

The Potential of Rambutan Seed Extract to Reduce Risk of Cardiovascular Disease in Diabetes Mellitus Type 2

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Abstract. This study aimed to find out the potential of ethanol extract in rambutan seed to reduce the risk factors of cardiovascular disease by using animal model on diabetes-type 2 (DM-2). The animals used for this study were 3-4 months old male wistar mice with 20-25 grams weight. The diabetes mellitus induction type 2 to the animal model was performed through the administration of high fat diet for 4 weeks followed by the daily dosage of Streptozotocin (STZ) 40 mg/kgbw and it was repeated for 4 times in the last week of induction. The administration of 70% ethanol extract of Binjai rambutan seed (*Naphelium lappaceum L.*) was done in Carboxy Methyl Cellulose 0.5% and metformin as positive control. The result indicated that 70% ethanol extract of Binjai rambutan seeds had a significant effect on the atherosclerosis index ($p < 0,01$) and all lipid profile parameters. The Binjai rambutan seed extract at 19.2 mg/kgbw dose was the most effective to decrease the atherosclerosis index, total cholesterol, triglyceride, low density lipoprotein, and malodialdehyde and to increase high density lipoprotein and super oxide dismutase of mice blood serum. It can be concluded that the rambutan seed extract at the dose of 19.2 mg/kgbw had the potential to reduce the risk of cardiovascular diabetes in mice by increasing lipid fraction and decreasing oxidative stress.

Keywords: atherosclerotic index, binjai rambutan seed (*Naphelium lappaceum L.*), diabetes mellitus, lipid profile

INTRODUCTION

Many cases of cardiovascular diseases in most developing countries including Indonesia cause morbidity and mortality and it requires substantial amount of treatment. Cardiovascular disease is non-Communicable Diseases (NCDs) that causes the main death in South East Asia. NCDs data profile in 2014

showed that 71% of 1,551,000 deaths in Indonesia were attributed to NCDs. Cardiovascular Disease (CVDs) was the biggest leading cause of death among 4 major NCDs with 37% of deaths followed by cancer (13%), DM (10%) and Chronic Respiratory Disease (CRD) was by 5% [1]. Chronic DM is also a source of various risk factors causing CVDs either traditional or non-traditional risk factors such as hypercholesterolemia, hypertension, insulin resistance and hyperglycemia.

The DM-2 patient prevalence dominates the cases of DM, approximately 80% of the DM cases. DM-2 is a disease caused by metabolic disorders which are characterized by symptoms of hyperglycemia because of the insulin resistance. Glucose limitation in the cells caused by the insulin resistance and leads to a disturbance on the lipid metabolic balance in most of human body tissue. Insulin resistance in fatty tissues obstructs lipogenesis and increases lipolysis. This will trigger glucotoxicity followed by lipotoxicity and leads to the increase of risk factors for cardiovascular disease, for example the increase of total cholesterol (TC), triglyceride (TG), and low density lipoprotein (LDL) levels and the decrease of high density lipoprotein (HDL) levels. Despite as a source of dyslipidemia, the increase of blood sugar levels can trigger oxidative stress and lead to the production of Reactive Oxygen Species (ROS) through the glucose autooxidation pathway. Atherosclerosis is as a cause of various cardiovascular diseases, it is resulted from chronic inflammatory reactions that involves Low Density Lipoproteine (LDL) and it is oxidized to foam cell macrophages in the sub endothelial vessel layer. Therefore, chronic DM patients have the potential to increase the LDL oxidation rate, thus having more risks of atherosclerosis and CVD compared to those with ordinary risk factors [2].

