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Original Article

Activity test of various mangosteen (*Garcinia mangostana*) pericarp extract fractions to decrease fasting blood cholesterol levels and lipid peroxidation activity in diabetic mice

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Abstract

The objectives of this study were to determine the effect of various fractions of mangosteen (*Garcinia mangostana*) pericarp extract to the changes of the fasting blood cholesterol and serum malondialdehyde (MDA) levels on diabetic mice (*Mus musculus*). Thirty 3-4 months old male mice strain BALB/c, weight 20-30 g were divided into six groups. The first group was KN as a non diabetic control group, KD as a diabetic control, KM as a group of diabetic mice treated with metformin, and NP, SP, and P as the treatment groups that were treated by using three different fractions from mangosteen pericarp extract, non polar, semi polar, and polar respectively. The induction of Diabetes mellitus was done by the injection of STZ, and the mice were given a high fat diet treatment to induce the hiperlipidemia condition using lard for three weeks. The blood cholesterol levels were measured in all groups before and after the injection of lard, and day 1, 7, and 14 of treatment as well. The serum MDA level as the indicator of lipid peroxidation were measured by using QuantiChrom TBARS Assay Kit (DTBA-100). The data of cholesterol levels were statistically analyzed by t-test, while the data of serum MDA levels were analyzed by variance analysis followed by Duncan test. The results showed that the polar fraction of mangosteen pericarp had effect to decrease the fasting blood cholesterol level in mice, however the non polar and semi polar fraction had no simmilar effect. All of the fractions has shown significant effect to decrease the serum MDA level in mice.

Key words: cholesterol, diabetes mellitus, *Garcinia mangostana*, malondialdehyde (mda), obesity.

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Introduction

Diabetes mellitus (DM) is a multisystem metabolic, characterized by hyperglycemic due to the abnormalities of insulin secretion, (American Diabetes Association, 2011). These abnormalities resulting the aberration in the metabolism of carbohydrates, fats and proteins. Estimated, more than 360 million people is affected by DM worldwide (6% of the population). It is predicted that in 2025 the number of people with diabetes will increase by five-fold (Sharma *et al.*, 2007). According to the World Health Organization (WHO), by the year of 2030, the number people with diabetes mellitus in Indonesia will raise to 28.3 million. One of the main causes of diabetes is obesity. Indonesia is one of the countries with the quite high number of obese people, along with the increases of the houshold incomes and the lifestyles changing toward the less healthy one. Obesity is defined as an abnormal condition where there is an excessive accumulation of fat (hyperlipidemia) in the body which may lead to the health risks. Obesity is a major factor of many chronic diseases, such as diabetes mellitus, cardiovascular disease and cancer (WHO, 2013).

The condition of hyperlipidemia in obesity may increase the oxidative stress in the body which can lead to various complications. Obese people also have an increased levels of cholesterol (hypercholesterolemia) that caused by the accumulation of excess fat in the body (Nagao, 2005). One of the many negative effects of obesity is insulin resistance, which is the inability of insulin to generate biological functions normally (the decreases of tissues sensitivities to the insulin), which lead to the inability of the glucose to be transported into the cell, resulting the glucose can not be processed into energy, so the energy had to be made from other sources such as fats and proteins. The condition of hyperglycemia that occurs for a long period may lead to the alteration of the functions and metabolism of fat. As a result of the formation of the energy from proteins and fats, the cholesterols that is formed as the result of fat metabolism can accumulate and lead to various blood vessels related disesases. The process of atherosclerosis will affect almost all of the blood vessels, causing various complications. Insulin inhibits the hormone-sensitive lipase in adipose tissue, so the reduction of insulin may cause the increase of free fatty acid in blood plasma (Faisal Baraas, 2003).

