Potential Activity of Rambutan (*Nepheliumlappaceum* L.) Fruit Peel Extract as Antidiabetic and Antihypercholesterolemia

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Abstract— **Rambutan (*Nephelium lappaceum* L.) fruit peel extract had been reported to have very strong antioxidant activity and had more with flavonoids and phenolic compounds. This study aims to determine the potential antidiabetic and antihypercholesterolemia activities of Rambutan fruit peel ethanol extract in vivo. This research design was pre and post-control group, 25 mice were divided into 5 groups. Group I (negative control) were given a solution of 0.5% CMC-Na, group II (positive control antidiabetic) was given Glibenclamide 0.45 mg/kg, III, IV, and V were given ethanol extract of rambutan peel with successive doses of 500, 250, and 125 mg/kgBW. While the antihypercholesterolemic testing, in group II given Cholesteramine 0.8 g/kg, whereas group III, IV and V given ethanol extract of rambutan skin with a dose of 500, 250, and 125 mg/kgBW, respectively. Treatment of mice conducted for 2 weeks. The results showed that the ethanol extract of Rambutan fruit peel with dose of 125, 250, and 500 mg/kgBW had blood glucose lowering activity in mice induced alloxan, the percentage decrease in blood glucose levels at 22.65 ± 2.10%, 49.05 ± 3.22%, 61.76 ± 4.26%. While the antihypercholesterolemia activity of the ethanol extract of Rambutan peel with dose of 125, 250, and 500 mg/kgBW had an activity to lower cholesterol levels 21.39 ± 6.61%, 31.15 ± 18.15%, and 60.75 ± 8.26%, respectively.**

Keywords— **Antihypercholesterolemia, antidiabetic, fruit peel, *in vivo*, rambutan (*Nephelium lappaceum* L.)**

I. INTRODUCTION

Diabetes mellitus (DM) and hypercholesterolemia is a degenerative disease often found the case in Indonesia, and showed an increasing trend each year prevalence number. Reported that about 50% of people with diabetes have not been diagnosed. Moreover only two-thirds of which have been diagnosed any treatment, both pharmacological and non-pharmacological. WHO (World Health Organization) and the IDF (International Diabetes Federation) predicts an increase in the prevalence of diabetes reaches 2-3 times in 2030, so a lot of research about the prevention and management of diabetes and its complications [1]. While cholesterol is fatty deposits found in the blood vessels can cause constriction of blood vessels due to blood vessel walls become thicker [2]. Cholesterol is normally produced by the body in the right amounts. However, changes in eating patterns that form of animal food and high fat, cholesterol is caused in large amounts in the blood. Increased levels of cholesterol in the blood is a major factor associated with atherosclerotic coronary heart disease which can lead to many deaths [3]

Currently, many on the market, drugs for lowering cholesterol and diabetes mellitus with either natural remedies or synthetic drugs. However, due to the use of modern drugs have side effects such as water retention with hyponatremia, gastrointestinal disorders [4], the tendency of people today prefer natural remedies as a natural remedy is believed to be safer, cheaper and easier to find the raw materials in the surrounding community, compared to synthetic drugs [5]. This phenomenon is of particular concern among the researchers of natural materials.

In 1980 WHO recommended that an examination of the plant which had the effect of hypoglycemia due to the use of modern drugs that are less safe [6]. One was the result of in vitro studies stating that ethanol extracts from durian rind [7] and rambutan fruit peel [8] had as antihyperglycemic activity. Based on the research of Batubara [9] of ethanol extract of the durian fruit skin had natural antioxidant activity. Durian had reported as an antioxidant and antiproliferative[10]. The content of polyphenols and flavonoids are high enough capable of inhibiting the rise in plasma lipids and plasma antioxidant activity in in vivo tests on Wistar mice burdened cholesterol [11]. Rambutan fruit peel contains flavonoids, tannins and saponins[12]. Ethanol extract of rambutan skin contains epigallocatechin-3-gallate [13] which has activity as antihiperglikemia[14] as well as powerful antioxidants [15]. Ethanol extract of rambutan skin are known to have a greater ability as an antioxidant to capture DPPH free radicals than vitamin E [16].

People with diabetes generally have high levels of oxidative stress as the effects of the imbalance between antioxidant protective and increased production of free radicals that can trigger complications ([17]. So as to prevent the occurrence of complications in diabetic condition should be given antioxidants [18]. Haruenkit et al. [10] explains that the content of polyphenols, flavonoids, flavanols, ascorbic acid and tannin acts as an antioxidant. Polyphenols and antioxidant phenols such as catechins can capture free radicals and reduce oxidative stress [19].

Based on the literature review and the above explanation, this paper will explain whether the ethanol extract of rambutan (Nepheliumlappaceum L.) fruit peel had a strong pharmacological activity as antidiabetic and antihypercholesterolemia were tested in vivo in male mice Wistar strain.

II. Materials and Method

Rambutan fruit peels were obtained from PasarGede, Surakarta. Herbarium voucher specimen was prepared and deposited in the Herbarium of Pharmacy Biology at Faculty of Pharmacy, UniversitasMuhammadiyah Surakarta, Indonesia. The peels were collected then cleaned, chopped into small pieces, dried, and then powdered with a blender. Dried powder is then weighed and ready to be extracted.

