RESEARCH ARTICLE | SEPTEMBER 21 2017

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AIP Conf. Proc. 1888, 020026 (2017)
https://doi.org/10.1063/1.5004303

Activity Assay of Mangosteen (*Garcinia mangostana* L.) Pericarp Extract for Decreasing Fasting Blood Cholesterol Level and Lipid Peroxidation in Type-2 Diabetic Mice

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Abstract. This study aimed to explore the activity of pericarp extract of mangosteen (*Garcinia mangostana* L.). Mangosteen pericarp contains various active compounds which are beneficial for human health. In-vivo antioxidant assay of pericarp extract was carried out using 3-4 month male mice of strain BALB/c weighed 30-40 g. The mice were divided into two groups: normal control (KN) group and STZ-induced diabetic group. STZ induction was performed using multiple low-dose method 30 mg/kg body weight treated daily for five consecutive days. Diabetic group was separated into two subgroups: diabetic control (KD), metformin control (KM), and crude extract treatment subgroups. The fasting blood glucose and the cholesterol level were measured before and after lard treatment, we also did it on the first, seventh, and fourteenth day of mangosteen pericarp crude extract treatment. The mice were treated with mangosteen pericarp crude extract for 14 days. The MDA level of the fasting blood serum was measured. The body weight and fasting blood cholesterol level before and after lard treatment were analyzed by t-test, whereas, the fasting blood cholesterol and the MDA level were analyzed using one-way variant analysis continued with Duncan test. The correlation between the increasing body weight and the fasting blood cholesterol level was determined by Pearson correlation test. The results of the study showed that the administration of mangosteen pericarp crude extract was able to reduce the fasting blood cholesterol and the malondialdehide level significantly.

INTRODUCTION

Diabetes mellitus is a multisystemic metabolic disorder featured by hyperglycemia caused by abnormality of insulin secretion, approximately six percent of the world population [1]. Diabetes mellitus (DM) can be classified into type-1 and type-2 diabetes mellitus [2]. One of the major causes of diabetes is obesity [3]. Obesity is defined as an abnormal condition where there is an accumulation of excessive fat in the body which can lead to health risks [4]. According to WHO, there are 42 million children under five years and 1.4 billion adults that are overweight and 500 million of them are obese [3]. The condition of hyperlipidemia in obesity may increase the oxidative stress in the body which can lead to various complications. Obese people also have high levels of cholesterol (hypercholesterolemia) caused by an accumulation of excessive fat in the body. One of the many negative effects of obesity is insulin resistance, which is the inability of insulin to generate biological functions normally. Obese people will develop a resistance to the cellular actions of insulin. It is characterized by a reduced ability of the insulin to support glucose intake in fat and muscle, leading to a condition of prolonged hyperglycemia [5].

Hyperglycemia condition has a direct impact to the increase levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS are highly reactive molecules that can cause oxidative stress. 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. Oxidative stress occur when there is an imbalance between the number of highly reactive molecules (ROS and RNS) and the antioxidants. The high levels of ROS will increase the expression of tumor necrosis factor (TNF-α) and aggravate the oxidative stress [6]. In many previous studies, it has been shown

6th International Conference and Workshops on Basic and Applied Sciences
AIP Conf. Proc. 1888, 020026-1–020026-6; https://doi.org/10.1063/1.5004303
Published by AIP Publishing. 978-0-7354-1571-3/$30.00

that β-cells dysfunction results from high levels of free fatty acids and glucose. β-cells are very sensitive to ROS and RNS because they do not have much free-radical scavenging enzyme, such as catalase and superoxide dismutase. These molecules (ROS and RNS) are highly reactive, can oxidize sulfhydryl groups of proteins, amino acids such as nitric tyrosine, and increase the lipid peroxidation [2].

Antioxidants are substances that inhibit the negative effects of the free radicals by acting as an electron donor, and thus preventing the damage of lipids, cell wall membrane, blood vessels, DNA, and other damage caused by reactive compounds, such as ROS and RNS. To reduce the negative effects of the free radicals, the extra antioxidants from the outside (exogenous), such as vitamin E, vitamin C, and other antioxidants obtained from consuming various kinds of fruits and vegetables containing high antioxidants can be beneficial [Evans]. Indonesia has enormous potential sources of natural medicines from natural ingredients. Indonesia is estimated to have about 30,000 species of plants and more than 7,000 species are known to be medicinal plants [7].

