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Research Article

Antidiabetic Activity of *Lactobacillus fermentum* Bacteria from *Dengke Naniura* Goldfish (*Cyprinuscarpio*) in Nicotinamide-Streptozotosin Induced Rats

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ABSTRACT

Objectives: This study aims to determine the effect of *Lactobacillus fermentum* on decreasing blood glucose levels, sRAGE, HbA1c levels.

Methods: Testing was done on male rats, which consisted of 7 groups. Group 1 as negative control was given Na-CMC0.5%, group 2 was given *Lactobacillus fermentum* isolate dose 0.5mL, group 3 was given *Lactobacillus fermentum* isolate dose 1mL, group 4 was given *Lactobacillus fermentum* isolate dose 1.5mL, group 5 was given *Lactobacillus fermentum* isolate dose 2 mL, group 6 as positive control was given glibenclamide dose 0.45 mg/kg, and group 7 as the normal group. Decreased KGD, sRAGE, HbA1C.

Results: The results of statistical analysis showed that the administration of *Lactobacillus fermentum* showed the effect of a decrease in blood glucose (KGD) rats that were not significantly different than the positive control group ($p < 0.05$). From the results of testing the *Lactobacillus fermentum* 2 mL treatment group gave the best results on decreasing sRAGE levels; decreased HbA1C.

Conclusion: Based on the results of this study *Lactobacillus fermentum* bacteria can reduce blood glucose, sRAGE, HbA1C levels in rats induced by Nicotinamide-Streptozotosin.

Keywords: *sRAGE, HbA1C, Insulin, Lactobacillus fermentum, reduction of KGD.*

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INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by chronic hyperglycemia with impaired carbohydrate, fat and protein metabolism, which occurs due to abnormalities in insulin secretion, insulin dysfunction or both. Chronic hyperglycemia in DM is associated with long-term damage, dysfunction or failure of several organs such as: eyes, kidneys, nerves, heart, and blood vessels [1].

High blood glucose levels in the long run in people with DM cause glycation (the bond between glucose and the amin group NH2 lysine residues) of blood protein. Glycation causes chemical changes from proteins, and other macromolecules that play a role in the pathogenesis of diabetes complications. The final glycation product, Advanced Glycation End Products (AGEs), is a collection of molecules formed from the nonenzymatic reaction of reduced sugars with amino acids from proteins, lipids and nucleic acids. The initial product of this reaction is called Schiff base, which spontaneously forms Amadori products in diabetes known as hemoglobin A1c (HbA1c) [2].

Based on the background, researchers are interested in testing the effect of *Lactobacillus fermentum* on blood sugar levels (KGD), sRAGE levels, HbA1c levels, and insulin expression in mice that are induced by

Nicotinamide-Streptozotosin. Tests will be carried out on mice induced with nicotinamide (NA)-streptozotosin (STZ). STZ triggers damage to pancreatic β cells (insulin secreting cells) while NA can partially protect pancreatic β cells from damage caused by STZ. The expected result of NA-STZ induction is partial pancreatic β-cell damage (40%), so that mice develop DM type 2 [3].

MATERIALS AND METHODS

Materials and Tools

Autoclave (Hirayama), desiccator (IaswerkWerti), glucometer and glukotes strip (Easy Touch® GCU), Laminar Air Flow (Labconco), microscope inverted (Olympus), microwave (Panasonic), micropipette (Eppendorf), electric balance (Easy Touch® GCU), Laminar Air Flow (Labconco), microscope inverted (Olympus), microwave (Panasonic), micropipette (Eppendorf), electric balance (Vibra AJ)), oven (Memmert), rotary evaporator (Stuart), centrifugator (Eppendorf), vortex (IKA), water bath. Aquabides, distilled water, 96% ethanol, fine test kits, ketamine-hameln (PT. Combiphar), MRS broth (Merck), nicotinamide, streptozotosin (NacalaiTasque, Kyoto, Japan).

RESEARCH METHODS

Making *Lactobacillus fermentum* isolates

The making of *Lactobacillus fermentum* bacterial isolate using the Surono method (2004) with the modification of making a mother starter. Preparation of the main starter was started by diluting the MRS broth as much as 5.2 grams with 100 ml aquadest, then sterilized by autoclaving at 121^{o}C for 15 minutes [2].