Animals used for testing were male Wistar mice, which obtained from Laboratory of Pharmacology of UniversitasMuhammadiyah Surakarta. The age of Wistar mice was approximately 2–3 months and weighted 175–225 g. This study was approved by Health Search Ethics Committee of Faculty of Medicine, UniversitasMuhammadiyah Surakarta.

A. Extraction of rambutan fruit peels

The extract of Rambutan fruit peels was prepared by maceration using solvent system with 96% ethanol:acetone (4: 1). A total of 2.0 kg of Rambutan fruit peels powders were soaked in 10 L of ethanol 96% and 2.5 L of acetone which kept away from sunlight and stirred for 3 days. The maceration then filtered with a Buchner funnel. The remaining pulps were re-maceration for another 2 times. The filtration of extracts was combined and concentrated using an evaporator to obtain dried extract.

B. Testing of preclinical antidiabetic condition [18]

Blood Sampling. Blood sampling was collected through the lateral vein in mice contained as much as 0.5 mL in the Eppendorf tubes, then centrifuged using mini spin for 20 minutes at 12,000 rpm to obtain the serum. Furthermore, the supernatant was taken using a micropipette inserted into as many as 10 uL cuvette then added 1000 mL GOD-PAP reagent mixture and incubated for 10 min at 37 °C. Then the absorbance of blank, standard and samples were determined using λ 500 nm visible spectrophotometer.

Treatment of Diabetic-Induced Mice.The animals used for testing were randomly divided into 5 groups, each consisting of 5 mice . Each rat was fasted blood drawn previously used for 12-15 hours and measured blood glucose levels baseline. Furthermore, all groups induced by alloxan monohydrateintraperitoneally 150 mg/kg. Four days after alloxan induced mice blood glucose levels were measured again, if there is an increase in blood glucose levels of mice up to ± 200 mg/dL was considered as diabetic mice . Then each group was treated as follows:

Group I: negative control, were given 0.5% CMC-Na.

Group II: positive control, given Glibenclamide dose of 0.45 mg/kg b.w.

Group III: Rambutan fruit peel extract dose of 500 mg/kg b.w daily.

Group IV: Rambutan fruit peel extract dose of 250 mg/kg b.w. daily.

Group V: Rambutan fruit peel extract dose of 125 mg/kg b.w. daily.

The treatment of the extract conducted for 15 days.

C. Antihypercholesterolemic condition testing

Preparation of Diet and High-Fat Feeding. The mice used for testing were fed by high-fat diet for 28 days. High-fat diet was made to consist of 50 mL of cooking oil, 10 g quail egg yolk, 0.1% PTU and water to 100.0 mL and other high-fat feed consist of 150 g standard feed (pellets), quail egg yolks 20 g, and 50 g margarine. Drinking water 0.1% PTU with dose of 2 mL/200 g b.w. was administered and always made new.

Treatment of Test Animals. Test animals used were white mice. They were divided into 5 groups. Each group consisted of 5 mice . All test animals first adapted to the standard and distilled water were fed ad libitum for 7 days. Before given the diet and a high-cholesterol feeding, the test animals were measured their total cholesterol levels. Then, they were given a high-fat diet for 4 weeks and treated with the extract for 2 weeks. Mice' blood samples of 1.5 mL were taken from the tail vein. High-fat feed were given as much as 30 g daily for 5 mice and high-fat diet with a dose of 2 mL/200g b.w, while the treatment of the extract conducted for 2 weeks (after hypercholesterolemia with total cholesterol levels > 150 mg/dL) in all groups as below:

Group I: 0.5% CMC-Na (negative control)

Group II: Cholestyramine 0.8 g/kg (positive control).

Group III: Rambutan fruit peels dose of 500 mg/kg daily.

Group IV: Rambutan fruit peels dose of 250 mg/kg daily.

Group V: Rambutan fruit peels dose of 125 mg/kg daily.

III. Results and Discussion

The Rambutan fruit peels were extracted using cold maceration with a mixture solvents of ethanol:acetone (4:1). Sanjaya [20] stated that the solvents mixture of ethanol and acetone can provide a good extracts because it was more selective, non-toxic, neutral, hot to less concentration and ethanol can be mixed with acetone in all comparisons. The percent yield for the extraction of Rambutan fruit peel was 27.16%.

A. Antidiabetic testing result

Induction of diabetic condition in mice in this study was conducted based on the method of destruction of the pancreas by giving diabetonicalloxan. Dose of alloxan monohydrate 150 mg/kg was given by intraperitoneal route which was able to make the diabetic condition in mice [18]. Induction of alloxan can caused an increasing blood glucose levels to ± 200 mg/dL which was considered as diabetic mice. The negative control, mice were treated with CMC-Na administration of 0.5%, while the mice administered with Glibenclamide dose of 0.45 mg/kgBW as the positive control, and the treatment were given the orange peel extract with dosage of 125 mg/kg, 250 mg/kg and 500 mg/kg for 10 days. The average

measurements of mouse blood glucose levels after the test was summarized in Table 1.

