Hypolipidaemic Effects of High Resistant Starch Sago and Red Bean Flour-based Analog Rice on Diabetic Rats

Sri Budi Wahjuningsih¹, Haslina Haslina¹, Marsono Marsono²

ABSTRACT

Introduction: Sago analog rice had known as an example of food with high resistant starch. Recent research shows that sago analog rice and red bean flour also had a low glycemic index (GI). However, Identification of hypolipidaemic mechanism based on the nutrigenomic analysis remains unknown.

Aim: This study aims to determine the effects of hypolipidaemic in diabetic rats with analog rice treatment.

Material and Methods: Thirty-five male Wistar rats were divided into 5 groups with different food treatment, such as standard dietary food (STD) group, and four groups of diabetic rats with standard dietary food (STDD), mentik wangi rice diet (MWRD), sago analog rice diet (SARD) and sago analog rice with 10% red bean flour (SARKBD).

Lipid profile was observed every week for a month. Measurement of insulin and blood glucose was performed twice at the beginning and end of treatment. Atherogenic index (AI) was also investigated. Then, the pancreas was collected for histological analysis.

Results: SARD group showed the highest effect of decreasing the total cholesterol (47.74%) which followed by SARKBD (34.62%). The triglyceride level in SARD group was also significantly decreased (31.14%), followed by SARKBD (19.32%). However, the HDL increase in SARD and SARKBD group were significantly decreased, respectively 32.89% and 22.19%. SARD atherogenic index levels lower than SARKBD; 1.00 and 2.06.

Conclusion: The improvement of insulin resistance by SARD and SARKBD were generated by role of resistant starch through the mechanism of bile acid binding, insulin sensitivity escalation and SCFA effect.

Keywords: diabetes mellitus, hypolipidaemic, lipid, rice, sago, starch.

1. INTRODUCTION

The increasing of the human welfare level in Indonesia was bringing negative impact to the society which indicated by the increasing of the degenerative disease level, for instance diabetes mellitus (DM). DM is one of non-infectious disease (NIDs) which caused the highest mortality rate. The number of DM patient was expected would be elevated to be 642 million people by 2040 if there is no serious prevention effort (1).

Specifically, WHO exhibited that in Indonesia, the number of patients with type 2 diabetes mellitus (T2DM) will be increased from 8.2 million people by 2000 to be 21.3 million people by 2030. It was assumed as the result of the increasing level of human welfare. Therefore, it changes the dietary habit of society (2, 3). Moreover, it is also affected by metabolic complication, such as hyperlipidemia (4), so that the dietary management for diabetes patient is proposed to prevent the glucose level and the cholesterol level in blood. However, those aims could be attained by consumption of hypoglycemic and hypocholesterolemic food.

Commonly, the diabetes patients are treated by the continuously chemical treatment, so that, it may cause the unexpected side effect. The diet management could be used as one of possible strategies for diabetes treatment. Interestingly, hypoglycemic food consumption proved that it could be decreased the cholesterol level of diabetes patient. Hence, it is suggested that diabetes patients should have low glycemic index (GI) food consumption, which enriched by 30-40% fiber and 35% saturated fat (5), in order to control the glucose and cholesterol level (6). Rice had known as one of food with high glycemic index (GI: 80). Since, rice is a staple food in Indonesia, therefore the consumption of rice.

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2. AIM

The aim of this study is to investigate the hypolipidaemic effect of rice analog derived from sago and red bean flour in STZ-NA induced diabetic rat model.

3. MATERIAL AND METHODS

Materials

This research was using Mentik Wangi rice, sago and red bean. Rice was commercially purchased from the market placed at Yogyakarta. The main materials in this research are starch of Sago varieties Meranti which obtained from Selat Panjang, Riau and red bean by local varieties which obtained directly from Temanggung.

Producing process of analog rice

Analog rice was produced in 2 types, such as sago analog rice (SARD) only and sago mixed with 10% of red bean flour (SARKBD) (18).

