

DOI: 10.5455/msm.2018.30.232-239

Received: July 09 2018; Accepted: August 24, 2018

© 2018 Sri Budi Wahjuningsih¹, Haslina Haslina¹, and Marsono Marsono²This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORIGINAL PAPER

Mater Sociomed. 2018 Dec; 30(4): 232-239

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

Sri Budi Wahjuningsih¹, Haslina Haslina¹, and Marsono Marsono²

¹Department of Processing, Agricultural Product, Faculty of Agricultural Technology, Semarang University, Semarang 50196, Indonesia

²Department of Food and Agricultural Products Technology, the Faculty of Agricultural Technology, Gadjah Mada University, Yogyakarta 55281

*Corresponding author: Sri Budi Wahjuningsih, Doctor, Semarang University, Jl. Arteri Soekarno-Hatta, Semarang 50196, Indonesia, <https://orcid.org/0000-0002-4396-1480>, Phone: +6281575544498, email: sribudiwahjuningsih@yahoo.com

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 glicemic 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 (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 (48.66%), followed by SARKBD (36.00%). The LDL level 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.

Keyword: diabetes mellitus, lipid profile, rice analog, sago, starch resistant

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 hypolesterolamic 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

should be controlled (7). One alternative way is by composing the rice analog from food with low glycemic index, such as sago and red bean.

Rice analog is demonstrated by extrusion technology which started with the pregelatinizing process to elevate the resistance starch level (8-10). Food with high resistance starch level tends to be resistant to the hydrolysis of amylase (11). Therefore, it hard to be digested and it essential for ameliorating the glucose level in diabetic patient (12, 13). Based on the previous report, it found that sago starch and sago starch mixed with 10% of red bean flour had resistance starch about 12.85% and 11.18%, respectively (14,15). However, their hypolipidemic effect remains unknown. In addition, another report showed that rice analog from red bean and whole grains were had a hypoglycemic character in diabetic mice (16, 17).

2. AIM

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

3. MATERIAL AND METHODS

Materials

This research was using Menthik 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 (SARKD) (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 AIN96M (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 230 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 microcapillar 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 1993. The detail of formula was presented in the Table 1.

Component	Diet (g/kg)*			
	STD/STDD	MWRD	SARD	SARKBD
Corn starch	620.7	-	-	-
MWRD	-	834	-	-
SARD	-	-	805	-
SARKBD	-	-	-	837
Casein	140	82	122.3	95
Sucrose	100	100	100	100
Soybean oil	40	35	32.8	27.5
CMC	50	39	28.3	8
Mineral mix	35	32.7	19	14
Vitamin mix	10	10	10	10
L- cysteine	1.8	1.8	1.8	1.8
Choline bitartrate	2.5	2.5	2.5	2.5

Table 1. Composition of the experimental animal diets * STD = Standard Diet (AIN 93M), MWRD = Menthik 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 standar diet, menthik wangi rice, sago based analog rice and sago and kidney bean flour based analog rice diets were fed to diabetic rats (STDD, 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:

$$\text{Total cholesterol level (} \frac{\text{mg}}{\text{dL}} \text{)} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \text{Standard concentration (} \frac{\text{mg}}{\text{dL}} \text{)}$$

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 proce-

ture 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.

$$\text{HDL level (} \frac{\text{mg}}{\text{dL}} \text{)} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \text{Standard concentration (} \frac{\text{mg}}{\text{dL}} \text{)} \quad (2)$$

Determination of low-density lipoproteins (LDL) level

LDL measurement was also conducted with precipitation by mixture with the reagent which contain of heparin and sodium citrate. LDL in the supernatant was measured using a kit reagent similar to th 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 centrifugated at 1074 xg for 10 minutes. Next, supernatant was isolated for total cholesterol analysis.

$$\text{LDL level (} \frac{\text{mg}}{\text{dL}} \text{)} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \text{Standard concentration (} \frac{\text{mg}}{\text{dL}} \text{)} \quad (3)$$

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 37C for 5 minutes, and absorbance was measured at 546 nm of wavelength. Calculation of triglyceride levels was determined by following formula:

$$\text{Triglycerida level (} \frac{\text{mg}}{\text{dL}} \text{)} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \text{Standard concentration (} \frac{\text{mg}}{\text{dL}} \text{)} \quad (4)$$

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):

$$\text{Atherogenic index (AI)} = \frac{(\text{Total cholesterol} - \text{HDL})}{\text{HDL}} \quad (5)$$

Measurement of insulin resistance

Insulin resistance was calculated by Homeostatic model assessment and insuli 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 concentration, 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 setioning was prepared by cutting the parafin blocks into 5µm of thickness using microtome. After that, the tissue section was placed on the 50°C hot plate for 15 minutes (25).