The hyperglycemia condition has direct impact to an increase in levels of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). ROS and RNS may directly oxidize and damage the DNA, proteins, and lipids. High levels of ROS and RNS may also indirectly damage the macromolecules. Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) is highly reactive molecules which can damage and cause the oxidative stress. Oxidative stress occurs when there is an imbalance between the number of highly reactive molecules (ROS and RNS) and the antioxidants. The high amount of ROS will increase the expression of tumor necrosis factor (TNF-α) and worsened the oxidative stress. In many previous studies, it is shown that β cells

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dysfunction is a result of high levels of free fatty acids and glucose. β cells are very sensitive to ROS and RNS, it is because these cells lack of the enzyme of free-radical scavengers (antioxidants) like catalase and superoxide dismutase. These molecules are highly reactive (ROS and RNS), can oxidize sulfhydryl groups of proteins, amino acids such as nitric tyrosine, and increase the lipid peroxidation (Evans *et al*., 2003).

Antioxidants are substances that inhibit the negative effects of the free radicals by giving the electrons thus preventing the damage of lipids, cell wall membrane, blood vessels, DNA, and other damage caused by reactive compounds such as ROS. To reduce the negative effects of the free radicals, we need extra antioxidants from the outside (exogenous), such as vitamin E, vitamin C and other antioxidants obtained from consuming various kinds of fruits and vegetables that contain high antioxidants. Indonesia has the enormous wealth in terms of potential sources of natural medicines from natural ingredients.

Indonesia has a highly abundant biodiversity, which contain a wide range of natural potential that can be utilized for the treatment of many diseases. One of the indigenous flora of Indonesia that is currently has a great potential to be developed as a raw material for medicinal purposes is mangosteen. Mangosteen fruit is called as the "Queen of Fruits" because the mangosteen fruit is very useful to treat various kinds of diseases such as cancer, heart disease, arthritis, diarrhea, tonsillitis, dysentery, in addition to that the pericarp extract of the fruit is also has benefits as an anti-hypertension, anti-inflammatory, anti-microorganism, anti-diabetic, even anti-HIV (Nugroho, 2012). The pericarp of the mangosteen fruit contains an active compound known as xanthones. In addition to having the role as an anti-hypertension and anti-inflammatory, xanthone is also served as a very powerful antioxidant. The xanthones found in the pericarp of mangosteen, are expected to repair the pancreatic β cells that were damaged, so that insulin can be produced optimally and able to improve the sensitivity of skeletal muscle cells to insulin in type 2 diabetes.

Based on the problems mentioned above, until now there is no scientific explanation for the activity of various fractions extract of mangosteen pericarp extract, which can lower the fasting blood cholesterol levels, and serum MDA (malondialdehyde) levels which is associated with the decreased levels of lipid peroxidation in mice. Therefore it is necessary to do a reasearch about "The Activity Test of The Mangosteen (*Garcinia mangostana*) Pericarp Extract to Decrease The Fasting Blood Cholesterol Levels and Lipid Peroxidation Activity in Mice with Type 2 Diabetes".

Methods

This research is an experimental study that was conducted at the Laboratory of Reproductive Biology, the Laboratory of Organic Chemistry of the Faculty of Science and Technology, and the Institute of Tropical Diseases (ITD), Airlangga University. Thirty male mice aged 3-4 months strain BALB/c, weight 20-30 g were used in this study.

The mangosteen fruit (Garcinia mangostana, L), n-hexan, chloroform, ethanol, STZ (streptozotocin, SIGMA S0130-1G) to induce diabetes in mice, citrate buffer solution pH 4.5, CMC (carboxymethylcellulose) as the solvent of the extract, standard antidiabetic drugs (Metformin HCl 100 mg/kg), anesthesia (ketamine and xylasin), and 10% D-glucose for glucose tolerant test, glucometers On Call Plus^{TM}, blood cholesterol meter *Easy Touch^{TM}*, *QuantiChrom TBARS Assay Kit* (DTBA-100) were used in this study

The extraction of the mangosteen pericarp

The extracts were made by using the pericarp of the mangosteen that were macerated using three different kinds of solvent. The solvents used were n-hexane (nonpolar fraction), chloroform (semi-polar fraction), and ethanol (polar fraction). After the maceration, the pulp and the extract solution were separated by using a vacuum filter. The solvent was evaporated using a rotary vacuum evaporator at 50 °C. Furthermore, each of the extracts were dried using a freeze dryer.

