



Effect of Sago Analogue Rice and Red Bean Diet to the Improvement of β -cell Pancreas in Streptozotocin-Nicotinamide (STZ-NA) Induced Diabetic Rats

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Abstract

The study aims to determine the effect of sago analogue rice and red beans in diabetic rats to repair pancreatic β -cells. Thirty-five males Wistar rats were divided into 5 groups: normal group diet (STD), the diabetic group (STDD) with a standard feed diet, the diabetic group with *mentik wangi* rice (MWRD), the diabetic group with sago analogue rice (SARD) and the diabetic group with sago analogue rice with the addition of 10% red bean flour (SARKBD). All groups were analysed for dietary interventions, blood glucose level, insulin level for HOMA- β and HOMA S indices and measurement of insulin level by using IHC analysis. In addition, short-chain fatty acids (SCFA) analysis was performed in the caecum. This study showed that decreasing blood glucose level shown in SARD and RASKBD groups. The pancreatic β -cell number indicated an increase in the SARD group compares to the STDD group. The pool total of SCFA in SARD group was the highest among of all groups, as well as the acetate, propionate and butyrate pools. These results indicate that the sago analogue rice diet could repair and increase the expression of pancreatic β -cell through absorption inhibition mechanisms and by increasing insulin sensitivity and the SCFA level.



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Keywords

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Introduction


Diabetes mellitus (DM) is a metabolic disease characterized by increasing blood sugar

(hyperglycemia) caused by a lack of insulin sensitivity.¹ More than 90% of Diabetes Mellitus Type 2 (DMT2) cases, which is diabetes caused by

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low insulin sensitivity.² According to the International Diabetic Federation,³ around 415 million people have DM and this may increase to 642 million in 2040. Basic Health Research also states that DM proportion at the age of > 15 years was increasing to 5,7%.⁴ Furthermore, 90-95% of cases of DM are type 2 DM and caused by the lack of insulin sensitivity and resistance of peripheral insulin.⁵

Type 2 diabetes mellitus (DMT2) was caused by the imbalance between free radicals and antioxidants that can reduce sensitivity to insulin (Evans *et al.*, 2002). Insulin resistance is indicated by the malfunction production of insulin by pancreatic β -cell, in other words, it shows the decrease of insulin sensitivity. The diabetes conditions are usually followed by hyperlipidemia cases.⁶ The (DMT2) case also could be controlled with diet management properly. Diet management involving resistant starch is one of the alternatives to improve glucose response in diabetics.⁷⁻¹⁰ Resistant starch (RS) is a fraction of the edible part of plants or their extracts, or synthetic analogue that are resistant to the hydrolysis of amylase digestive enzymes in the small intestine.¹⁰ Both of analogue sago rice and sago rice with the addition of red beans can be used as a new ingredient in DM diet therapy because they are rich in resistant starch. Sago analogue rice and red beans were expected to reduce insulin resistance and decrease blood glucose level as well as improve lipid profiles in diabetic rats.⁶ The purpose of this study is to determine the effects of sago analogue rice and kidney beans on the improvements of pancreatic β -cell in the diabetic rats induced by Streptozotocin-Nicotinamide (STZ-NA).

Materials and Methods

Materials

The sago starch (*Metroxylon* spp.) Meranti variety was obtained from Selat Panjang, Riau and local varieties of red beans (*Phaseolus vulgaris*) were taken from farmers in Parakan Subdistrict, Temanggung Regency. The Mentik wangi rice was obtained from a supermarket in Yogyakarta.

Animal model

In this study, 2-3 months male Wistar rats (200-250g) were obtained from the Experimental Animal Laboratory, UPT LPPT, Universitas Gadjah Mada, Yogyakarta. The rats were conditioned with a 12-

hour dark/light cycle, an adequate air ventilation in the cage at the temperature of 25°C. They were then divided into five groups and each group was treated with different diet formulation. The groups consisted of: one normal control group (STD) which follow standard AIN93M feed diet¹¹ and four diabetic induced groups which were treated with different kind of diets: standard diet (STDD), mentik wangi rice (MWRD), 100% sago rice (SARD) and 10% red bean sago rice (SARKBD). The food and water were given in ad libitum. Diabetes induction was carried out with nicotinamide (NA) in 0.9% buffer saline (NaCl) at a dose of 230 mg/kg intraperitoneally, fifteen minutes later the rat was induced with Streptozotocin 60 mg/kg (Gupta and Sharma, 2012). After induction, the rat was given a 5% glucose solution in water for 24 hours to reduce the possibility of death due to hypoglycemic effects (Saeed *et al.*, 2012). This conditions and handling were approved by the Ethics Commission for Preclinical Research, Gadjah Mada University No. 00070/04/LPPT/X/2016.