Animal studies

A Wistar rats (Male, 2-3 old months, weight 200-250g) were used in this research. During the experiment, rats were placed in the cage with good ventilation, room temperature around 25°C and uncontrolled lightening. This research had obtained a legal permission from an Ethical committee of Preclinical Research No. 00070/04/LPPT/X/2016, Gajah Mada University. During the experiment, Wistar rats lived in the cage with excellent condition, including good lighting, ventilation, and room temperature.

Bioassay assessment in vivo

Around 35 Wistar rats aged 2-3 months with weight 200-250 gram were dividing into 5 groups. In detail, the group I was control with fed by AIN93M (19). Group II until V were given by different dietary, such as standard (STTD), mentik wangi rice dietary (MWRD), sago analog rice diet (SARD) only, sago analog rice with 10% red bean flour (SARKBD). Food and drink were given by ad libitum.

The diabetes induction was generated by intraperitoneal injection of 250 mg/kg Nicotinamide (NA) within buffer saline NaCl 0.9%. After 15 minutes of injection, rats were injected again with 60 mg/kg of Streptozocin (STZ) (20). After injection, rats were provided with 5% glucose in water for 24 hours to avoid the hypoglycemic (21). Then, blood was aspirated from the vena retroorbital by microcapiller technic. Then, five days after induction, blood glucose level, lipid level and insulin level were measured. Blood glucose more than 200mg/kg was indicated that rats successfully induced as diabetic rats. Generally, rats were treated with particular food for 4 weeks and blood glucose was measured in every week. The level of insulin was analyzed at the first week and last week of diabetes condition.

Formulation of food

The formulation of food was based on the formula of AINM 1995. The detail of formula was presented in the Table 1.

3.1 Formulation of food


equation 1: \[ \text{component} \times \text{diet (g/kg)} \]

Table 1. Composition of the experimental animal diets * STD = Standard Diet (AIN 93M), MWRD = Mentik Wangi Rice Diet, SARD = Sagu Analog Rice, SARKBD = Sagu Kidney Bean Analog Rice (10% kidney bean). STD Diet was fed to healthy rats (STD) and standard diet, menthik wangi rice, sago based analog rice and sago and kidbean flour based analog rice diets were fed to diabetic rats (STD, MWRD, SARD and SARKBD rats). These apply to all the tables and figures where they appear.

Determination of total cholesterol level

The blood lipid profiles were analyzed based on the total cholesterol level, triglycerides, HDL, and LDL levels in serum measured by kits (DiaSys diagnostic systems GmbH, Alte Strasse 9 Holzheim Germany). This kit had a number of specific enzymes that convert the substrate into a chromophore which easily to be detected by spectrophotometry.

The cholesterol level analysis procedure uses the oxidase-p-aminophenozone (CHOD-PAP) cholesterol method. Samples or standards were taken as 10 µl and mixed with 1000 µl of reagent kit. The mixture was incubated at 37°C for 5 minutes, and then absorbance was measured at a wavelength of 546 nm. Total cholesterol levels were calculated as follows:

Determination of high-density lipoproteins (HDL) level

HDL measurement started by precipitation of low-density lipoproteins (LDL) and chylomicrons. Precipitation was conducted by the addition of phosphotungstic acid (PTA) and magnesium ions (MgCl2). After centrifugation, HDL in the supernatant is measured using a kit which used for measuring the total cholesterol (oxidase-p-aminophenozone/
CHOD-PAP cholesterol). In detail, the precipitation procedure was about adding 200 µl of blood serum with 500 µl of precipitation reagent diluted in aquabides with ratio 4:1, then incubated for 10 minutes at room temperature. After that, centrifuge that mixture solution with 4000 rpm for 10 minutes. Furthermore, supernatant was collected for further total cholesterol analysis.