1 ml of pure *Lactobacillus fermentum* culture was grown in 10 ml of sterile MRS broth, then incubated at 37^{o}C for 24 hours then after growing, re-grown in sterile MRS broth media and incubated at 37^{o}C for 12 hours 5% (V/V) with 24 hours after growth, re-growing in sterile MRS broth media and incubated at 37^{o}C for 12 hours 5% (V/V) with 24 hours density of 10^{7}-10^{9} CFU/mL [4].

Making Streptozotosin Solution (STZ)

A total of 65 mg of STZ was dissolved in 10 mL of 0.9% NaCl solution [5].

Making Nicotinamide Solution

110 mg of nicotinamide was dissolved with 10 ml of 0.9% NaCl, then injected intraperitoneally in mice at a dose of 110 mg/kg bb [5].

Preparation of Test Animals

The animals used are male white rats with weights ranging from 180-200 grams. Before the experiment, the rats were kept for 2 weeks in a good cage to adjust their environment, that is, with 12 hours of dark light and 12 hours of light [1].

Induction of Test Animals

42 male mice weighing 180-200 g that had been fasted for 18 hours, weighed, determined fasting KGD, induced with 110 mg/kg bbnicotinamide solution and streptozotosin 65 mg/kg intraperitonially. Rats were measured their blood glucose levels on the 5th day. Rats are considered diabetic if fasting blood glucose levels are ≥ 200 mg/dL and can be used for testing [6].

Testing the Antidiabetic Activity of *Lactobacillus fermentum*

Test animals used in this experiment were Wistar-induced white rats nicotinamide 110 mg/kg bb and streptozotosin 65 mg/kgBB divided into 7 groups and each group consisted of 6 animals, among others:

Group 1 : Normal (without treatment)

Group 2 : 0.5% Na-CMC suspension

Group 3 : Administration of 0.5 mL *Lactobacillus fermentum* isolate

Group 4 : Administration of 1.0 mL *Lactobacillus fermentum* isolate

Group 5 : Administration of 1.5 mL *Lactobacillus fermentum* isolate

Group 6 : Administration of 2.0 mL *Lactobacillus fermentum* isolate

Group 7 : Administration of glibenclamide 0.45 mg / kg bb

Each group was given an oral test, then blood glucose levels were measured at week 1 through week 4, at week 4 test animals were first anesthetized using ketamine then dissected, blood was taken from 3-3.5 mL from the heart to obtain plasma used for the determination of sRAGE levels, and HbA1c levels. Mouse pancreatic organs were taken for examination of insulin expression [7].

Measurement of sRAGE and HbA1c levels by ELISA Method

Prepared tools and materials, washed plates with wash buffer 2 times, added 100 µl standard, zero sample and control into each well, closed and incubated for 90 minutes at 37°C. Wash the plates with wash buffer 2 times add 100 µl of biotin solution into each well and incubate for 60 minutes at 37^{o}C. Wash the plate with wash buffer 3 times then add 100 µl of SABC solution into the well and incubate for 30 minutes at 37^{oC} temperatures protected from light. Wash the plates using wash buffer 5 times, add 90 µl TMB substrate to each well and incubate 15-30 minutes at 37^{o}C, protected from light. Observation of color changes (some solutions in the well will turn blue according to concentration). A 50 µl stop solution was added to each well and color changes were observed which appeared yellow. Absorption was read with a microplate reader at a wavelength of 450 nm and the levels were calculated [8].

RESULTS AND DISCUSSION

Effect of *Lactobacillus fermentum* isolates on sRAGE levels in rats

Measurement of sRAGE levels was carried out using the Rat sRAGE Kit with the ELISA method, which was read absorbed by a microplate reader at a wavelength of 450 nm. This method is based on the principle of measurement of antibody antigens that are both relative and quantitative. The sRAGE level is obtained by measuring the absorbance in the presence of 5000ng/ml standard solution; 2500ng/ml; 1250ng/ml; 625ng/ml; 312,5ng/ml; 156.25ng/ml; and 78.1ng/ml. Standard curves as shown in **Figure 1.**