The induction of diabetes type 2 in mice

The mice were induced with lard for 3 weeks with a dose of 0.3 ml orally prior to the induction of STZ. This stage was intended so that the mice were in a condition of a high fat diet. Mice were induced with diabetic condition using a multiple low dose streptozotocin (STZ) that is expected to induce type II diabetes as described at Novelli *et al (*2010).

The administration of mangosteen pericarp extract

The mice were divided into six groups. The first group was KN as a non diabetic control group, KD as a group of diabetic mice that was not treated by either mangosteen pericarp extract or metformin, KM as a group of diabetic mice that was treated by metformin (100 mg/kg BW), and NP, SP, and P as the treatment groups that were treated by using three different fractions from mangosteen pericarp extract, non polar, semi polar, and polar respectively. The fractions were given orally for 14 days with the dose equivalent to 100 mg/kg BW of crude extract.

Blood cholesterol and serum MDA levels measurement

The blood cholesterol levels were measured in all groups before and after the administration of lard, also on the day 1, 7, and 14 of treatment as well. The serum MDA level as the indicator of lipid peroxidation were measured by using QuantiChrom TBARS Assay Kit (DTBA-100).

Data Analysis

The data of the fasting blood cholesterol levels before and after the administration of lard were statistically analyzed by t-test, while the data of the fasting blood cholesterol and serum MDA levels that had normal distribution and a homogenous variances were analyzed by variance analysis followed by Duncan test ($\alpha = 0.05$). Pearson correlation test was used to determine the relationship between fasting blood cholesterol levels wand the levels of serum malondialdehyde (MDA) ($\alpha = 0.05$).

Results

The data of the body weight, fasting blood cholesterol levels before and after the administration of lard is shown in figure 1 and 2. The result of the fasting blood cholesterol and the serum MDA levels after the administration of the various fractions of the mangosteen pericarp extract is shown in figure 3 and 4.

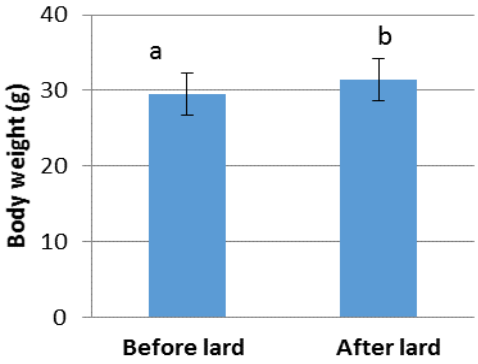

Figure 1. Diagram showing the effect of lard to changes in body weight (g) in diabetic mice. The letters located above the diagram of each group shows the results of t-test at α = 0.05. Different letters indicate a significant differences.

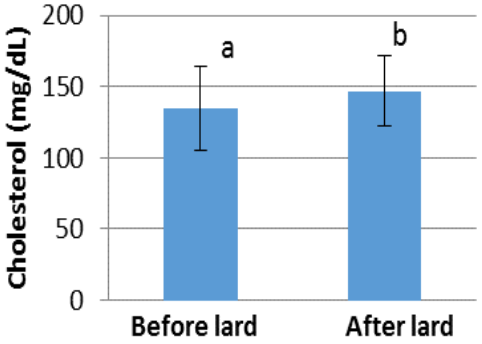

Figure 2. Diagram showing the effect of lard to changes in fasting blood cholesterol levels (mg / dL) in diabetic mice. The letters located above the diagram of each group shows the results of t-test at α = 0.05. The same letters indicate no significant differences and different letters indicate a significant differences.