**Determination of low-density lipoproteins (LDL) level**

LDL measurement was also conducted with precipitation by mixture with the reagent which contain of hepargin and sodium citrate. LDL in the supernatant was measured using a kit reagent similar to the total cholesterol measurement (CHOD-PAP). About 200 µl of blood serum was mixed with 500 µl of precipitation reagent which diluted in aquabides with ratio 4:1, then incubated for 10 minutes at room temperature. Then, mixture solution was centrifuged at 1074 g for 10 minutes. Next, supernatant was isolated for total cholesterol analysis.

**Determination of triglyceride (TG)**

Analysis of triglycerides was investigated by glycerol phosphate oxidase-aminophenozone (GPO-PAP) method. About 10 µl of sample or standard were mixed with 1000 µl of kit reagent. The mixture was incubated at 37°C for 5 minutes, and absorbance was measured at 546 nm of wavelength. Calculation of triglyceride levels was determined by following formula:

\[
\text{Triglyceride level (mg/dL)} = \frac{\text{Sample absorbance} \times \text{Standard concentration}}{\text{Standard absorbance}}
\]

**Determination of atherogenic index (AI)**

The atherogenic index can be used to measure the risk of coronary heart disease (CHD). A low atherogenic index indicates a high HDL-C ratio. Higher HDL-C and LDL-C and a lower atherogenic index are protection against CHD. The atherogenic index (IA) is calculated based on formula below (22):

\[
\text{Atherogenic index (AI)} = \frac{(\text{Total cholesterol} - \text{HDL})}{\text{HDL}}
\]

**Measurement of insulin resistance**

Insulin resistance was calculated by Homeostatic model assessment and insulin resistance (HOMA-IR) index obtained from multiplying the glucose levels during fasting (mg/dL) (unpublished data) with the insulin levels during fasting (ng/mL) then divided by 405 (23, 24).

**Preparation of histopathological sample**

Tissue samples were collected and fixed using 10% formalin. Then, transfer the samples into alcohol with serial concentrations, such as 70%, 80%, 95% and absolute alcohol to remove water from the tissue. After that, purified the sample with xylol before embedded into block paraffin. Then, tissue sectioning was prepared by cutting the paraffin blocks into 5µm of thickness using microtome. After that, the tissue section was placed on the 50°C hot plate for 15 minutes (25).

**Hematoxilin-Eosin (HE) staining**

Hematoxilin-Eosin staining method was conducted in several processes. It started with deparaffinization process by dipping in the tissue sample into xylol I, xylol II and xylol III for 3 minutes, respectively. After that, the tissue samples were transferred into rehydration process by serial ethanol concentrations, for instance absolute, 95%, 80%, and 70% for 2 minutes in each. Soak the samples into Harri’s Hematoxilin for 10 minutes and rinse it with tap water for 10 minutes. Furthermore, the samples were immersed in eosin for 10 minutes, and then dehydrated with serial ethanol concentration from 70% to absolute. For clearing process, the samples were put into xylol I, II, III. After the coloring process is complete, the adhesive is dripped (Canada balsam) and covered with a glass cover and then dried (25, 26).

**Statistical analysis**

Statistical analysis of all data was conducted using ANOVA by Statistical Analysis System (SAS) version 9.2. The significant results were further analyzed by Duncan’s multiple range test (DMRT) with 5% significance level.

### 4. RESULTS

The level of total cholesterol, triglycerides, low-density lipoproteins (LDL), and high-density lipoproteins (HDL)

Complications in patients with type 2 diabetes mellitus were indicated by dyslipidemia or lipid metabolic disorder. Generally, it characterized by increased cholesterol level, LDL levels, and triglycerides then decreased the HDL levels. The lipid profile measurement in rats was exhibited the similar result. Based on the Tables 2, 3, 4 and 5 it showed that four treatment groups of STZ-NA induced diabetic rats