One of the indigenous plants of Indonesia that has a great potential as a raw material for medicine is mangosteen. Mangosteen is a fruit that comes from the Southeast Asian region, especially Indonesia, Malaysia, Thailand and Myanmar. Mangosteen fruit has been dubbed as the "Queen of Fruits" due to its ability to treat various kinds of diseases such as cancer, heart disease, arthritis, diarrhea, and dysentery. Also the pericarp extract of the fruit is also beneficial as an anti-hypertension, anti-inflammatory, anti-microorganism, anti-diabetic, even anti-HIV [8]. The pericarp of the mangosteen fruit contains active compounds known as xanthones. Xanthone is a very powerful antioxidant. Xanthones found in the pericarp of mangosteen, can repair damaged pancreatic β-cells, so insulin can be produced optimally and it may be able to improve the sensitivity of skeletal muscle cells to insulin in type-2 diabetes [9].

The objectives of this study were to prove that the administration of lard orally may increase the body weight and fasting blood cholesterol levels in mice, and to prove that the administration of the crude extract of mangosteen pericarp can decrease blood cholesterol levels and malondialdehyde (MDA) levels in blood serum of type-2 diabetic mice. The results of this study are expected to give information about the potential of the local natural resources to help people with diabetes. The information may be useful for increasing economic value of the tropical fruits, especially the mangosteen fruit.

MATERIALS AND METHODS

This study was an experimental study which was conducted at the Laboratory of Reproductive Biology, Faculty of Science and Technology, and the Institute of Tropical Diseases (ITD), Universitas Airlangga. The samples of the study were 24 male mice of strain BALB/c, aged 3 – 4 months and weighed 25 – 40 grams. The materials used in the study include crude pericarp of mangosteen (*Garcinia mangostana* L), streptozotocin (SIGMA, cat. S0130-1G), lard, citrate buffer solution pH 4.5, CMC (carboxymethylcellulose) as extract solvent, standard antidiabetic drugs (Metformin HCl 100 mg/kg), and anesthesia (ketamine and xylasin).

The extracts were made by using the pericarp of the mangosteen macerated using the 96% ethanol as solvent. After the maceration, the pulp and the extract solution were separated using a vacuum filter. The solvent was evaporated using a rotary vacuum evaporator at 50° C. Each of the extracts was then dried using a freeze dryer.

Next, the mice were induced orally with lard for three weeks with a dose of 0.3 mL before the induction of STZ. This stage was done to make the mice in a condition of a high-fat diet. The mice were induced with diabetic condition using a multiple low-dose of streptozotocin (STZ) that was expected to induce type-2 diabetes. The twenty four mice were later divided into six groups consisted of four mice respectively: KN as a non diabetic control group; KD as a group of diabetic mice (fasting blood glucose >170 mg/dL) 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 of BW); and P1, P2, and P3 as the treatment groups that were treated by using three different concentrations of the mangosteen pericarp crude extracts, 50 mg/kg BW, 100 mg/kg BW, and 200 mg/kg BW respectively. The extracts were given orally for 14 days with a dose referring to the results of the toxicity tests with LD_{50} = 630.95 mg/kg BW [11].

The blood cholesterol levels and the body weight were measured in all groups before and after the administration of lard, as well as on day one, day seven, and day fourteen of the crude extracts treatment. The blood

cholesterol levels were measured by using the blood cholesterol meter Easy Touch^{TM}. The serum MDA level as the indicator of lipid peroxidation was measured by using QuantiChrom TBARS Assay Kit (DTBA-100).

RESULTS AND DISCUSSION

The results of measurements of the body weight, the fasting blood cholesterol levels before and after the administration of lard are shown in figure 1. Meanwhile, the results on the body weight and the fasting blood cholesterol levels after the administration of the mangosteen pericarp extract on day one, day seven, and day fourteen are shown in figure 2. In addition, the results of the serum MDA levels after the administration of the mangosteen pericarp extract on day fourteen are shown in figure 3.