Hematoxiline-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 Hematoxylin 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. .RESULT

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 had elevated the cholesterol levels, LDL, and triglycerides. However, it decreased the HDL levels.

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).

OBSERVATION	STD	STDD	MWRD	SARD	SARKBD
Prior to induction	33.77 ^c ± 2.84	24.53 ^a ± 3.18	33.48 ^b ± 2.69	34.54 ^c ± 4.18	30.21 ^b ± 1.62
After induction	37.47 ^a ± 3.79	76.32 ^c ± 1.37	76.32 ^c ± 1.99	75.14 ^b ± 1.87	72.47 ^b ± 4.21
Week-1	38.98 ^a ± 3.71	79.66 ^d ± 1.45	72.12 ^c ± 2.54	58.20 ^b ± 1.81	60.65 ^b ± 2.30
Week-2	42.81 ^a ± 3.76	82.78 ^e ± 1.58	69.91 ^d ± 2.67	56.52 ^b ± 1.71	59.55 ^c ± 1.97
Week-3	43.48 ^a ± 3.47	84.81 ^e ± 1.42	68.84 ^d ± 2.49	53.43 ^b ± 1.67	58.27 ^c ± 2.45
Week-4	42.53 ^a ± 3.50	84.01 ^e ± 1.54	69.40 ^d ± 2.44	50.43 ^b ± 1.82	56.39 ^c ± 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 ^b ± 2.09	81.51 ^b ± 4.07	69.51 ^a ± 2.99	68.28 ^a ± 5.97	66.92 ^a ± 2.33
After induction	75.11 ^a ± 5.44	129.13 ^b ± 2.44	127.01 ^b ± 1.81	126.50 ^b ± 3.03	126.50 ^b ± 3.96
Week-1	76.95 ^a ± 4.89	130.56 ^d ± 2.84	123.05 ^c ± 2.18	99.17 ^b ± 3.89	102.71 ^b ± 4.17
Week-2	78.39 ^a ± 4.96	130.96 ^e ± 2.85	121.09 ^d ± 2.34	98.73 ^b ± 4.77	111.44 ^c ± 2.75
Week-3	79.38 ^a ± 4.90	132.63 ^e ± 2.58	119.07 ^d ± 2.06	91.16 ^b ± 4.53	108.73 ^c ± 2.23
Week-4	80.76 ^a ± 4.40	133.37 ^e ± 2.20	117.24 ^d ± 2.46	86.66 ^b ± 3.88	102.06 ^c ± 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 ^a ± 2.83	55.28 ^a ± 2.96	63.61 ^c ± 2.51	60.16 ^b ± 3.40	60.45 ^b ± 2.42
After induction	60.64 ^b ± 4.02	25.46 ^a ± 1.51	25.07 ^a ± 9.15	24.39 ^a ± 2.01	25.46 ^a ± 1.84
Week-1	59.29 ^d ± 4.65	25.07 ^a ± 2.13	24.39 ^a ± 1.55	40.32 ^b ± 1.94	34.22 ^c ± 3.61
Week-2	58.31 ^d ± 4.49	23.93 ^a ± 2.14	25.09 ^a ± 1.28	41.35 ^c ± 2.27	35.31 ^b ± 3.51
Week-3	54.88 ^e ± 4.33	23.33 ^a ± 2.03	27.03 ^b ± 1.49	44.60 ^d ± 1.56	37.41 ^c ± 11.98
Week-4	54.01 ^e ± 4.52	22.52 ^a ± 2.01	28.91 ^b ± 1.23	47.51 ^d ± 2.35	39.78 ^c ± 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).

