Molecular Genetics Laboratory, Faculty of Science and Technology, Universitas Airlangga. The ethical clearance for this study was obtained from the Committee of Animal Care and Use, Faculty of Veterinary Medicine, Universitas Airlangga (701-KE). The used samples were adult male mice of BALB/C strain aged 3-4 months with weights ranging from 30 to 40 g. The study materials consist of alpha-mangostin and STZ (purchased from Sigma), buffer citrate solution pH 4.5, phosphate-buffered saline (PBS), solvent extract of carboxymethylcellulose (CMC), standard antidiabetic drugs (Metformin HCl 100 mg/kg bw),

lard, xylazine and ketamine, and glucose (10% D-glucose in aquadest). The main tools used were mice cage made in plastic with lid of gauze wire, drinking bottle, feeding plate, husk, microscope, Petri dish, analytical scale, injection needles which have lead tackle at the end, 1 mL insulin injection needle for diabetic induction, Accu-Check® Active Test, EasyTouch® GCU Multi-Function Monitoring System, glass tools, and rotary vacuum evaporator (Buchi).

The study samples consisted of 24 male mice, distributed to the normal control group (KN) and diabetic group (induced by STZ). The fasting blood glucose and fasting blood cholesterol were measured before and after the STZ induction. The measurement of fasting blood glucose was performed on the 7th and 14th day after induction of STZ. Meanwhile, the measurement of blood glucose levels was performed using a glucometer to determine the diabetic condition of mice. Only the mice which had the fasting blood sugar level of more than 140 mg/dL were used as a diabetic mice group. The grouping of experimental animals was performed as follows: non-diabetic mice were used as the normal control group (KN), the diabetic mice induced by STZ were divided into 2 control groups, namely the diabetic control group (KD), the diabetic control group which was given Metformin HCl of dose 100 mg/kg bw (KM), and, the last one, was the alpha-mangostin treatment group. Furthermore, the alpha-mangostin treatment group was divided into 3 subgroups, which were P1 which was given 2 mg/kg bw, P2 which was given 4 mg/kg bw, and P3 which was given 8 mg/kg bw. All treatments were administered for 14 days.

The measurements of fasting blood glucose and fasting blood cholesterol levels were performed in all groups of mice before and after STZ induction, the measurement of which then continued on the 1st, 7th, and the 14th day of the alpha-mangostin treatment. The measurement of fasting blood glucose was done by using Accu-Check® Active Test, while the measurement of cholesterol levels was performed using EasyTouch® GCU Multi-Function Monitoring System. The measurements of fasting blood glucose and cholesterol levels were performed after the mice were fasted for 6-8 hours.

3 RESULTS AND DISCUSSION

The mean of mice's fasting blood glucose level and fasting blood cholesterol level data, before and after STZ induction, are presented in Figure 1. The mean of mice's fasting blood glucose and fasting blood cholesterol level data after alpha-mangoostin treatment is presented in Figure 2, while the mean data of diameter of the islets of Langerhans after the administration of alpha-mangostin is shown in Figure 3.

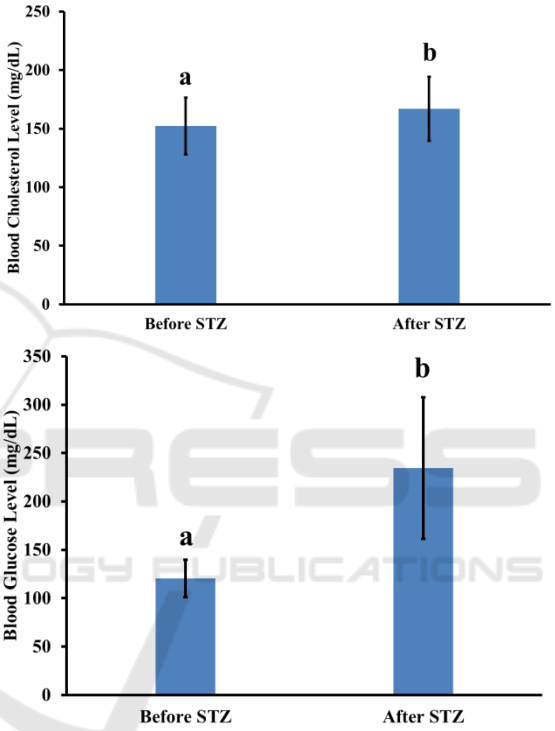

Figure 1: Blood glucose and cholesterol level (mg/dL) before and after STZ induction. The different letter indicated a significant difference.