| Observations | STD     | STDD    | MWRD    | SARD    | SARKBD  |
|--------------|---------|---------|---------|---------|---------|
| Prior to induction | 89.82b ± 1.92 | 89.44b ± 2.43 | 85.52a ± 2.17 | 86.96a ± 1.39 | 89.35b ± 2.00 |
| After induction  | 87.12a ± 2.47 | 182.88b ± 4.02 | 182.31b ± 3.10 | 181.65b ± 3.05 | 185.81b ± 4.68 |
| Week – 1       | 88.76a ± 2.08 | 186.26e ± 4.46 | 173.59d ± 2.47 | 143.45b ± 2.37 | 151.40c ± 2.40 |
| Week–2         | 82.28a ± 2.67 | 186.47e ± 4.03 | 171.93d ± 2.93 | 134.78b ± 2.03 | 147.50c ± 3.02 |
| Week–3         | 83.39a ± 2.66 | 187.45e ± 4.61 | 170.69d ± 3.03 | 116.71b ± 1.78 | 144.12c ± 2.68 |
| Week–4         | 84.09a ± 2.59 | 188.35e ± 4.89 | 165.96d ± 2.84 | 94.93b ± 1.98 | 121.48c ± 2.45 |

Table 2. Cholesterol Total of standard and treatment dietary food of control and diabetics rat group. STD = Standard Diet (AIN 93M), MWRD = Menthik Wangi Rice Diet, SARD = Sagu Analog Rice, SARKBD = Sagu Kidney Bean Analog Rice (10% kidney bean).
had elevated the cholesterol levels, LDL, and triglycerides. However, it decreased the HDL levels.

In detail, elevated cholesterol levels after STZ-NA induction in the rat group of STD, STDD, MWRD, SARD, and SARKBD were about 51.09%, 53.09%, 52.12%, 51.91%, and not significantly different, respectively (Table 2). The increase of LDL levels in STDD, MWRD, SARD, and SARKBD after STZ-NA induction were 67.86%, 56.13%, 54.03%, and 58.31%, respectively (Table 3). Otherwise, HDL levels decreased into 53.94%, 60.59%, 59.46%, and 57.88%, respectively for each group: STDD, MWRD, SARD, and SARKBD (Table 5). Furthermore, the triglycerides level was observed. The result which presented in Table 4 showed that there was an increase in triglycerides levels in rats after being induced with STZ-NA. Group of diabetic rats with standard food (STDD) exhibited the higher level of triglyceride into 36.88%, while another group such as MWRD, SARD and SARKBD showed increased level into 45.27%, 46.02%, and 47.10%.

However, in the end of treatment, all groups of diabetic rats which treated with analog rice experienced a decreased cholesterol levels, whereas the control group (STDD) still exhibited high levels of cholesterol. After 4 weeks of treatment, diabetic rats with sago analog rice (SARD) had the highest reduction of total cholesterol level about 47.74%, followed by SARKBD and MWRD group with 34.62% and 8.97%, respectively. The treatment of sago analog rice (SARD) also highly reduced the LDL levels about 32.89%, followed by 22.19% in SARKBD and 9.07% in MWRD.

Furthermore, the sago analog rice also decreased the

| OBSERVATION | STD | STDD | MWRD | SARD | SARKBD |
|-------------|-----|------|------|------|--------|
| Prior to induction | 33.77± 2.84 | 24.53± 3.18 | 33.48± 2.69 | 34.54± 4.18 | 30.21± 1.62 |
| After induction | 37.47± 3.79 | 76.32± 1.37 | 37.82± 1.99 | 75.14± 1.87 | 72.47± 4.21 |
| Week–1 | 38.98± 3.71 | 79.66± 1.45 | 72.12± 2.54 | 58.20± 1.81 | 60.65± 2.30 |
| Week–2 | 42.81± 3.76 | 82.78± 1.58 | 69.91± 2.67 | 56.52± 1.71 | 59.55± 1.97 |
| Week–3 | 43.48± 3.47 | 84.81± 1.42 | 68.84± 2.49 | 53.43± 1.67 | 58.27± 2.45 |
| Week–4 | 42.53± 3.50 | 84.01± 1.54 | 69.40± 2.44 | 50.43± 1.82 | 56.39± 2.15 |