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 group 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 triglyceride 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 triglyceride levels around 31.14%, followed by SARKBD and MWRD respectively at 19.32% and 7.69%. However, the

OBSERVATION	STD	STDD	MWRD	SARD	SARKBD
Prior to induction	0.64c ± 0.09	0.62c ± 0.11	0.35a ± 0.06	0.45b ± 0.08	0.48b ± 0.08
After induction	0.44a ± 0.12	6.20b ± 0.35	6.28b ± 0.26	6.49b ± 0.65	6.34b ± 0.69
Week - 1	1.39a ± 2.33	6.38d ± 0.63	5.67cd ± 1.39	2.64ab ± 0.30	4.13bc ± 1.69
Week - 2	0.42a ± 0.13	6.84e ± 0.64	5.87d ± 0.36	2.27b ± 0.18	3.21c ± 0.42
Week - 3	0.53a ± 0.14	7.08e ± 0.65	5.33d ± 0.36	1.62b ± 0.09	2.88c ± 0.33
Week - 4	0.57a ± 0.15	7.41e ± 0.70	4.75d ± 0.22	1.00b ± 0.05	2.06c ± 0.16

Table 5. Index Atherogenic of standard and treatment dietary food of control and diabetics rat group. STD = Standard Diet (AIN 93M), MWRD = Menthik Wangi Rice Diet, SARD = Sagou Analog Rice, SARKBD = Sagou Kidney Bean Analog Rice (10% kidney bean).

OBSERVATION	STD	STDD	MWRD	SARD	SARKBD
Result	0.79c±0.56	9.13a±3.31	5.61b±0.81	1.96c±0.27	2.30c±0.80

Table 6. HOMA IR of standard and treatment dietary food of control and diabetics rat group. STD = Standard Diet (AIN 93M), MWRD = Menthik Wangi Rice Diet, SARD = Sagou Analog Rice, SARKBD = Sagou Kidney Bean Analog Rice (10% kidney bean).

OBSERVATION	STD	STDD	MWRD	SARD	SARKBD
PANKREAS-B	14.20 ^A ±8.44	4.60 ^A ±1.95	11.40 ^A ±6.91	15.40 ^A ±9.34	13.00 ^A ±8.83
PANKREA-S	8.40 ^A ±3.98	5.00 ^A ±3.39	7.40 ^A ±5.13	10.00 ^A ±6.82	11.80 ^A ±7.63
PANKREA-K	23.20 ^{AB} ±11.65	6.40 ^C ±2.88	16.80 ^{BC} ±7.16	27.20 ^{AB} ±13.97	33.60 ^A ±16.92

Table 7. Observations of Langerhans. STD = Standard Diet (AIN 93M), MWRD = Menthik Wangi Rice Diet, SARD = Sagou Analog Rice, SARKBD = Sagou Kidney Bean Analog Rice (10% kidney bean).

different result demonstrated in the measurement of HDL level. The sagou 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 dyslipid-

emia, 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 Langerhans islands in the SARKBD was higher as compared to MWRD group and the control group. Data in Table 6 demonstrated that HOMA-IR at the end of the treatment of SARD and SARKBD group was slightly higher than HOMA-IR in the control group (STD). This proved that the 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. Therefore, it will inhibit the lipolysis process. However, the role of insulin in increasing the synthesis of fatty ac-