Analogue Rice Production

Analogue rice was used in 2 different formulas; analogue rice from sago starch (SARD) and sago analogue rice with 10% red bean flour (SARKBD).¹²

Food Formulation

Food formulation refers to formula of AINM 1993. The details of the formula can be found in the previous study.⁶

In Vivo Analysis

Five days after STZ-NA induction, the blood glucose and fasting insulin level were measured using glucose oxidase phenol aminophenazone or GOD-PAP methods.¹³ The blood was aspirated from vena retroorbital by microcapillary technique. The criterion of rat diabetic is fasting blood glucose level more than 200 mg/dL. The intervention period of the treatment was 4 weeks and blood glucose level were analyzed each week.

HOMA Analysis

The Homeostatic model assessment and insulin resistance (HOMA-IR) index were analyzed according to Matthews *et al.*,¹⁴ while Homeostasis Model of Assessment- β (HOMA- β) was analyzed according to Hirata *et al.*,¹⁵ methods.

Immunohistochemistry Analysis

Immunohistochemistry assays were performed on formalin-fixed paraffin in pancreatic tissue. The organ was deparaffinized in xylol and rehydrated with graded alcohol and then washed in phosphate buffer solution (PBS) pH 7.4. The endogenous peroxidase blocking was performed with 3% H₂O₂ solution in distilled water for 30 minutes, it was then washed with PBS solution. The blocking was performed for 5 minutes with goat serum and dripped anti-insulin primary antibody (1: 300) for overnight in the 4°C, after that, it was washed in PBS. The secondary antibodies used were biotinylated goat anti-polyvalent and was performed for 10 minutes and the conjugate of the streptavidin peroxidase enzyme was added. The substrate and chromogen 3,3 diaminobenzidine (DAB) were added, afterwards, the mixture was incubated for 10 minutes at room temperature in the dark. The slide was then washed with distilled water solution and was treated with counterstain with hematoxylin for 10 seconds.

The result of immunohistochemistry staining was visualized and analyzed using BX53 (Olympus) with 200X Magnifications.

SCFA Analysis

The short-chain fatty acid (SCFA) level were analyzed by the gas chromatography method in the caecum. The caecum was weighed then centrifuged at 10,000 rpm for 15 minutes. The supernatant was separated and injected into the GCMS column (Shimadzu GC 8A brand, with FID detector). The caecal SCFA pool was concentrated in the caecum ($\mu\text{mol/g}$) by the mass of the caecal contents (g) and was expressed in micromoles per 100 g of body weight.¹⁶

Statistical Analysis

Statistical analysis was conducted using variance (ANOVA) with IBM SPSS Statistics version 20 and DMRT test with 5% confidence level.

Table 1: Glucose level changes (%) in every week treatment of STD, STDD, MWRD, SARD and SARKBD rat groups

Treatment	STD	STDD	MWRD	SARD	SARKBD
Glucose changes 1	1.01 ^a ± 0.35	155.09 ^b ± 3.64	155.66 ^b ± 5.09	156.22 ^b ± 6.69	161.12 ^b ± 10.83
Glucose changes 2	5.77 ^c ± 1.92	4.21 ^c ± 7.79	-22.79 ^b ± 7.49	-57.27 ^a ± 5.19	-55.69 ^a ± 10.26
Glucose changes 3	1.29 ^d ± 0.57	1.57 ^d ± 3.97	-12.78 ^c ± 1.11	-20.29 ^a ± 0.48	-16.01 ^b ± 4.25
Glucose changes 4	0.93 ^d ± 0.23	1.27 ^d ± 0.49	-2.93 ^c ± 0.42	-14.21 ^a ± 0.81	-11.22 ^b ± 1.65
Glucose changes 5	0.50 ^d ± 0.28	1.55 ^e ± 0.51	-1.82 ^c ± 0.64	-28.99 ^a ± 0.99	-13.45 ^b ± 1.31

Note : Data were presented as mean ± STDEV of three repetitions samples (n=3). Different superscripts in the same row showed significant different ($p > 0.05$).

Results and Discussion

The level of Blood Glucose

The blood glucose level of rat induced by STZ-NA were not significantly different in all groups, the changes ranging from around 150-160% in first week period with STDD, MWRD, SARD and SARKBD were 155.09%, 155.66%, 156.22%, and 161.12%, respectively. (Table 1).