Table 2. LDL of standard and treatment dietary food of control and diabetics rat group. STD = Standard Diet (AIN 93M), MWRD = Menthik Wangi Rice Diet, SARD = Sagu Analog Rice, SARKBD = Sagu Kidney Bean Analog Rice (10% kidney bean).

| OBSERVATION | STD | STDD | MWRD | SARD | SARKBD |
|-------------|-----|------|------|------|--------|
| Prior to induction | 78.66± 2.09 | 81.51± 4.07 | 69.51± 2.99 | 68.28± 5.97 | 66.92± 2.33 |
| After induction | 75.11± 5.44 | 129.13± 2.44 | 127.01± 1.81 | 126.50± 3.03 | 126.50± 3.96 |
| Week–1 | 76.95± 4.89 | 130.56± 2.84 | 123.05± 2.18 | 99.17± 3.89 | 102.71± 4.17 |
| Week–2 | 78.39± 4.96 | 130.96± 2.85 | 121.09± 2.34 | 98.73± 4.77 | 111.44± 2.75 |
| Week–3 | 79.38± 4.90 | 132.63± 2.58 | 119.07± 2.06 | 91.16± 4.53 | 108.75± 2.23 |
| Week–4 | 80.76± 4.40 | 133.37± 2.20 | 117.24± 2.46 | 86.66± 3.88 | 102.06± 2.24 |

Table 3. Triglycerides of standard and treatment dietary food of control and diabetics rat group. STD = Standard Diet (AIN 93M), MWRD = Menthik Wangi Rice Diet, SARD = Sagu Analog Rice, SARKBD = Sagu Kidney Bean Analog Rice (10% kidney bean).

| OBSERVATION | STD | STDD | MWRD | SARD | SARKBD |
|-------------|-----|------|------|------|--------|
| Prior to induction | 54.85± 2.83 | 55.28± 2.96 | 63.61± 2.51 | 60.16± 3.40 | 60.45± 2.42 |
| After induction | 60.64± 4.02 | 25.46± 1.51 | 25.07± 9.15 | 24.39± 2.01 | 25.46± 1.84 |
| Week–1 | 59.29± 4.65 | 25.07± 2.13 | 24.39± 1.55 | 40.32± 1.94 | 34.22± 3.61 |
| Week–2 | 58.31± 4.49 | 25.93± 2.14 | 25.09± 1.28 | 41.35± 2.27 | 35.31± 3.51 |
| Week–3 | 54.88± 4.33 | 23.33± 2.03 | 27.03± 1.49 | 44.60± 1.56 | 37.41± 11.98 |
| Week–4 | 54.01± 4.52 | 22.52± 2.01 | 28.91± 1.23 | 47.51± 2.35 | 39.78± 2.34 |

Table 4. HDL of standard and treatment dietary food of control and diabetics rat group. STD = Standard Diet (AIN 93M), MWRD = Menthik Wangi Rice Diet, SARD = Sagu Analog Rice, SARKBD = Sagu Kidney Bean Analog Rice (10% kidney bean).
triglyceride levels around 31.14%, followed by SARKBD and MWRD respectively at 19.32% and 7.69%. However, the different result demonstrated in the measurement of HDL level. The sago analog rice diet (SARD) group had increased levels of HDL around 48.66%, followed by SARKBD about 36.00% and MWRD about 13.28%.

**Atherogenic index (AI)**

As mentioned, the diabetic complications followed by dyslipidemia which promote the atherosclerosis related to coronary heart disease. Therefore, to predict the risk of atherosclerosis, there is calculation model to calculate an atherogenic index (AI). Results showed that the increased of AI after diabetes induction in STDD, MWRD, SARD, and SARKBD groups was as follow: 90.00%, 94.43%, 93.07%, 92.43% and not significant result (Table 6). However, after 4 weeks of treatment, diabetic rats with SARD treatment was highly reduce the AI about 84.59%, followed by SARKBD and MWRD respectively at 67.51% and 24.36%. The AI of SARD group was 1.00 and SARKBD was about 2.06, those results were closed to the control group with 0.57.