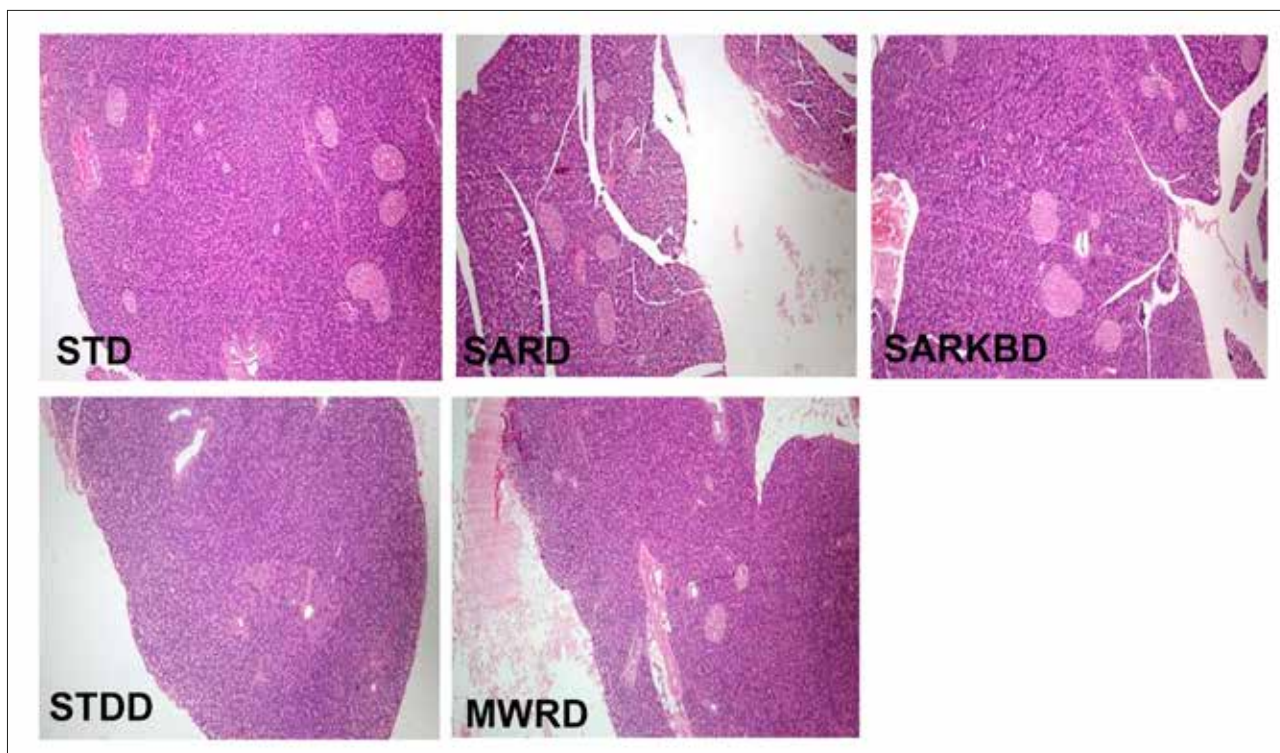


Figure 1. Langerhans Islet of standard and treatment dietary food of control and diabetics rat group by Hematoxylen- Eosin staining. STD = Standard Diet (AIN 93M), MWRD = Menthik Wangi Rice Diet, SARD = Sagou Analog Rice, SARKBD = Sagou Kidney Bean Analog Rice (10% kidney bean).

ids 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 sagou 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 levels, triglyceride levels and increase HDL levels in mice (17, 42-47).

The RS characterized as hypolipidemic which had ability to reduce the cholesterol abundant by providing a substrate to produce a short chain fatty acids (SCFA), especially propionate and butyrate. Those SCFA prevents the synthesis of cholesterol in the liver which caused the increased excretion of bile acids (48). Specifically, cholesterol is also the result of

initial metabolism in the formation of bile acids and plays a role in fat removal (49,50). It supported by the recent result stated that the hypocholesterolemic affected to the food diet with high RS. So that, it inhibits the absorption of bile acids, then their excretion increased (44). However, there are several food components which reduce the cholesterol by HMG-CoA reductase inhibition (51).

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.

• Conflict of Interest

- The Authors declare that there is no conflict of interest.
- **Authors' Contributions**
- All the authors were involved during data collection, analysis, documentation of the collection and writing the publication. S.B.W, and M.M contributes to conception and design, S.B.W, H.H and M.M contributes to acquisition of data, analysis and interpretation of data and all author role for final approval of the version to be published.
- **Acknowledgment**
- This research was supported of financial DGHE, Indonesia of Decentralization Program No. 110.1/USM.H9/L/2018.