The STDD group showed positive changes in every week of treatment, while the MWRD, SARD and SARKBD showed the negative changes. The positive changes indicate that the group still have

diabetes condition. Negative blood glucose level was demonstrated by the MWRD, SARD and SARKBD with an average percentage of 10.08%, 30.19%, and 24.09%, respectively. The highest alteration of blood glucose was shown by the SARD group. Feed intervention on all group of diabetes-induced rats demonstrated a decrease in blood glucose levels at the end intervention period.

STZ-NA is one of the diabetic agents that has a toxic ability to destroy pancreatic β -cell. It contains Nicotinamide (NA) which is a derivative of vitamin B3 (niacin) that has an antioxidant capacity to reduce

toxic effects from STZ.^{9,17} In β cells, STZ attenuates the glucose oxidation and insulin synthesis, also decrease the glucose transport and glucokinase activity. SARD group showed the highest changes in blood glucose levels in every week of treatment, and this is followed by SARKBD and MWRD group. This research showed that dietary intervention groups can lower blood glucose due to the presence of resistant starch. RS in sago rice (SARD and SARKBD) was 12.25% and 11.80%, respectively.⁹ Meanwhile, the content of RS in fragrance rice was only 10.72%. According to Marsono *et al.*,¹⁸

RS could reduce blood glucose level because it cannot be digested in the intestine which impacts to the decrease of the amount of glucose availability. Besides, the viscous characteristic of RS able to inhibit small intestine absorption. In addition, the Glycemic index of sago and red bean-based analogue rice was 40.7 and 50.4⁹ while, rice was 80.^{18,19} Similarly, this was also supported by the research of Okoniewska and Witwer²⁰ which states that RS in the small intestine can reduce the glycemic and insulin response in diabetics and hyperinsulinemic patients.

Table 2: HOMA β and HOMA S level of STD, STDD, MWRD, SARD and SARKBD rat groups.

Treatment	STD	STDD	MWRD	SARD	SARKBD
Homa β	2.84 ^b \pm 0.94	0.40 ^a \pm 0.15	0.38 ^a \pm 0.05	0.83 ^a \pm 0.07	0.46 ^a \pm 0.15
Homa S	1.07 ^c \pm 0.40	0.11 ^a \pm 0.03	0.19 ^a \pm 0.03	0.54 ^d \pm 0.06	0.54 ^b \pm 0.20

Note : Data were presented as mean \pm STDEV of three repetitions samples (n=3). Different superscripts in the same row showed significant different (p>0.05).

HOMA β and HOMA S Level

The index data of HOMA β and HOMA S are shown in Table 2 in which it shows that SARD group has

the highest index among other groups at the end of intervention.

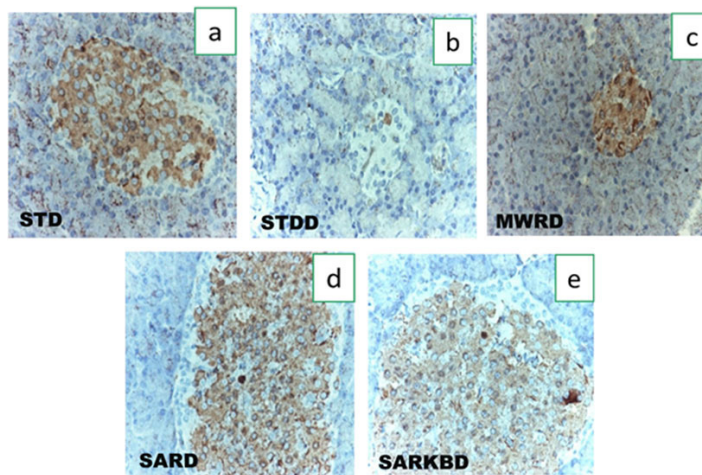


Fig.1: The insulin expression of pancreatic Langerhans beta cells with specific antibody and was visualized using BX53 microscope with 200X Magnification

The high value of HOMA β and HOMA S in the treated group showed that the β -cell function and insulin sensitivity were still high. In this study,

treatment with Sago and red bean analogue rice showed a decrease in blood glucose levels in every week of treatment and this occurs through the

increase of insulin sensitivity. Some previous studies reported that RS could improve insulin sensitivity by increasing the glucose uptake from the blood to muscle.²¹⁻²⁵

Immunohistochemistry Analysis

The expression profile of insulin in the islets of Langerhans is shown in Figure 1. The insulin expression profile was higher in SARD and SARKBD group compare to STDD group. While, the number of pancreatic β -cell for SARD, SARKBD, and STDD, were 92.42%, 85.62% and 21.97% respectively.

