RESEARCH ARTICLE

Hypoglycemic Activity and Safety Assessment of *Pediococcus acidilactici* Strain DNH16 in Experimental Type 2 Diabetes Mellitus Rats Induced with Streptozotocin

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Received date: Nov 14, 2023; Revised date: Dec 25, 2023; Accepted date: Dec 28, 2023

Abstract

BACKGROUND: Type 2 diabetes mellitus (T2DM) cases tend to rise throughout the year in all parts of the world. The α-glucosidase inhibitors are frequently used to both prevent and treat T2DM. This α-glucosidase inhibitor activity is seen in some probiotic species, and a certain strain of *Pediococcus acidilactici* exhibits promising characteristics as an α-glucosidase inhibitor. This study was conducted to assess the hypoglycemic activity and safety of *P. acidilactici* strain DNH16 (PADNH16) in experimental rats with T2DM induced by streptozotocin.

METHODS: The experiment was conducted *in vivo* using Wistar rats. Acarbose was employed as a positive control, and *Lactobacillus casei* was used as a comparative. For 24 days, post-prandial blood glucose levels were assessed every 3 days, followed by serum biochemical levels measurement of liver, kidney, and lipid profiles. The pancreas was histopathologically examined utilizing the Hematoxyline-Eosin staining procedure.

RESULTS: Administration of PADNH16 to T2DM rats lowered post-prandial blood glucose levels and gave hypoglycemic benefits comparable to acarbose and commercial probiotics. PADNH16 dosing did not affect serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), urea, or creatinine levels, showing that PADNH16 was not hazardous to liver or kidney function. The lipid profile assessment revealed that the values, including high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides, were comparable to the control group. The pancreatic histopathology revealed that injection of PADNH16 caused no alterations to pancreatic β cells.

CONCLUSION: *P. acidilactici* isolate DNH16 has a hypoglycemic effect on T2DM rats, but does not affect liver function enzymes, kidneys, lipid profiles and does not provide significant changes in pancreatic β cells.

KEYWORDS: diabetes mellitus, inhibitor α-glucosidase, *Pediococcus acidilactici* strain DNH16

Indones Biomed J. 2023; 16(1): 31-9

Introduction

Globally, the prevalence of type 2 diabetes mellitus (T2DM), a chronic metabolic disorder, has been rising consistently. The pattern above is rapidly escalating into an epidemic in certain nations across the globe. Projections indicate that the number of afflicted individuals will double within the following decade, primarily attributable to the aging population. This will further strain healthcare providers, particularly in developing countries.(1) Diabetes prevalence has increased in recent years, and the number

of diabetic individuals is expected to reach 642 million by 2040.(2) An upward trend in the national prevalence of diabetes among individuals aged 15 and older between 2013 and 2018 was reported. The majority of reported cases occurred in individuals aged 45 and above. Based on blood glucose testing, the Indonesian Ministry of Health estimated the empirical prevalence of diabetes to be 6.9% in 2013, whereas self-reported diabetes diagnosis indicated a prevalence of basically 2.1%.(3)

There are numerous diabetes medications available today. Metformin is typically used as a first-line treatment. Alternatives to metformin exist when it is not possible to control blood sugar levels by dietary and lifestyle modifications alone. These alternatives include sulfonylureas and glinides, pioglitazone, glucosidase inhibitors, glucagon-like peptide-1 agonists, and dipeptidyl peptidase 4 (DPP-4) inhibitors.(4) The development of T2DM is significantly influenced by prolonged postprandial hyperglycemia; therefore, α-glucosidase inhibitors (α-GIs) are frequently employed in both the prevention and management of this condition. Oral α-GIs have been suggested to potentially postpone the digestion of carbohydrates, thereby ameliorating hyperglycemia and diabetic complications.(5) Acarbose therapy has been found to have notable impacts on glycemic control and insulin levels, but no significant effects on lipids. However, it has been reported that this therapy may lead to gastrointestinal disorders, including abdominal aches and bloating.(6)

Probiotics are living microorganisms that bestow a beneficial effect on the host when administered in sufficient quantities. The majority of scientific publications adhere to this definition of probiotics.(7) The selection of the correct food matrix for transporting the probiotics into their host to produce synbiotic foods assures their viability during shelf life. It allows them to overcome physical and chemical barriers in the gastrointestinal system. Resistance to simulated gastric juice is crucial for probiotic bacteria to colonize their host's colon efficiently.(8) Several previous research studies have found that probiotics have the potential to alleviate hyperglycemia via the α-GI mechanism.(9–11)

A prior investigation isolated a probiotic strain from the traditional food from North Sumatera, Indonesia, known as *Dali Ni Horbo*. This particular strain identified as *Pediococcus acidilactici* DNH16 (PADNH16) exhibits promising characteristics as an α-GI.(12) Therefore, the hypoglycemic activity of *P. acidilactici* DNH16 *in vivo* was assessed using streptozotocin (STZ)-induced experimental rats, and the effects of this probiotic administration on liver and kidney function were evaluated.