REFERENCES

1. International Diabetes Federation. International Diabetes Federation Diabetes Atlas 7th Edition. www.diabetesatlas.org. (Accessed: Desember 6, 2017).
2. Naik SR, Niture NT, Ansari AA, & Shah PD. Anti-diabetic activity of embelin: involvement of cellular inflammatory mediators, oxidative stress and other biomarkers. *Journal Phytomedicine*. 2013; 20(10): 797-804.
3. PERKENI. Pengelolaan dan pencegahan diabetes melitus tipe 2 di Indonesia. *Perkumpulan Endokrinologi Indonesia*. 2015.
4. Renard CB, Kramer F, Johanson F, Lamharzi N, Tannock LR, Von Herrath MG, Chait A, Bornfeldt K. Diabetes and diabetes-associated lipid abnormalities have distinct effect on initiation and progression of atherosclerotic lesion. *J Clin Invest*. 2004; 114: 659-668.
5. The British Diabetic Association's. Dietary recommendations for diabetics for the 1980s. A policy statement by the British Diabetic Association. *Hum Nutr: Application Nutrition*. 1982; 378-394.
6. Franz MJ. Medical nutrition therapy for diabetes mellitus and hypoglycemia of nondiabetic origin. 2012; 30: 764-809.
7. Marsono Y. Glycemic index of selected Indonesian starchy foods. *Indonesian Food and Nutrition Progress*. 2001; 8: 15-20.
8. Gomes LDOF, Santiago RDAC, Carvalho AV, Carvalho RN, Oliveira IGD & Bassinello PZ. Application of extruded broken bean flour for formulation of gluten-free cake blends. *Food Sci Technol (Campinas)*. 2015; 35(2): 307-313.
9. Mishra A, Mishra HN, Rao PS. Preparation of rice analogues using extrusion technology. *Int J Food Sci Technol*. 2012; 47(9): 1789-1797.
10. Riaz MN. *Extruders in food applications*. CRC Press Inc, Boca Raton, US, 2000..
11. Englyst HN, Kingman SM, Cummings JH. Classification and measurement of nutritionally important starch fractions. *Eur J Clin Nutr*. 1992; 46(2): S33-S50.
12. Behall KM, Hallfrisch J. Plasma glucose and insulin reduction after consumption of bread varying in amilose content. *Eur J Clin Nutr*. 2002; 56: 913-920.
13. Wolever TMS, Mchling C. High-carbohydrate-low-glycemic index dietary advice 570 improves glucose disposition index in subjects with impaired glucose tolerance. *571 Br J Nutr*. 2002; 87: 477-487.
14. Wahjuningsih SB, Marsono Y, Praseptianga D, Haryanto B. Resistant starch content and glycaemic index of sago (*Metroxylon spp.*) starch and kidney bean (*Phaseolus vulgaris*) based analogue rice. *Pakistan J Nutr*. 2016; 15(7): 667-672.
15. Wahjuningsih SB. Karakterisasi Sifat Fungsional dan Identifikasi Nilai Indeks Glikemik serta Sifat Hipoglikemik Beras Analog Berbasis Pati Sagu (*Metroxylon spp.*) dan Tepung Kacang Merah (*Phaseolus vulgaris*). Disertasi. Universitas Sebelas Maret, Surakarta, 2017.
16. Wahjuningsih SB, Haslina, Untari S, Wijanarka A. Hypoglycemic Effect of Analog Rice Made from Modified Cassava Flour (Mocaf), Arrowroot Flour and Kidney Bean Flour on STZ-NA Induced Diabetic Rats. *Asian J Clin Nutr*. 2018; 10(1): 8-15.