Phenolic of Rambutan seed methanol extract is 124 mg GAE/g DW (Garlic Acid Equivalent, GAE; Dried Weight, DW) and DPPH scavenging activity is 383 mg VCEAC/g DW (Vitamin C Equivalent Antioxidant Capacity, VCEAC) [3]. It was also reported that

rambutan seed extract from the cultivar Sichompu of rambutan inhibited ROS formation [4]. Dry rambutan seed contains of a phytochemical with antioxidant characteristic (in mg/100g) such as saponin (2.10 ± 0.05), alkaloid (1.95 ± 0.02), HCN (0.00 ± 0.00), tannin (0.28 ± 0.01), phytate (0.77 ± 0.03), phenol (0.41 ± 0.09), oxalate (0.19 ± 0.01), and flavonoids (1.63 ± 0.32) [5], there are pretty much alkaloid in the rambutan seed extract. A previous study on the alkaloid test could inhibit 3T3 the preadipocyte differentiation. It was also reported that in vivo test in mice with HFD showed that the component could inhibit adiposity, obesity and body fat accumulation, which was characterized by the decreased levels of TC, TG, and LDL and the increase of HDL [6].

Atherogenic index (AI) of Plasma is $LDLc/HDLc$ that can be used as a regular monitoring index of CVD in every day practice, especially for people with cardiovascular risk factors [7]. Based on the previous studies, it is predicted that 70% of ethanol extract in rambutan seed is anti hyperlipidemic and rich of antioxidant. Thus, this study aimed to determine the potential of ethanol extract in rambutan seed for decreasing the atherogenic index in mice with DM-2. The supporting parameters in this study included the decrease of lipid serum profile including LDL and HDL levels, oxidative stress levels including SOD and MDA.

METHOD

Research Design

This study employed Completely Randomized Design with 6 treatments and 4 repetitions. It used 24 mice (*Mus musculus L.*) Strain Balb-C, they were 2-3 months old and their weight were 20-30 g, around 20 mice were induced with DM-2. The mice with DM-2 were divided into 5 groups. For the positive control, they were given metformin dose 39 mg/kgbw (DMmet), and for the negative control without treatment (DMd0). One group was given rambutan seed extract at a dose of 19.2 mg/kgbw (DMd19) while another group was given rambutan seed extract at a dose of 23,4 mg/kgbw (DMd23). It was repeated until 4 times for each group. Meanwhile, a group of 4 mice was normal nonDM (nDM). The nDM and DMd0 groups were given 0.5% CMC as placebo. The treatments of ethanol extract in rambutan seed, metformin and CMC were given orally at a dosage of 0.5 ml for 30 days.

Herbal Extraction, animal model induction and blood serum preparation

Rambutan seed extraction was done by using a maceration method with 70% ethanol solvent referred to Vongsak et al. [8]. The induction of DM-2 to the animal model was performed by giving the High Fat Diet (HFD) followed by the injection of STZ in multiple low dosages referred to Zang et al., [9]. The Streptozotocin used was Bio-world, USACat 714992 CAS 18883-66-4.

Blood was taken from the heart of the fasting mice to which dislocation of the neck was previously performed. Blood was centrifuged at 3000 rpm for 15 minutes to obtain a clear supernatant. The measurements of SOD and MDA were performed immediately on fresh serum, while the remaining serum was stored at -80°C for the measurement of lipoprotein level.

Techniques of Measurement

FBG level was measured by using Accutrend Plus Cholesterol Meter from Roche. The cLDL Reagent precipitation measurement to determine the cLDL level was performed using CHOD-PAP method on photometric systems, DiaSys, Germany. Meanwhile, the HDLc measurement was performed using Differential Precipitation Enzymatic Colorimetric Test Endpoint from Glory Diagnostic, with Reagent from Glory Diagnostic, Spanyol, GD-HDLP80 and GD-HDLP180. Measurement of serum cHDL level by using Differential Precipitation Enzymatic Colorimetric Test Endpoint method.

SOD activity was determined spectrophotometrically by using Superoxide Dismutase (SOD) Assay Kit Catalogue No: E-BC-K020. SOD activity was measured by the WST-1 method, the measurement procedure followed by the protocol attached to the kit and the measurement used a spectrofotometer at λ 450nm. The level of MDA was measured by TBARS method. TBA from Sigma Aldrich. Spectrophotometric determination of MDA-TBA with UV-Vis spectrophotometer at λ 533 nm at room temperature, the principal measurement refers to Setin et al. [10]. AI was calculated by dividing LDLc with HDLc level.