The high expression of pancreatic β -cell could affect the alteration of insulin level, increase insulin sensitivity and improve the β -cell function.^{26,27} This result showed that SARD and SARKBD interventions have high expression profile values compared to

other diet groups. This indicates that the sago rice diet can enhance insulin production and sensitivity.

On the other hand, STDD group showed the lowest expression of insulin in pancreatic Langerhans beta cells. In this case, it is argued that STZ-NA induction can damage the pancreatic Langerhans beta cells and standard food is not capable enough to repair the defect.

The sago analogue rice can be a promising diet treatment for improving insulin sensitivity by increasing insulin expression and regeneration in pancreatic Langerhans β -cell.²⁷ We expected that this is due to the effect of resistant starch characteristic on Sago analogue rice that impact insulin sensitivity.

Table 3: The Pool SCFA of STD, STDD, MWRD, SARD and SARKBD rat groups

Pool SCFA	STD	STDD	MWRD	SARD	SARKBD
Acetate	47.27 ^b ± 4.49	20.44 ^a ± 1.21	51.93 ^c ± 1.81	148.12 ^e ± 3.13	84.79 ^d ± 0.53
Propionate	21.89 ^b ± 2.08	10.26 ^a ± 0.61	28.28 ^c ± 0.99	58.88 ^e ± 1.24	38.56 ^d ± 0.24
Butyrate	7.85 ^b ± 0.74	3.04 ^a ± 0.18	9.38 ^c ± 0.33	30.90 ^e ± 0.65	15.56 ^d ± 0.09
Total	77.01 ^b ± 7.31	33.74 ^a ± 2.00	89.58 ^c ± 3.13	237.89 ^e ± 5.02	138.92 ^d ± 0.87

Note : Data were presented as mean ± STDEV of three repetitions samples (n=3). Different superscripts in the same row showed significant different (p>0.05).

SCFA Analysis

The pool of short chain fatty acids (SCFA) level of all group are shown in Table 3. The SARD group showed the highest pool SCFA level of acetate, propionate, butyrate and in total. Meanwhile, the STDD group was the lowest concentration among other groups. Furthermore, the STD group showed the lower amount of SCFA than SARKBD and MWRD group. After all, the STDD group was significantly different (p <0.05) with SARKBD, MWRD, STDD and STD.

The enhancement of insulin sensitivity can be caused by the production of SCFA through RS fermentation. Canfora *et al.*,²⁸ explained that acetate and propionate from RS fermentation products can increase buffering capacity which decreases the fatty acids in the muscles.²⁸ This can cause a decrease of lipid storage and increase insulin sensitivity.

The mechanism of blood glucose reduction was due to the ability of SCFA to reduce blood glucose through several mechanisms. Gao *et al.*,²⁹ stated that the products of RS fermentation bring a hipolipidemic effect on STZ-NA induced-rats. This is also supported by Luo *et al.*,³⁰ which mention that high fatty acids can inhibit the process of glucose utilization in muscle tissue and insulin resistance disorder. Other researchers reported that the increase in SCFA concentration in humans suppress the formation of free fatty acids and thus, improves insulin sensitivity.^{31,32}

According to Aluko,³³ the high level of free fatty acid can cause disruption of GLUT4 activity and impact on the increase of blood glucose level. Whereas, the SCFA can increase fatty acid β oxidation to

avoid high free fatty acids level and induce the activity of GLUT4 to increase.³⁰ Furthermore, in this study, butyrate was found in the high level in sago analogue rice and red bean group treatment. The butyrate and acetate are fatty acid that can increase AMPK (AMP-activated protein kinase) for stimulating glucose absorption. According to Wronkowska *et al.*,¹⁶ the resistant starch diet produced the highest SCFA concentrations including butyrate compared to the standard diet. The butyrate can increase energy production and improve the mitochondrial function to increase insulin sensitivity and it also improves glucose metabolism.^{29,34} Another study, Gao *et al.*,²⁹ reported that butyrate also induces PGC-1 α (PPAR Gamma Coactivator -1 α) in the muscle so that improve the mitochondrial function, increase fatty acid oxidation and reduce the risk of decreased insulin sensitivity.

Conclusion

This study concludes that sago analogue rice and sago analogue rice with 10 % red bean flour in diabetic rats have an effect to improve and increase pancreatic Langerhans β -cell expression. The mechanism involves the role of resistant starch in the Sago analogue rice that reduce absorption, increase insulin sensitivity and improve the better result for total pool of SCFA alteration.

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Conflict of Interest

There are no conflict interests

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