17. Aggarwal D, Sabikhi L, Lamba H, Chaudhary N, Kapila R. Whole grains and resistant starch rich, reduced-calorie biscuit diet as a hypoglycaemic, hypolipidaemic and insulin stimulator in streptozotocin-induced diabetic rats. *Int J Food Sci Technol*. 2017; 52: 118-126.
18. Wahjuningsih SB, Marsono Y, Praseptianga D, Haryanto B. A study of sago starch and red bean flour-based analog rice development as functional food. eproceedings of the 3rd international conference on agricultural and food engineering "sustaining agriculture, preserving life", Department of Process and Food Engineering, Department of Biological and Agricultural Engineering, UPM, 23-25 Agustus 2016, Kuala Lumpur, Malaysia, 2016; 71-76.
19. Reeves PG, Nielsen FH, Fahey GCJr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on reformulation of the AIN-76A rodent diet. *J Nutr*. 1993; 123(11): 1939-1951.
20. Gupta R, Sharma SB. Effect of germinated Glycine max seeds on glycemic control in STZ+NAD induced type 2 diabetic models: a preliminary study. *JEIM*. 2012; 2(2): 155-160.
21. Saeed S, Mosa-Al-Reza H, Fatemeh AN, Saeideh D. Antihyperglycemic and antihyperlipidemic effects of guar gum on streptozotocin-induced diabetes in male rats. *Pharmacogn Mag*. 2012; 8(29): 65-72.
22. Balasinska B. Hypocholesterolemic effect of food dietary evening primerose (*Oenothera paradoxa*) cake extract in rats. *Food Chemistry*. 1998; 63(4): 453-459.
23. Gandhi GR, Pautu V, Stalin Antony, Irudayaraj SS, Ignacimuthu S, Paulraj MG. Polyphenol-rich *Cyamopsis tetragonoloba* (L) Taub. beans show hypoglycemic and Beta-cells protective effects in type 2 diabetic rats. *Journal Food and Chemical Toxicology*. 2014; 66: 358-365.
24. Hirata A, Maeda N, Hiuge A, Hibuse T, Fujita K, Okada T, Kihara S, Funahashi T, Shimomura I. Blockade of mineralocorticoid receptor reverses adipocyte dysfunction and insulin resistance in obese mice. *Cardiovasc Res*. 2009; 84(1): 164-172.
25. Ndaong NA. Efek Pemaparan Deltamethrin Pada Broiler Terhadap Aktivitas Enzim Alanine Amino Transferase, Aspartat Aminotransferase, Gambaran Histopatologi Hepar Dan Feed Conversion Ratio, Tesis. Fakultas Kedokteran Hewan. Universitas Gadjah Mada, Yogyakarta, 2013.
26. Selan YN. Morfologi Dan Momfometri Saluran Pencernaan Kalong Kapauk (*Pteropus vampyrus*) Beserta Distribusi Sarafnya. Tesis. Fakultas Kedokteran Hewan. Universitas Gadjah Mada, Yogyakarta, 2013.
27. Akah PA, Alemji JA, Salawu OA, Okoye TC, Offiah NV. Effects of *Vernonia amygdalina* on biochemical and hematological parameters in diabetic rats. *Asian J Med Sci*. 2009; 1:108-113.
28. Vijayaraghavan K. Treatment of dyslipidemia in patients with type 2 diabetes. *Lipids in Health and Disease*. 2010; 9: 1-12.
29. Sone H, Nakagami T, Nishimura R, Tajima N, MEGA Study Group. Comparison of lipid parameters to predict cardiovascular events in Japanese mild-to-moderate hypercholesterol-