Data Analysis

Data of the effect of 70% of ethanol extract in rambutan seed on the levels of LDLc, HDLc, MDA, SOD dan AI which met the parameter data rules (being distributed and having homogen variance values) were analyzed using One Way Anova test, to find out the significant mean. When the result was significant, further test of Duncant Multiple Range Test (DMRT) was performed with $p=0,05$. If the data did not meet the parameter rules, Mann-Whitney U-Test was performed. The statistic test was done using SPSS program ver.16.0

RESULT

HFD diet and STZ injection succeeded in increasing the blood sugar as well, FBG level was increased from $101.75 \pm 31.521 \text{ mg/dL}$ in normal mice to $420.19 \pm 20.347 \text{ mg/dL}$ in mice with DM. The mean of DM-2 FBG level were at least 285mg/dl (complete data not shown). Statistic result of LDL and HDL serum level shown at figure 1, SOD and MDA level at figure 2, and AI at figure 3.

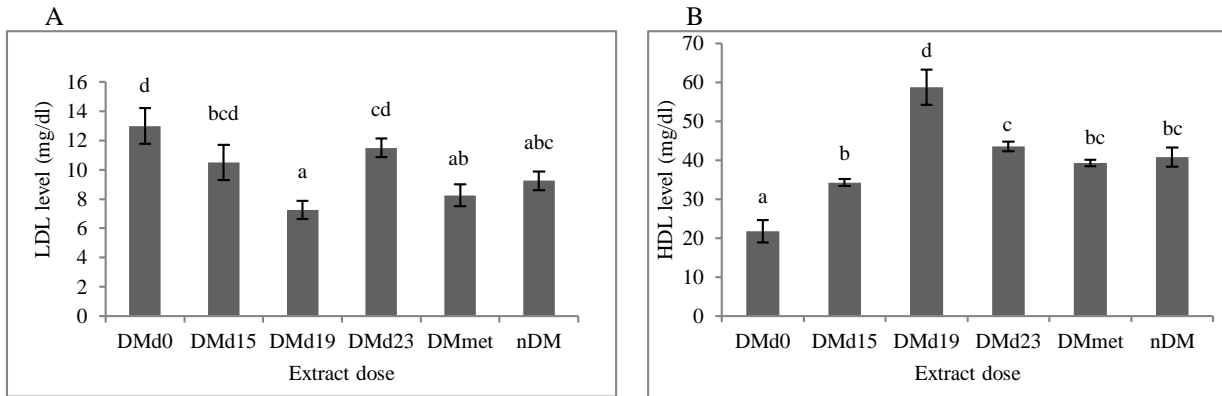


Figure 1: The Effect of Rambutan Seed Extract on Characteristic Mice Serum A. LDL B. HDL
 Note: Different label is significant in DMR t test $\alpha=5\%$.

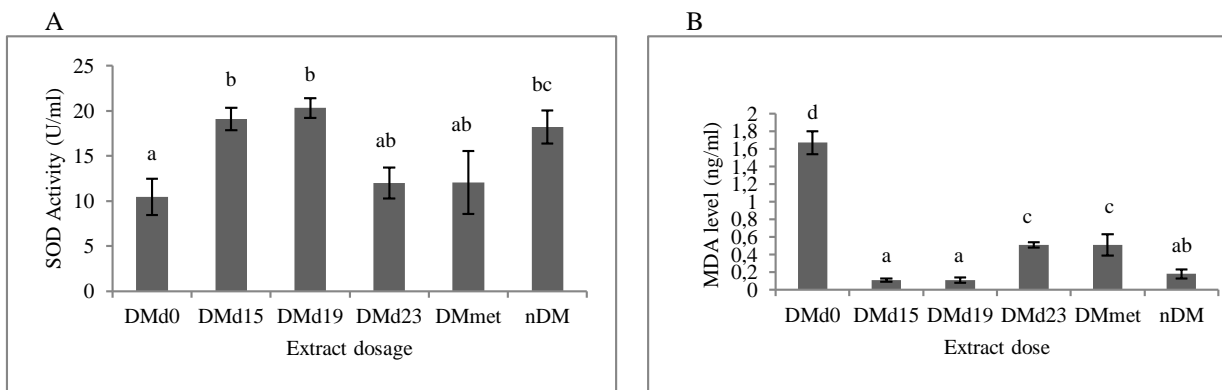


Figure 2: The Effect of Rambutan Seed Extract on the Characteristic Mice Serum A. SOD B. MDA
 Note: Different label is significant in SOD DMRT test 5% and MDA U-Mann Whitney test $\alpha=5\%$.