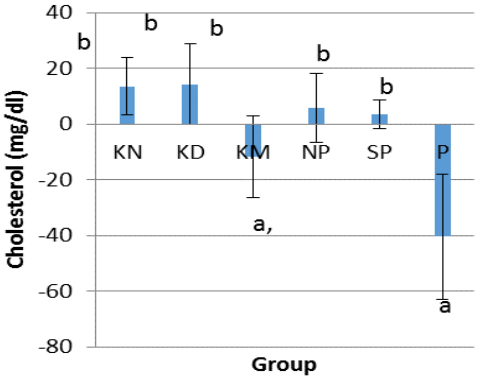

Figure 3. Diagram showing the effect of various fractions of mangosteen pericarp extract to changes in fasting blood cholesterol levels in diabetic mice. The same letters indicate no significant differences and different letters indicate a significant differences α = 0.05.

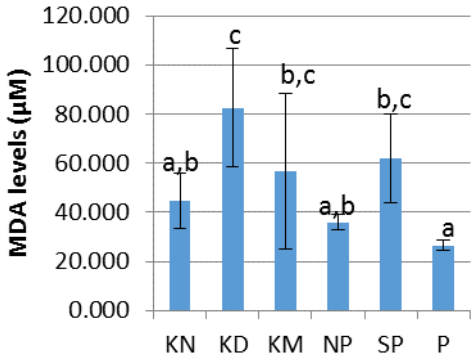

Figure 4. Diagram showing the effect of various fractions of mangosteen pericarp extract to changes in serum MDA levels in diabetic mice. The same letters indicate no significant differences and different letters indicate a significant differences α = 0.05.

Discussion

The data of body weight were statistically analyzed by t-test, the result showed that the lard administration was able to increase the body weight in mice significantly. Therefore, the administration of lard is capable of causing obesity which is characterized by an increase in body weight of mice. Obesity is an abnormal condition where there is an excessive accumulation of fat in the body which may lead to many chronic diseases, such as diabetes mellitus, cardiovascular disease and cancer. The main cause of obesity is the excess of the energy intake that does not comply with the energy expenditure over the long term. The data of cholesterol levels before and after the administration of lard were statistically analyzed by t-test, the result showed that the lard administration was able to increase increase the cholesterol levels in mice significantly.

From the result of the body weight, it is proved that the administration of lard for 21 days was able to increase the body weight of the mice from 29.5 ± 2.7 g before the administration of lard to 31.4 ± 2.8 g after the administration of lard. The administration of lard was also increase the blood cholesterol levels of the mice from 134.9 ± 29.4 mg/dL to 146.8 ± 24.8 mg/dL after the administration of lard. Obese people have hypercholesterolemia that caused by the accumulation of excess fat in the body (Nagao, 2005). One of the many negative effects of obesity is insulin resistance, which is the inability of insulin to generate biological functions normally. They will develop a resistance to the cellular actions of insulin that is characterized by a reduced ability of the insulin to support glucose uptake in fat and muscle tissues, leading to a prolonged condition of hyperglycemia (Husen and Winarni, 2014).

After 14 days of the experiment, the normal control group (KN) and the diabetic control group (KD) showed an increase levels of fasting blood colesterol compared to the diabetic-metformin group (KM) that showed a decrease levels of fasting blood cholesterol. While in the mice group treated with the fraction of non-polar and semi-polar (NP and SP) extract of the mangosteen pericarp, both treatment groups showed an increase in fasting blood cholesterol levels, that is simmilar to the increase in the normal control group. The increase levels of the fasting blood cholesterol in the normal control

group, was presumably beacause of the increase stress as the result of the administration of CMC, thus affecting the fasting blood cholesterol levels when measured. The increase of the fasting blood cholesterol levels in non polar and semi polar fractions treated group showed that the non polar and the semi polar fractions of the mangosteen pericarp extract were not able to reduce the cholesterol levels in diabetic and hiperlipidemia mice (Husen and Winarni, 2013).