Methods

Bacterial Cultures

PADNH16 was cultured into 9 mL of sterile MRS broth in test tubes and incubated for 24 hours anerobically using an anaerobic jar, at 37°C. For long-term storage, PADNH16 culture was stored in 20% glycerol stock at -25°C.(13) For *in vivo* testing, PADNH16 was diluted to $1x10^9$ CFU/mL. PADNH16 solution was obtained by dissolving PADNH16 cells in sterile physiological NaCl.

Animal and Experimental Design

Twenty-five Wistar rats weighed 150-180 grams were obtained from the Faculty of Medicine, Universitas Prima Indonesia. All rats were kept in a temperature-controlled environment (25°C) with a 12-hour light/dark cycle and free access to water and food. All rats were acclimatized to the laboratory environment for 7 days before the experiment. All rats were then randomly assigned to one of 5 groups, each group with 5 rats. STZ was used to induce T2DM in experimental rats. STZ was injected intraperitoneally into fasting rats at 45 mg/kgBW.(14) After 72 hours of the STZ injection, blood glucose levels were determined, and rats with blood glucose levels higher than 200 mg/dL were used in the experiments as rats with T2DM.(15) Normal control (NC) group included non-diabetic rats that were normally feed and given 0.25 mL of 3% sucrose. T2DM Group included diabetic untreated rats that were normally fed and given 0.25 mL of 3% sucrose. Positive Control (PC) group included diabetic rats that were normally fed and treated with 0.25 mL acarbose 100 mg/kgBW. PADNH16 group included diabetic rats that were normally fed and treated orally with 0.25 mL of $1x10^9$ CFU/mL PADNH16. *Lactobacillus casei* (LC) group included diabetic rats that were normally fed and treated orally with 0.25 mL of $1x10^9$ CFU/mL *L. casei*.

Measurement of post-prandial glucose levels was done every three days. Blood samples were taken every 3 days until the end of the experiment on day-24. The study protocol has been approved by The Health Research Ethics Commission of Universitas Prima Indonesia (No. 034/KEPK/UNPRI/V/2023).

Serum Biochemical Analysis

At the end of the experiment, the rats were anesthetized by placing a towel that had been soaked in volatile anesthetics into the nose of the rats, then the procedure of drawing blood from the heart was carried out. Four mL of blood was

collected into a tube and centrifuged to obtain serum. The rat should be immediately euthanized post-blood collection.(15) The safety of probiotics for consumption was determined by determining the rats' liver function parameters, with serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT), as well as the kidney function parameters, with urea, creatinine, and lipid profiles. SGOT/SGPT levels were determined using SGOT and SGPT kits by Sigma Aldrich (St. Louis, MO, USA). Triglyceride levels were determined using the glycerol phosphate oxidase-p-aminophenazone (GPO-PAP) method, while high-density lipoprotein (HDL), low-density lipoprotein (LDL) and cholesterol levels were determined using the cholesterol oxidase-p-aminophenazone (CHOD-PAP) method. The creatinine and urea levels were determined using DiaSys Creatinine and Urea FS kit (DiaSys Diagnostic Systems GmbH, Waterbury, CT, USA).

Histopathological Examination

Rats were sacrificed after 24 days, and each pancreas was extracted and fixed in a formalin solution. After that, the samples were immersed in a xylol solution for around 2 minutes. They were then rehydrated for 30 minutes in a sequence of alcohols (70, 80, 90, 95, and 100%). After being cleared for 1.5 hours with xylene until the xylene's color became pale, they were cleared again with xylene/paraffin replacement (3:1, 1:1, and 1:3, v/v) for 30 minutes each in a 45°C oven. They were then penetrated twice daily with hard paraffin and embedded in paraffin. Eosin was used to stain sections of these embedded samples. A 400x magnification histological investigation was performed using a light microscope.(16) An anatomic pathologist was assigned to interpret the pancreatic histopathology results based on the shape of the islets of Langerhans, the shape of acinar cells, and whether there was congestion and interstitial hemorrhage.