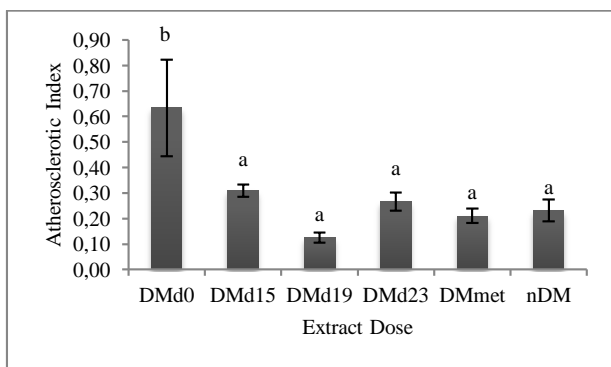


Figure 3. The Effect of Rambutan Seed Extract on Atherosclerotic Index
 Note: Different label is significant in AI DMRT test $\alpha=5\%$.

DISCUSSION

The FBG of mice that received HFD and STZ injection in this research was increasing the blood sugar level up to 55% in diabetic category, $FBG \geq 285 \text{mg/dl}$. This is in line with the required mice with DM by Alacron-Aguilar at $FBG > 200 \text{mg/kgbw}$ [11]. The level of

FBG in mice with DM and administered with rambutan seed extract decreased significantly. The results of this study support the results of Soeng in 2015 in which the rambutan seed extract of 70% ethanol on pre-adipocyte 3T3 cell culture is able to decrease α -glucosidase activity, thereby decreasing the blood glucose level. In addition, Soeng et al. also reported an anti-obesity effect of rambutan seed extract by decreasing G6PDH activity and TG levels. Moreover, 70% ethanol extract of rambutan seed is able to give much better effect than hexane fraction at the same concentration [12].

The animal model of DMd0 showed high glucose levels, experiencing hyperlipidemia, increased MDA and decreased SOD. These data are consistent with the patient serum profile, the DM patients experienced hyperlipidemia and decreased SOD compared to normal individuals [13] and DM patients with high HBA1c (glycated hemoglobin) showed increasing MDA and decreasing SOD [14]. In DM-2 patients, the lipid profile significant increases except HDL cholesterol. It tends to decrease. Similarly, there is a significant decrease in antioxidant enzymes such as reduced glutathione, glutathione peroxides, glutathione reductase, and superoxide dismutase, except catalase as compared to the control subjects. Other findings showed that the level of

lipid peroxide (MDA) increased as per the increase in the blood glucose concentration [15].

The high ratio of LDL/HDL is associated with the risk of atherosclerosis. The higher the ratio, the higher the risk of atherosclerosis, which then known as the Atherosclerosis Index (AI). In this study, the administration of rambutan seed extract significantly decreases AI compared to DM mice. The value is similar with in mice receiving standard drug and normal mice. Hyperlipidemia accompanied by oxidative stress potentially triggers lipid peroxidation and endothelial dysfunction. Low Density Lipoprotein Cholesterol (LDLc) is a material which turns into non-self material if oxidized thus stimulating the inflammatory reaction and chronically having been able to develop into atherosclerosis [16].

CONCLUSION

Based on the result, it is concluded that administration of 70% alcohol extract of rambutan seed potentially lowers the risk of CVD in the DM patients because it decreases not only the level of blood glucose as the main factor, but also other proatherogenic conditions such as lowering the levels of LDL atheroma materials and improving oxidative stress by increasing the SOD enzymatic antioxidant.

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