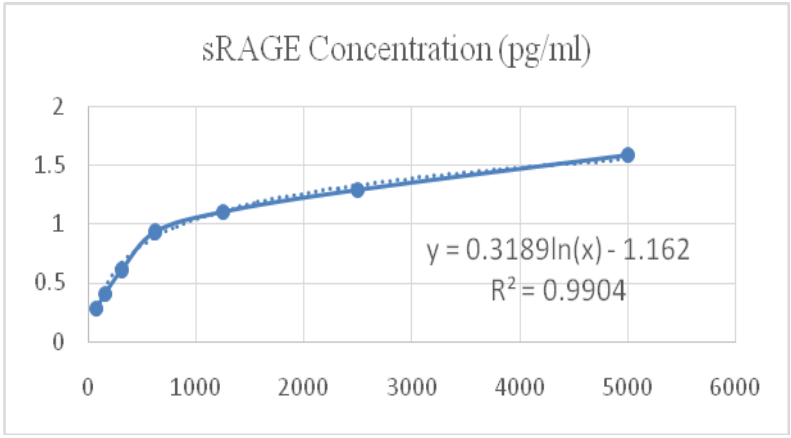

Figure 1: Standard Curve of sRAGE

The sRAGE level is calculated by substituting the absorbance value (y) of the sample at a wavelength of 450 nm into the logarithm regression equation y = ax + b, which is obtained from the standard sRAGE curve so that the sRAGE concentration value (x) is obtained.

The results of the sRAGE concentration were then carried out statistical analysis using One Way Analysis of Variant (ANOVA) obtained significant differences in measurement results ($p<0.05$) between treatment groups. The results of the HbA1C test in rat blood plasma can be seen in **Table 1**.

Table: 1. Concentrations of sRAGE in rat blood

Treatment group	Average Concentration sRAGE± SEM (ng/ml)
Na-CMC 0,5%	575,17 ± 0,64[bc]
Provision isolate of *Lactobacillus fermentum* 0.5 mg / kg bb	132,86 ± 0,05[abc]
Provision isolate of *Lactobacillus fermentum*1 mg/kg bb	109,83 ± 0,15[abc]
Provision isolate of *Lactobacillus fermentum*1,5 mg/kg bb	95,68 ± 0,09[abc]
Provision isolate of *Lactobacillus fermentum*2 mg/kg bb	80,32 ± 0,05[abc]
Glibenklamide 0,45 mg/kg bb	88,19 ± 0,09[ac]
Normal	68,75 ± 0,04[ab]

Information: [a]Sig (p) <0.05 there is a significant difference with the Na-CMC group, [b]Sig (p) <0.05 there is a significant difference with the Glibenclamide group, [c]Sig (p) <0.05 there is a significant difference with the Normal group

Effect isolateof *Lactobacillus fermentum* on HbA1c levels in rats

The measurement of HbA1C was carried out using the Rat HbA1C Kit by the ELISA method, which was read absorbance by a microplate reader at a wavelength of 450 nm. This method is based on the principle of measurement of antibody antigens that are both relative and quantitative. The HbA1C level results are obtained by measuring absorbance in the presence of a standard solution of 100ng/ml; 50ng/ml; 25ng/ml; 6.25ng/ml; 3,125ng/ml; 1,562ng/ml. Standard curves as shown in **Figure 2**.

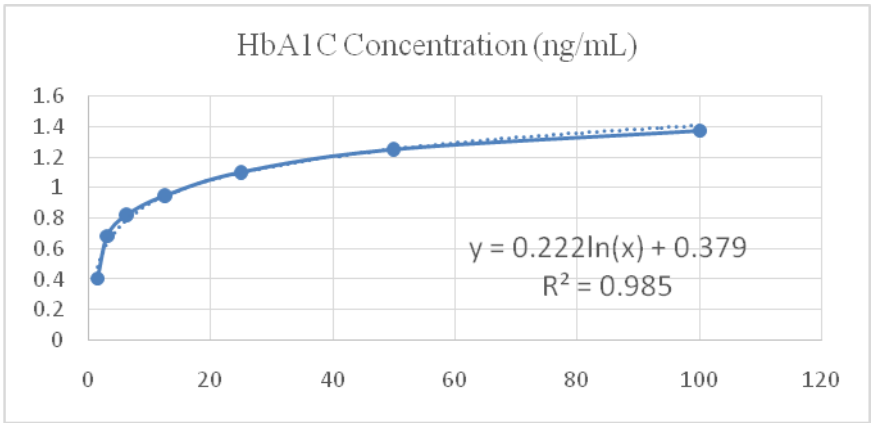

Figure: 2. HbA1C Standard Curve

The HbA1C level is calculated by substituting the absorbance value (y) of the sample at 450 nm wavelength into the logarithm regression equation y = ax + b, which is obtained from the standard HbA1C curve so that the HbA1C concentration value (x) is obtained.