TABLE I
THE MEAN OF BLOOD GLUCOSE LEVELS OF RATS, BASELINE, PRE AND POST TREATMENT

Groups	Glucose levels (mg/dL), n = 3			Decreasing percentage
	Baseline	Post alloaxan induction	Post Treatment of extracts	
1	79.60±22.01	217.80±15.27	227.80±21.58	-
2	66.60±6.88	213.60±13.94	131.60±29.57	41.90±13.05
3	113.80±8.70	200.80±7.46	86.60±9.63	61.75±4.26
4	91.00±21.20	250.80±26.88	115.40±7.30	49.05±3.22
5	74.20±9.15	228.80±6.53	171.20±5.89	22.65±2.10

The ethanol extract of Rambutan peel dose of 500 mg/kgBW had activities of percentage decrease stronger than Glibenclamide 0.45 mg/kgBW. Ability in lowering blood glucose levels of the ethanol extract of rambutan peel was suspected because of the flavonoid compounds. Presumed mechanism of action of flavonoids which was a way to regenerate and stimulate the release of insulin by pancreatic β cells [21]. There are three mechanisms of a decrease in blood glucose levels from the plant as a potential antidiabetic, namely: 1) the adstringen ability that can precipitate proteins intestinal mucous membrane and form a layer that protects the intestine, thereby inhibiting the intake of glucose, 2) accelerating the release of glucose from the circulation to expedite filtration and renal excretion, 3) accelerating the release of glucose by the increase in metabolism or enter into fatty deposits, which process involves the pancreas to produce insulin [20]. Rambutan fruit peel extract contains flavonoids and tannins [12], there are quercetin, geraniin[13] and epigallocatechin-3-gallate (EGCG), which has an activity antihyperglychemia[14] as well as powerful antioxidants [15]. The content of flavonoids such as catechin, quercetin and EGCG, polyphenols and tannins, suspected that the mechanism of reduction in blood glucose levels in testing animals through the inhibition of glucose absorption, stimulates the release of insulin and indirectly through a mechanism of antioxidant processes. Other reported mechanisms of decrease in blood glucose levels in testing animals, which was due to geraniin on rambutan fruit peel extract had the ability to prevent the formation of AGEs [22].

In this study proved that the ethanol extract of Rambutan fruit peel was able to decrease the blood glucose levels of mice induced alloxan. However, the mechanism of the antidiabetic activity of the ethanol extract of Rambutan fruit peel was not known certainly. It is necessary to test the molecular pharmacology to determine the mechanism of decrease in blood glucose levels that occurs. In addition it is necessary to ensure the active compound what is most responsible for its pharmacological activity.

B. Antihypercholesterolemia testing result

Testing blood cholesterol lowering effects of rambutan fruit peel extract using three different doses ranking. Positive controls were treated with cholestyramine administration of 0.8 g / kg / day. The positive control treated cholesteramine form of 0.8 g/Kg BW daily. Sujono and Sutrisna [9] stated that cholesteramine which was given 800 mg/kg bw reduced cholesterol by 52.97 ± 1.12% when administered for 30 days. In this study, cholesteramine given for 15 days had reduced blood cholesterol by 34.20% ± 10.42. The CMC-Na 0,5% was used as negative control and it does not affect the blood cholesterol levels.

TABLE II
THE MEAN CHOLESTEROL LEVELS DECREASE AFTER BEING GIVEN THE EXTRACT

Groups	Cholesterol levels (mg/dL), n = 3			Decreasing percentage
	Induction for 4 weeks	After giving of extract	Induction for 4 weeks	
I	58.60±4.16	116.80±10.23	77.20±16.02	34.20±10.48
II	61.00±11.87	145.80±23.40	126.60±32.17	13.44±15.45
III	78.80±18.75	203.20±43.41	80.20±24.71	60.75±8.26
IV	62.20±5.36	133.60±30,01	88.20±15,64	31.15±18,15
V	61.20±6.34	180.20±12,68	141.00±2,92	21.39±6.61

Based on data of antihypercholesterolemiatesting results in Table 2, Rambutan fruit peel extract at a dose of 500 mg/kg bw had the strongest activity to reduce cholesterol levels in the blood of mice, which was 60.75% more powerful than the positive control (cholestyramine, a dose of 800 mg / kgBW). This showed that the ethanol extract of rambutan fruit peel was very potent as the antihypercholesterolemia herbal medicine ingredient. Based on the review of the literature has never reported the results of research and known exactly what compounds which act as lowering levels of cholesterol in the extract of rambutan peel, and also unknown pharmacological mechanism.

IV. CONCLUSIONS

Ethanol extract of rambutan fruit peel had a high potential as an antidiabetic and antihypercholesterol acitivities. At doses of 125, 250, and 500 mg/kgBW might be lowers blood glucose levels were 22.65±2.10%, 49.05±3.22%, 61.76±4.26%, respectively. While on antihypercholesterol activity at the same dosage, had the ability to decrease cholesterol levels in the blood, respectively for 21.39±6.61%, 31.15±18.15%, and 60.75±8.26%.

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