**Homeostatic model assessment and insulin resistance (HOMA-IR)**

In general, diabetes mellitus usually begins with the problem of insulin resistance or loss of insulin sensitivity. It is characterized by high insulin levels in the HOMA-IR value (30, 31). High HOMA-IR indicates lower insulin sensitivity due to a decrease insulin response in target tissue (32).

Moreover, the diabetic complications such as dyslipidemia, hypertension and coronary heart disease are also associated with a high insulin resistance (33). The HOMA-IR index data in Table 7 show that at the end of the treatment, the SARD group demonstrated the lowest HOMA-IR value about 1.96, and then followed by SARKBD with 2.30 and MWRD with 5.61. The STDD rats didn’t show of insulin decrease. It was closely supported the pancreatic histopathology observations (Table 7 and Figure 1).

The tissue staining showed that the number of Landergans islands in the SARKBD was higher as compared to MWRD group and the control group. Data in Table 6 demonstrated that HOMA-IR of SARD and SARKBD treatment could reduce the insulin resistance and increase the number of pancreatic β-cells.

### 5. DISCUSSION

Diabetes mellitus is generally characterized by elevated blood glucose levels and dyslipidemia with high total cholesterol, triglycerides, LDL and low HDL level (29). In diabetes, the metabolism of fat and carbohydrates occurred in the liver and fat tissue. Insulin played an essential role in the synthesis of fatty acids and triglycerides in fat tissues.
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Therefore, it will inhibit the lipolysis process. However, the role of insulin in increasing the synthesis of fatty acids in the liver tissue also stimulate the secretion of very-low-density lipoprotein (VLDL), and enzyme HMG-KoA reductase (34, 35). However, in diabetes, the decrease in insulin response causes the removal of fat as an energy source through the lipolysis mechanism (36, 37). Lipolysis increased resultant high level of Acetyl-coA which promote the ketone bodies and blood cholesterol level (38). It supported by several studies shown that diabetic rats had higher cholesterol levels (17, 39-41). This research exhibited similar result that after the treatment, the groups of STZ-NA induced diabetic rats had elevated the cholesterol levels, LDL, and triglycerides.

Interestingly, the result observed that all groups of diabetic rats with sago analog rice (SARD) treatment had decreased cholesterol level at the end of treatment. The decreased levels of total cholesterol were found as well as the decreased of LDL and triglycerides, but an increase of HDL levels. This result is related to the levels of analog rice resistant starch in each dietary. The highest levels of resistant starch showed affected to the decreased of total cholesterol levels. The resistant starch (RS) value in SARD and SARKBD group was 12.25% and 11.80% (14) while the RS value of MWRD group was only 10.72%. This result is related to the previous report stated that resistant starch had ability to reduce the blood cholesterol levels, LDL, and triglycerides.

Of note, all the results indicated that treatment of food with high resistant starch could reduce the total cholesterol levels and increase the HDL levels. The increased of HDL is the most important criteria of anti-atherogenic (17, 52). In addition, the food, fiber diet can reduce the atherogenic index (AI), where the physiological properties of dietary fiber are also possessed by resistant starch (RS) (17).

Regarding to the insulin resistance in diabetes mellitus, the insulin resistance was commonly measured by homeostasis model assessment and insulin resistance (HOMA-IR). Previous research reported that RS is essential since it can decrease the insulin resistance (53-55), therefore it increases glucose uptake from blood and decreases the blood glucose level. In addition, the increased insulin sensitivity can also enhance by the presence of short chain fatty acid (SCFA). An acetate and propionate are the main of SCFA fermented the RS products (56).

6. CONCLUSION

This research concludes that the effect of decreased lipid profile and improvement of insulin resistance by analogous SARD and SARKBD rice diets is due to the role of resistant starch through the mechanism of bile acid binding, increased insulin sensitivity and influence of SCFA fermented.
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