Results

Effect of PADNH16 Administration on Reducing Post-Prandial Glucose Levels

In general, there was a decrease in blood glucose levels in rats with T2DM conditions that were given PADNH16 probiotics. The same pattern was shown in the PC and LC rat groups.

As presented in Table 1, after three days of rats induced with STZ, there was a significant increase in post-prandial blood glucose levels (p<0.001) between groups T2DM, PC, PADNH16, and LC compared to the NC group. On day-3, there was still a highly significant difference (p=0.000) between the average blood glucose levels of PADNH16 group rats and NC group rats, which were 193.20±9.36 mg/dL and 103.40±7.30 mg/dL respectively. The average blood glucose level of PADNH16 group rats also did not show a significant difference compared to the average blood glucose level of T2DM group rats (p=0.171). On day-12, there was a significant decrease in

Table 1. The changes in post-prandial blood glucose levels in each treatment group.

Time	Post Prandial Glucose Level (mg/dL)				
	NC	T2DM	PC	PADNH16	LC
Day-0	110.40±3.84^{*b*c*d*e}	222.80±11.43^{*a}	217.20±12.61^{*a}	208.40± 6.42^{*a}	207.20±6.09^{*a}
Day-3	103.40±7.30^{*b*c*d*e}	210.40±11.41^{*a*c*d*e}	194.40±18.00^{a}	193.20±9.36^{*a}	190.40±11.86^{*b}
Day-6	97.00±4.18^{*b*c*d*e}	227.60±15.19^{*a*c*d*e}	161.20±22.87^{*a*b}	182.80±14.13^{*a*b}	184.80±9.20^{*a*b}
Day-9	111.60±9.18^{*a*b*c*d*e}	220.60±13.16^{*a*c*d}	151.60±27.33^{*a*b}	170.00±22.13^{*a*b}	188.20±20.71^{*a}
Day-12	108.00±4.18^{*bce}	222.60±5.63^{*acde}	144.40±18.17^{ab}	148.20±33.98^{b}	173.20±22.67^{ab}
Day-15	113.20±14.37^{*b}	277.00±89.98^{*acde}	145.20±12.87^{b}	139.00±27.45^{b}	156.00±23.39^{b}
Day-18	110.40±9.78^{*bc}	240.80±26.02^{*abcd}	139.20±11.12^{*ab}	136.80±28.55^{b}	151.00±32.96^{b}
Day-21	109.40±8.79^{*b*e}	237.80±16.67^{*a*cd}	127.80±10.75^{*b}	139.40±26.78^{b}	129.20±21.07^{*b}
Day-24	103.60±4.03^{*b}	214.20±9.75^{*a*cde}	108.80±5.97^{*b}	133.40±28.80^{b}	114.20±10.73^{b*}

Values are the mean±SD. The p-value for blood glucose levels on days 0, 3, 9, and 15 were determined based on Bonferroni post hoc non-parametric analysis results. The p-value for blood glucose levels on days 6, 12, 18, 21, and 24 were determined based on the Games-Howell Post-Hoc non-parametric analysis results. ^{a}significant difference to NC group (p<0.05); ^{b}significant difference to T2DM group (p<0.05); ^{c}significant difference to PC group (p<0.05); ^{d}significant difference to PADNH16 group (p<0.05); ^{e}significant difference to LC group (p<0.05); ^{*a}significant difference to NC group (p<0.001); ^{*b}significant difference to T2DM group (p<0.001); ^{*c}significant difference to PC group (p<0.001); ^{*d}significant difference to PADNH16 group (p<0.001); ^{*e}significant difference to LC group (p<0.001).

blood glucose levels in PADNH16 group rats compared to T2DM group rats (p=0.034).

The decrease in post-prandial blood glucose levels continued to occur gradually until the end of the experiment (day-24). From day-24, there was no significant difference in post-prandial blood glucose levels in groups PC, PADNH16, and LC group compared to the NC group. In contrast, there was still a significant difference in post-prandial glucose levels between groups NC, PC, PADNH16, and LC against group T2DM. There was a significant difference in blood glucose levels (p=0.011) in the PADNH16 rat group compared to the T2DM rat group with mean blood glucose levels of 133.4±28 mg/dL and 214.2±9.75 mg/dL respectively, while the average blood glucose levels of rats in the PC, LC and NC groups showed a very significant decrease (p=0.000) compared to the average blood glucose levels of rats in the T2DM group.