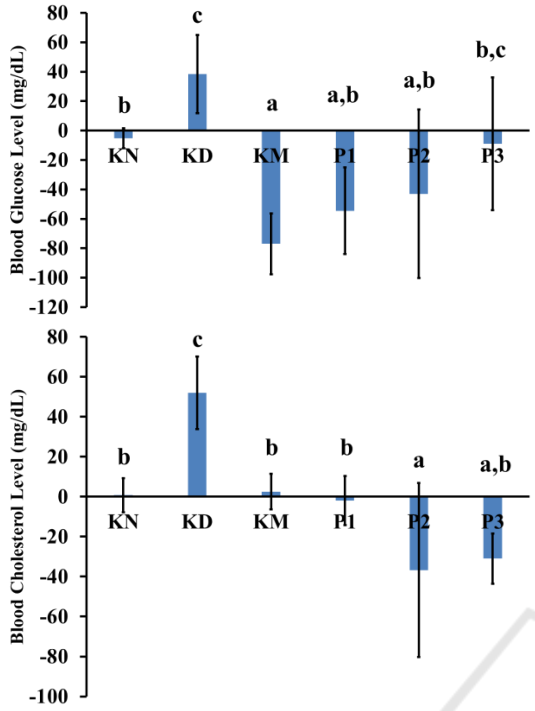

Figure 2: Blood glucose level and cholesterol level changes of each mice groups after treatments. The same letters above diagrams indicated an insignificant difference, while different letter indicated a significant difference. KN: normal control group; KD: diabetic group without metformin HCl; KM: diabetic group with metformin HCl 100 mg/kg bw; P1: diabetic treatment group with alpha-mangostin 2 mg/kg bw; P2: diabetic treatment group with alpha-mangostin 4 mg/kg bw; P3: diabetic treatment group with alpha-mangostin 8 mg/kg bw.

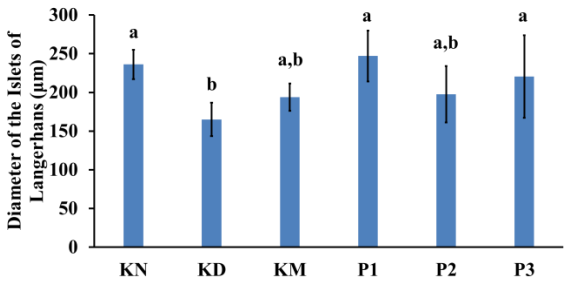

Figure 3: Diameter of the islets of Langerhans of each mice group after treatments. Same letters above the diagrams indicate insignificant difference, while different letters indicate significant difference. KN: normal control group; KD: diabetic group without metformin HCl; KM: diabetic group with metformin HCl 100 mg/kg bw; P1: diabetic treatment group with alpha-mangostin 2 mg/kg bw; P2: diabetic treatment group with alpha-mangostin 4 mg/kg bw; P3: diabetic treatment group with alpha-mangostin 8 mg/kg bw.

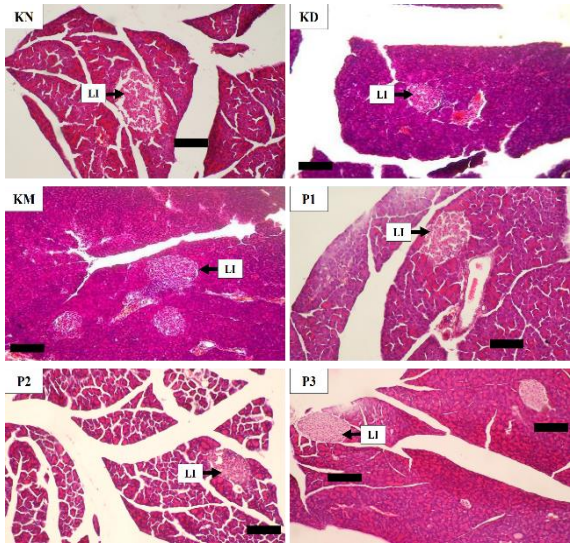

Figure 4: Histological structure of pancreatic gland and diameter of the islets of Langerhans of each mice group after treatments. KN: normal control group; KD: diabetic group without metformin HCl; KM: diabetic group with metformin HCl 100 mg/kg bw; P1: diabetic treatment group with alpha-mangostin 2 mg/kg bw; P2: diabetic treatment group with alpha-mangostin 4 mg/kg bw; P3: diabetic treatment group with alpha-mangostin 8 mg/kg bw; LI: diameter of the islet of Langerhans; Bar: 100 µm.