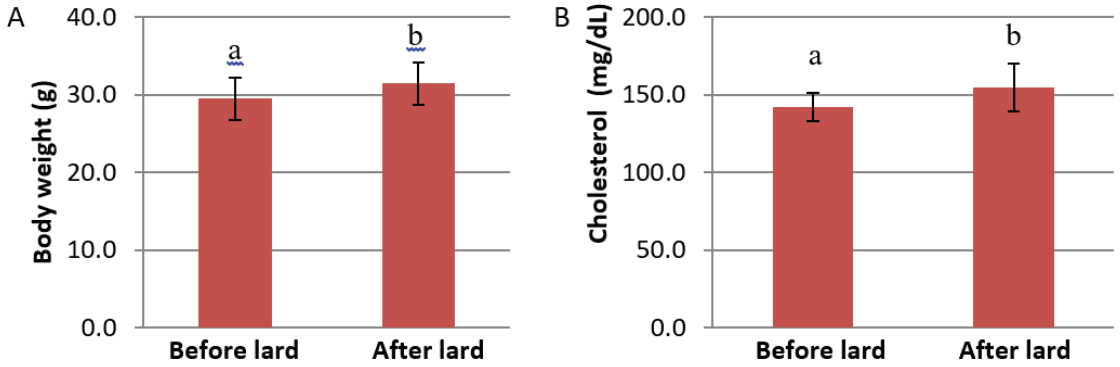

FIGURE 1 The diagram showing the effects of lard on the changes of the (A) body weight (g) and (B) the fasting blood cholesterol levels (mg/dL) in the diabetic mice. The letters located above the diagram of each group show the results of t-test at $\alpha = 0.05$. Different letters indicate a significant difference.

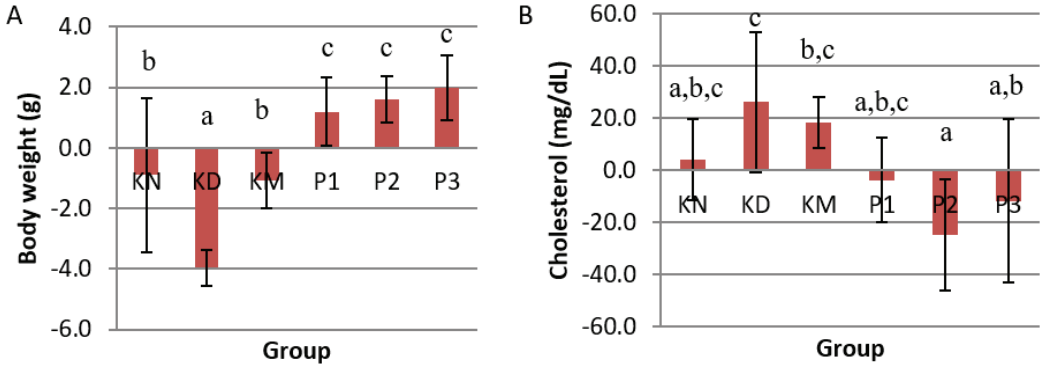

FIGURE 2 The diagram showing the effects of the mangosteen pericarp extract on the changes of (A) the body weight (g) and (B) the fasting blood cholesterol levels (mg/dL) in the diabetic mice. KN is a non-diabetic control group, KD is a group of diabetic mice (fasting blood glucose >170 mg/dL) that was not treated by either mangosteen pericarp extract or metformin, KM is a group of diabetic mice that was treated by metformin (100 mg/kg of BW), and P1, P2, and P3 are the treatment groups that were treated by using three different concentrations of the mangosteen pericarp extracts 50 mg/kg BW, 100 mg/kg BW, 200 mg/kg BW respectively. The same letters indicate no significant differences and the different letters indicate a significant difference of $\alpha = 0.05$.

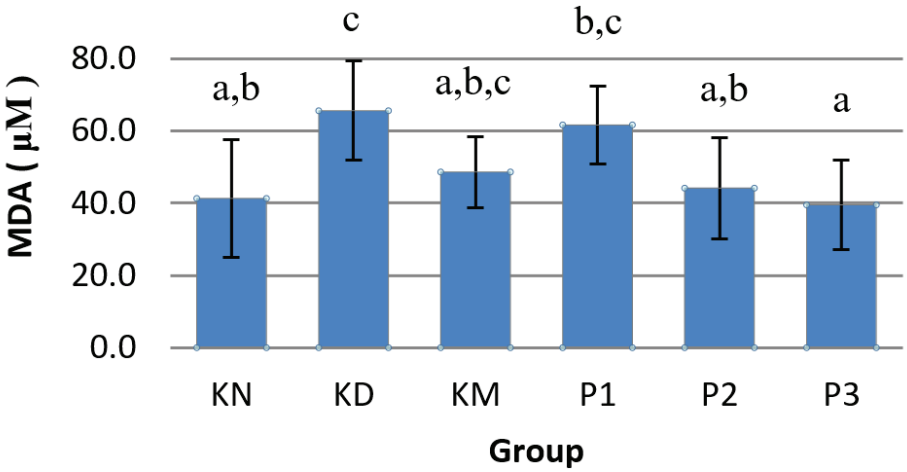

FIGURE 3 The diagram showing the effects of the mangosteen pericarp extract on the serum MDA levels in the diabetic mice. KN is a non-diabetic control group, KD is a group of diabetic mice (fasting blood glucose >170 mg/dL) that was not treated by either mangosteen pericarp extract or metformin, KM is a group of diabetic mice that was treated by metformin (100 mg/kg of BW), and P1, P2, and P3 are the treatment groups that were treated by using three different concentrations of the mangosteen pericarp extracts 50 mg/kg BW, 100 mg/kg BW, 200 mg/kg BW respectively. The same letters indicate no significant differences and the different letters indicate a significant difference of $\alpha = 0.05$.