- emic patients with and without type 2 diabetes: Subanalysis of the MEGA study. *Diabetes Res Clin Pract.* 2016; 113: 14–22.
30. Al-Jada DN, Ahmad MN. Effect of dietary fats on the development of insulin resistance in relation to PPAR γ activation and leptin concentration in rats. *Journal of American Science.* 2014; 10(8): 59–66.
 31. Calvo-Ochoa E, Hernandez-Ortega K, Ferrera P, Morimoto S, Arias C. Short-term high-fat-and-fructose feeding produces insulin signaling alterations accompanied by neurite and synaptic reduction and astroglial activation in the rat hippocampus. *J Cereb Blood Flow Metab.* 2014; 34: 1001–1008.
 32. DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care.* 2009; S157–S163.
 33. Liese AD, Roach AK, Sparks KC, Marquart L, D'Agostino Jr RB, Mayer-Davis EJ. Whole-grain intake and insulin sensitivity: the Insulin Resistance Atherosclerosis Study. *Am J Clin Nutr.* 2003; 78: 965–971.
 34. Mahfouz MM, and Kummerow FA. Cholesterol-rich diets have different effects on lipid peroxidation, cholesterol oxides, and antioxidant enzymes in rats and rabbits. *J Nutr Biochem.* 2000; 11: 293–302.
 35. Wilson JD, Foster DW. *William textbook of endocrinology* 7th edition. WB. Saunder Co, Philadelphia, 1985.
 36. Sajilata MG, Singhal RS, Kulkarni PR. Resistant starch – A review. *Compr Rev Food Sci Food Saf.* 2006; 5: 1–17.
 37. Aluko RE. *Functional feed and nutraceutical.* Springer, USA, 2012.
 38. Kaplan A, Szabo LL. *Clinical Chemistry, Interpretation and Techniques* 2nd edition. Lea and Febiger, Philadelphia, 1983.
 39. Marsono Y. Sifat Hipoglisemik dan Hipolipidemik Kacang Kapri dan Kedele Pada Tikus Spreque Dawley Diabetik Induksi Alokasan. *Jurnal Agritech.* 2002; 22(4): 137–143.
 40. Hartoyo A, Muchtadi D, Astawan M, Dahruhsyah, dan Winarto A. Pengaruh ekstrak protein kacang komak (*Lablab purpureus* (L.) Sweet) pada kadar glukosa dan profil lipida serum tikus diabetes. *Journal of Food Technology and Indutstry.* 2011; 22(1): 58–63.
 41. Erukainure OL, Osaretin AT, Ebuehi, Adeboyejo FO, Okafor EN, Muhammad A, Elemo G.N. Fiber-enriched biscuit enhances insulin secretion, modulates b-cell function, improves insulin sensitivity, and attenuates hyperlipidemia in diabetic rats. *Pharma Nutrition.* 2013; 1: 58–64.
 42. Han K, Sekikawa M, Shimada K, Sasaki K, Ohba K, Fukushima M. Resistant starch fraction prepared from kintoki bean affects gene expression of genes associated with cholesterol metabolism in rats. *Exp Biol Med.* 2004; 229: 787–792.
 43. Higgins JA, Higbee DR, Donahoo WT, Brown IL, Bell ML, Bessesen DH. Resistant Starch Consumption Promotes Lipid Oxidation. *Nutr Metab J.* 2004; 1 (8): 1–11.
 44. Park OJ, Nam E K, Chang MJ, and Kim WK. Resistant Starch Supplementation Influences Blood Lipid Concentrations and Glucose Control in Overweight Subjects. *J Nutr Sci Vitaminol.* 2004; 50: 93–99.
 45. Damat, Marsono Y, Haryadi, Cahyanto MN. Efek hipokoles-terolemik dan hipoglikemik pati-garut butirat pada tikus Sprague Dawley. *Majalah Farmasi Indonesia (MFI), Fakultas Farmasi, UGM. Jurnal Terakreditasi.* 2008; 19(3).
 46. Ha AW, Han GJ, dan Kim WK. Effect of retrograded rice on weight control, gut function, and lipid concentrations in rats. *Nutr Res PracT.* 2012; 6(1): 16–20.
 47. Nugraheni M, Hamidah S, Auliana R. Pengaruh Konsumsi Crackers Kentang Hitam (*Coleus Tuberosus*) Kaya Resistant Starch Tipe 3 Terhadap Profil Lipida Tikus Yang Menderita Hiperkolesterolemia. *Jurnal Penelitian Sainstek.* 2016; 21(1): 21–31
 48. Weitkunat K, Schumann S, Petzke KJ, Blaut M, Loh G, Klaus S. Effects of dietary inulin on bacterial growth, short-chain fatty acid production and hepatic lipid metabolism in gnotobiotic mice. *J Nutr Biochem.* 2015; 26(9).
 49. Lehninger AL. *Principles of biochemistry* 2nd edition. Worth Publishers, New York, 1982.
 50. Bertolotti M, Crosignani A, Del Puppo M. The use of stable and radioactive sterol tracers as a tool to investigate cholesterol degradation to bile acids in humans in vivo. *Molecules.* 2012; 17: 1939–1968.
 51. Chen JJ, Lii CY, Lu S. Physicochemical and morphological analyses on damaged rice starches. *J Food Drug Anal.* 2003; 4: 283–289.
 52. Cedo L, Metso J, dan Santos D. Consumption of polyunsaturated fat improves the saturated fatty acid-mediated impairment of HDL antioxidant potential. *Mol Nutr Food Res.* 2015; 59: 1987–1996.
 53. Robertson MD, Bickerton AS, Dennis AL, Vidal H, Frayn KN. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. *Am J Clin Nutr.* 2005; 82: 559–567.
 54. Johnston KL, Thomas EL, Bell JD, Frost GS, Robertson MD. Resistant starch improves insulin sensitivity in metabolic syndrome. *Diabet Med.* 2010; 27(4): 391–397.
 55. Raso GM, Simeoli R, Russo R, Iacono A, Santoro A, Paciello O, Ferrante MC, Canani RB, Calignano A, Meli R. Effects of sodium butyrate and its synthetic amide derivative on liver inflammation and glucose tolerance in an animal model of steatosis induced by high fat diet. *PLOS ONE.* 2013; 8(7): 1–13.
 56. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Endocrinol (Nature Reviews).* 2015; 11(10): 577–591.