Lard was administered for 3 weeks to obtain hyperlipidemic condition within the mice. Hyperlipidemic state has a great chance to cause insulin resistance, so it is very easy to occur after administration of STZ. This study began with the administration of lard followed by STZ. The measurements of fasting blood glucose level and fasting blood cholesterol level (mg/dL) were done before and after the STZ injection. The data is presented in Figure 1, it shows that STZ injection with a dose of 30 mg/kg body weight for five constitutive days was able to significantly increase the mice's blood glucose levels significantly, from a mean blood glucose level of 120.625±19.354 to 183.500±39.419. STZ is a nitric oxide (NO) donor and NO was found to bring about the destruction of pancreatic islet cells and contribute to DNA damage in the cells (Kröncke et al., 1995). This condition indicates that STZ as an oxidant agent is capable of destroying pancreatic islet β-cells, which leads to the decrease of insulin production. Therefore, it generates an increased level of fasting blood glucose (Husen et al., 2016). Meanwhile, on the blood cholesterol levels, the STZ injection was also able to increase the mice's blood cholesterol level significantly from a mean of 152.40±24.294 before injection to 167.000±27.325 after injection. This

means that STZ is a highly reactive free radical which is able to increase ROS and RNS levels in cells, especially for insulin-sensitive cells and tissues such as the pancreatic gland. In the current work, diabetes was induced in laboratory mice via intraperitoneal injection of STZ. Streptozotocin (STZ) is considered to be toxic to insulin producing beta cells within pancreas, and thus it is widely used to induce experimental diabetes in laboratory animals (Aldahmash et al., 2015). Ansori et al. (2018) showed pathological changes in kidney of STZ-induced diabetic mice.

The increased levels of blood cholesterol after STZ injection caused by the prolonged hyperglycemic conditions were the result of the cellular damage of pancreatic islet β-cells and the decrease in insulin levels in blood. These conditions led to the increase of gluconeogenesis and lipolysis in striated muscle and fat tissues, as well as fat mobilization of adipose tissue, which caused the increased level of cholesterol in blood. The breakdown of fatty tissue both within the striated muscle cells and within the tissues of the body can lead to the increased levels of cholesterol in the blood (Husen et al., 2016; Husen et al., 2017a). In the prolonged hyperglycemic conditions, the administration of exogenous antioxidant compounds such as alpha-mangostin was expected to provide hope for ameliorating of the pancreatic islet β-cells damaged by free radicals, such as ROS and RNS.

Based on this study, it was found that the administration of alpha-mangostin antioxidants affects average fasting blood glucose levels in the diabetic mice. The dose of 2 mg/kg body weight had the highest response compared to the other treatment groups with doses of 4 and 8 mg/kg body weight. Those results showed a condition in which the lowest dose of the alpha-mangostin antioxidant active substance was able to provide a more positive response, compared to the larger dose groups. It has been proven that the antioxidant compounds of alpha-mangostin is a powerful antioxidant and has the ability to restore the homeostatic condition of glucose levels in the blood, called hormesis. Hormesis is a term in toxicology that demonstrates the phenomenon of response to the low doses stimulation and inhibition at the high doses and results in a curve formed of inverted J or U (Husen et al., 2017b).

The glucose cannot be processed into energy because of the hyperglycemic condition. Therefore, the energy must be made from other sources, such as fat and protein. Energy is obtained through the increased catabolism of protein and fat. Along with these conditions, there is a stimulation of lipolysis and the increase levels of free fatty acids and blood glycerol. This leads to the increasing production of acetyl-CoA by the liver, which in turn is converted to acetoacetic acid and ultimately reduced into β-hydroxybutyric acid or decarboxylated into acetone (Husen et al., 2016; Husen et al., 2017a). Due to the formation of energy from proteins and fats, the cholesterol levels formed in the chain of fat and protein metabolism increase. In patients with DM, hyperglycemic conditions lead to the increased production of ROS and RNS due to the increase of NADPH oxidation in endothelial tissue. ROS and RNS are highly reactive molecules that can directly oxidize and destroy DNA, proteins, and lipids and can cause an oxidative stress. An oxidative stress occurs when there is an imbalance between the number of highly reactive molecules (ROS and RNS) with the existing antioxidants (Husen et al., 2018; Ansori et al., 2018).

Interestingly, this study has proved that most diabetic mice have high cholesterol levels, as shown in the KD group, due to the interference of fat metabolism which causes high levels of acetate as one of the cholesterols formed in one reaction catabolism. Excessive energy sources lead to an excessive acetate formation, and fat in the body will increase as well. The increased fat metabolism causes the occurrence of abnormal fat metabolism with cholesterol deposits in the blood vessel wall. This condition can lead to an atherosclerosis and a decrease protein in the body. Various diseases are often associated with the increased cardiovascular risk parameters such as hypertriglyceridemia, hypercholesterolemia, and high-density lipoprotein (HDL) (Höhn et al., 2014).

4 CONCLUSIONS

We found that the administration of STZ can increase the fasting blood glucose levels and the fasting blood cholesterol levels in STZ-induced diabetic mice significantly. In addition, the administration of alpha-mangostin can reduce the average of fasting blood glucose level and fasting blood cholesterol level, as well as ameliorate the pancreatic islet β-cells damaged by STZ administration. Therefore, we concluded that alpha-mangostin is a promising antidiabetic and antilipidemic agent due to its antioxidant activity.

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