The results of the HbA1C concentration were then performed statistical analysis using One Way Analysis of Variant (ANOVA), which resulted in a significant difference in measurement results ($p <0.05$) between

treatment groups. The results of the HbA1C test on rat blood plasma can be seen in **Table 2**.

Table: 2 HbA1C concentrations in rat blood

Treatment group	Average Concentration HbA1C ± SEM (ng/ml)
Na-CMC 0,5%	43,12 ± 0,19[bc]
Provision isolate of *Lactobacillus fermentum* 0,5 mg/kg bb	10,45 ± 0,04[abc]
Provision isolate of *Lactobacillus fermentum*1 mg/kg bb	6,56 ± 0,02[abc]
Provision isolate of *Lactobacillus fermentum*1,5 mg/kg bb	6,79 ± 0,02[abc]
Provision isolate of *Lactobacillus fermentum*2 mg/kg bb	4,26 ± 0,05[abc]
Glibenklamid 0,45 mg/kg bb	3,96 ± 0,01[ac]
Normal	3,12 ± 0,01[ab]

Information: [a]Sig (p) <0.05 there is a significant difference with the Na-CMC group, [b]Sig (p) <0.05 there is a significant difference with the Glibenclamide group, [c]Sig (p) <0.05 there is a significant difference with the Normal group

The results of analysis of variance (ANOVA) showed a significant difference between treatment groups on the concentration of HbAlc (ng / ml) with a significance value of p <0.05. The results showed that the negative control group (CMC-Na) was 43.12±0.19 ng/ml showing an increase in HbAlc compared to the normal group. This is caused by the diabetogenic effect of STZ which causes necrosis of β-pancreatic cells causing hyperglycemia. At the time of high blood sugar levels, the nonenzymaticglycation process will increase, where the glycation itself will cause the concentration of free radicals to also increase which triggers an increase in HbAlc levels [3].

Oral administration of the *Lactobacillus fermentum* bacterial isolate was able to reduce HbA1C levels in hyperglycemic mice, but it did not match the decrease in HbA1C levels in the glibenclamide group. The HbAlc level in the *Lactobacillus fermentum* bacterial isolate group 0.5 mg/kg bb was 10.45 ± 0.19 ng/ml, in the *Lactobacillus fermentum* bacterial isolate group 1 mg/kg bb was 6.56 ± 0.02 ng/ml, in the *Lactobacillus fermentum* bacterial isolate group 1.5 mg/kg bb is 6.79 ± 0.02 ng/ml, in the *Lactobacillus fermentum* bacterial isolate group 1.5 mg/kg bb is 6.79 ± 0.02 ng/ml, the four groups showed significant differences between the glibenclamide group and the normal group. Thus, it can be concluded that the one who gave the biggest decrease in HbA1c was *Lactobacillus fermentum* 2ml>*Lactobacillus fermentum* 1.5 ml>*Lactobacillus fermentum* 1ml>*Lactobacillus fermentum* 0.5 ml.

The decrease in HbAlc reflects the effect of decreased blood glucose by increasing insulin secretion due to the regeneration of B-pancreatic cells. *Lactobacillus fermentum* decreases protein levels by inhibiting the enzyme α-glucosidase in the small intestine. Inhibition of the enzyme a-glucosidase causes a decrease in the rate of digestion of carbohydrates into monosaccharides that can be absorbed by the small intestine, thereby reducing hyperglycemia. Decreased hyperglycemia contributes to decreased HbAlc levels [9].

CONCLUSION

Based on the results of the study, the bacterium *Lactobacillus fermentum* can reduce blood glucose, sRAGE, HbA1C levels in rats induced by Nicotinamide-Streptozotosin.

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