The results of the measurement on the body weight and the fasting blood cholesterol indicate that there were statistically significant differences before and after the administration of lard. The administration of lard for 21 days was able to increase the body weight of the mice from 29.4875 ± 2.7372 g before the administration of lard to 31.4458 ± 2.8042 g after the administration of lard. The fasting blood cholesterol levels also increased from 141.9583 ± 9.1864 mg/dL before the administration of lard to 154.6250 ± 15.1939 mg/dL after the administration of lard. The obesity is characterized by an increase in the body weight. Obesity is an abnormal condition where there is an excessive accumulation of fat (hyperlipidemia) along with an increased level of cholesterol (hypercholesterolemia). One of the many adverse 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 [10].

The diabetic group of KD and KM showed a decrease in the body weight with the average of 3.95 g for KD group, and 1.08 g for KM (metformin treated) group. The decrease of the body weight is one of the characteristics of diabetes. However, all of the groups that were treated with the crude extract showed a positive increase in the body weight. It proves that mangosteen pericarp extract is able to increase the body weight of diabetic mice significantly [11].

After 14 days of the experiment, the diabetic control group showed an increase level of the fasting blood cholesterol. It means that most diabetic people tend to have high cholesterol levels. As it can be seen in the diabetic control group (KD), the fat metabolism disorder has resulted in the high levels of acetate in the body which is one of the precursors of cholesterol formed in catabolism reaction. This is in line with the opinion of Corwin [12] that the excessive amount of energy sources can lead to the high levels of acetate and the accumulation of fat in the body. The increase of fat metabolism leads to an abnormal fat metabolism accompanied with cholesterol deposits in the walls of blood vessels causing atherosclerosis symptoms 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).

The concentration of the serum MDA can be used as an indicator of the oxidative stress. The products produced as a result of lipid peroxidation are MDA, 4-hydroxy-2-noneal (HNE), 4-hydroxy-2-hexenal (4-HHE). MDA is one of the final products of the 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 [12].

The results of this study showed that the induction of the STZ was able to increase the ROS and RNS levels in the diabetic control group mice that were indicated by the increase levels of the serum MDA [13]. In the diabetic

control group, the mice showed the signs of hyperglycemic that were characterized by the increase in the fasting blood glucose levels in this group. This result is in line with the opinion of Powers and Jackson [14] that the hyperglycemia condition may lead to an increase in ROS and RNS due to the increased oxidation of NADPH on endothelial tissue. Reactive oxygen species and RNS may directly oxidize and damage the DNA, proteins, lipids, and can 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. When the oxidative stress occurs, the antioxidant produced by the body is inadequate to eliminate the negative effects of the free radicals. This condition destabilizes the normal oxidation-reduction chain, leading to oxidative damage to tissues. Many reports from the previous studies showed that β-cells dysfunction had resulted from high levels of free fatty acids and glucose. β-cells are very sensitive to ROS and RNS because these cells are lack of free-radical scavenging enzymes (antioxidants) like catalase and superoxide dismutase [6]. This tissue damage also depends on several factors, such as the molecular targets, the level of stress that occurs, the mechanisms that involved, as well as the timing and the nature of the system being attacked [15].

From this study, it can be concluded that the administration of lard can increase the body weight and the fasting blood cholesterol levels in mice significantly and the administration of the mangosteen pericarp extract can re-increase the body weight of mice that has been decreased as result of the STZ-induced diabetic significantly. The mangosteen pericarp extract is also able to decrease the fasting blood cholesterol levels in diabetic mice significantly. From the results of this study, it is advisable to do outreach to the community about the benefits of the mangosteen pericarp extract to reduce the negative effects of the wide range of metabolic diseases, especially diabetes mellitus. In addition, it is also necessary to do outreach to the community about the potential of the mangosteen as the traditional medicine, which has been proven more effective and more efficient in fighting free radicals compared to vitamin C and vitamin E.

ACKNOWLEDGMENT

This work was supported and funded by Direktorat Riset dan Pengabdian Masyarakat, Direktorat Jenderal Penguatan Riset dan Pengembangan, Kementerian Riset, Teknologi, dan Pendidikan Tinggi, Republik Indonesia. The author would like to thank Theo Herdika Putra, S.Si. and R. Joko Kuncoroningrat Susilo, S.Si. who have given much help in this study.

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