From the result of this study, it can be showed that most of the people with diabetes tend to have raised cholesterol levels as can be seen in the diabetic control group (KD), as a result of their fat metabolism disorder that causing the high levels of acetate in their body that is one of the precursor of cholesterol that formed in catabolism reaction. This is in line with the opinion of Corwin (2009) that the excess amount of energy sources can lead to high levels of acetate and the accumulation of the body fat. The increase of the fat metabolism lead to abnormal fat metabolism accompanied with the cholesterol deposits in the walls of blood vessels causing the symptoms of atherosclerosis and reduced protein in the body. A wide range of diseases is often associated with increased cardiovascular risk parameters such as hypertriglyceride, hypercholesterolemia and low high-density lipoprotein (HDL) (Garber, 2002).

The serum MDA levels is shown in figure 4. Differences between the mean of each treatment could be determined after the Duncan test. Based on the Duncan test of the mean of serum MDA levels of mice between treatment groups, it is showed that the KN group was significantly different when compared to KD, but it was not significanly different when compared to KM, NP, SP and P, KN groups. This indicates that the serum MDA levels in KD group was increased significantly. All of the fractions has shown significant effect to decrease the serum MDA level in mice.

From the result of this study, it is showed that the induction of the STZ were able to increase the ROS and RNS levels in the diabetic control group mice which is indicated by the increase levels of the serum MDA. In the diabetic control group the mice showed the signs hyperglycemic that is characterized by an increase in fasting blood glucose levels in this group. This result is in line with the opinion of Powers and Jackson (2008), that the hyperglicemia condition may lead to an increase in reactive oxygen species (ROS) and reactive nitrogen species (RNS), due to the increased oxidation of NADPH on endothelial tissue. ROS and RNS may directly oxidize and damage the DNA, proteins,lipids, and can cause the oxidative stress.

The free radicals such as ROS and RNS will cause the lipid peroxidation of the cell membranes. These lipid peroxides will be formed in the longer chain and may damage the cell membrane organization. This peroxidation will affect the membrane fluidity, cross-linking of the membrane, as well the structure and function of membrane (Powers and Jackson, 2008). There is products that were produced as a result of lipid peroxidation such as malondialdehyde (MDA), 4-hydroxy-2-noneal (HNE), 4-hydroxy-2-hexenal (4-HHE) (Catala, 2006). MDA is one of the final product of peroxidation of polyunsaturated fatty acid (PUFA) in the cell. The concentration of MDA can be used as an indicator of cell or tissue damage due to the increased activity of lipid peroxidation.

Antioxidants are substances that inhibit the negative effects of the free radicals by giving the electrons thus preventing the damage of lipids, cell wall membrane, blood vessels, DNA, and other damage caused by reactive compounds such as ROS. To reduce the ROS and RNS negative effect, the antioxidants can be acquired trough the food. We need extra antioxidants from the outside (exogenous), such as vitamin E, vitamin C and other antioxidants obtained from consuming various kinds of fruits and vegetables that contain high antioxidants such as mangosteen. The pericarp of the mangosteen contains several compounds with the pharmacological activity, for example anti-inflammatory, antihistamine, antibacterial, antifungal and antidiabetic. The key compound that plays a role in antioxidant activity is known xanthones that are found mainly in the pericarp of the mangosteen fruit.

It can be informed from this study that the administration of lard orally was able to increase the body weight and the fasting blood cholesterol levels of the mice. The results showed that the polar fraction of mangosteen pericarp had effect to decrease the fasting blood cholesterol level in mice, however the non polar and semi polar fraction had no simmilar effect. All of the fractions has shown significant effect to decrease the serum MDA (malondialdehyde) level in mice with type 2 diabetes, and there is a positive correlation between the fasting blood cholesterol level and the serum MDA levels.

From the results of this study it is advisable to do an outreach to the community about the benefits of the mangosteen pericarp extract to reduce the negative effects of the wide range of degenerative and metabolic diseases, especially diabetes mellitus.

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