Safety Assessment of Probiotic

Serum biochemical measurements of liver and kidney function and lipid profile were conducted at the end of the experiment to assessed the safety of the probiotic used. The measurement results showed no significant difference between groups for SGPT, SGOT, and urea levels. There was a significant difference in creatinine between the NC group, T2DM, and PADNH16 against the LC group, where LC group rats showed the lowest creatinine value. There was a significant increase in triglyceride levels in T2DM group rats compared to groups NC, PC, PADNH16, and LC. While for HDL, LDL, and cholesterol levels, there were no significant differences between groups (Table 2).

The administration of PADNH16 isolate only induced changes in triglyceride levels. There was a highly significant decrease in triglyceride levels in PADNH16 group rats compared to T2DM group rats with p=0.000.

Histopathological Analysis

The results of pancreatic histopathology of each representative group of experimental rats were shown in Figure 1. In the NC group, the islets of Langerhans were regular in shape, namely oval round, acinar cells have an oval, round shape, with bluish cytoplasm with dark nuclei, there was no congestion, and there was also no interstitial hemorrhage (Figure 1A). In T2DM group, the islets of Langerhans had an irregular shape, indicating that damage had occurred. The acinar cells have irregular shapes, with pale cytoplasm and dark nuclei. This indicated that the acinar cells in the rat pancreas are also damaged. There were signs of congestion in the pancreatic blood vessels. This congestion was characterized by dilated and reddish-colored blood vessels. This indicated that there was increased blood flow to the pancreas (Figure 1B). In PC group, the islets of Langerhans and acinar cells had a more regular shape, with bluer cytoplasm and darker nuclei, with no signs of congestion or interstitial bleeding. Overall, the histopathology images of the pancreas of T2DM rats treated with acarbose above showed that the pancreas of the rats had improved. The administration of acarbose can repair the damage caused by T2DM (Figure 1C). In the PADNH16 group the islets of Langerhans and acinar cells had a more regular shape with bluish cytoplasm and darker nuclei. However, there were congested blood vessels, no signs of

Table 2. Statistical analysis of liver, kidney and lipid profile parameters.

Parameters	NC	T2DM	PC	PADNH16	LC
SGOT (U/L)	106.20±16.11	152.60±25.20	106.60±18.83	119.20±4.08	113.00±5.61
SGPT (U/L)	58.80±8.92	59.60±9.18	50.80±9.31	55.80±9.09	72.20±25.11
Urea (mg/dL)	48.40±6.30	45.60±4.33	48.80±2.58	47.20±4.71	53.40±3.97
Creatinine (mg/dL)	0.54±0.11^{e}	0.62±0.13^{e}	0.79±0.31	0.59±0.05^{e}	0.24±0.02^{abd}
Trygliceride (mg/dL)	47.60±7.33^{*bd}	95.00±16.24^{*a*c*d*e}	68.20±12.57^{*b*e}	50.40±6.22^{*b}	42.80±4.54^{*b*c}
Cholesterol (mg/dL)	53.40±3.78	51.20±3.27	58.40±15.20	57.40±3.28	50.40±1.67
HDL (mg/dL)	27.60±2.90	26.40±3.84	34.80±7.22	29.80±5.06	33.80±1.48
LDL (mg/dL)	7.40±2.07	15.60±6.50	9.00±1.00	9.80±1.09	7.80±3.03

Significant differences in data between groups were determined based on p-value. The p-values for SGPT, serum urea, triglyceride, and HDL levels were determined based on Bonferroni post hoc non-parametric analysis results. The p-values for SGOT, creatinine, cholesterol, and LDL levels were determined based on the results of Games-Howell Post-Hoc non-parametric analysis. [a]significant difference to NC group (p<0.05); [b]significant difference to T2DM group (p<0.05); [c]significant difference to PC group (p<0.05); [d]significant difference to PADNH16 group (p<0.05); [e]significant difference to LC group (p<0.05); [*a]significant difference to NC group (p<0.001); [*b]significant difference to T2DM group (p<0.001); [*c]significant difference to PC group (p<0.001); [*d]significant difference to PADNH16 group (p<0.001); [*e]significant difference to LC group (p<0.001).

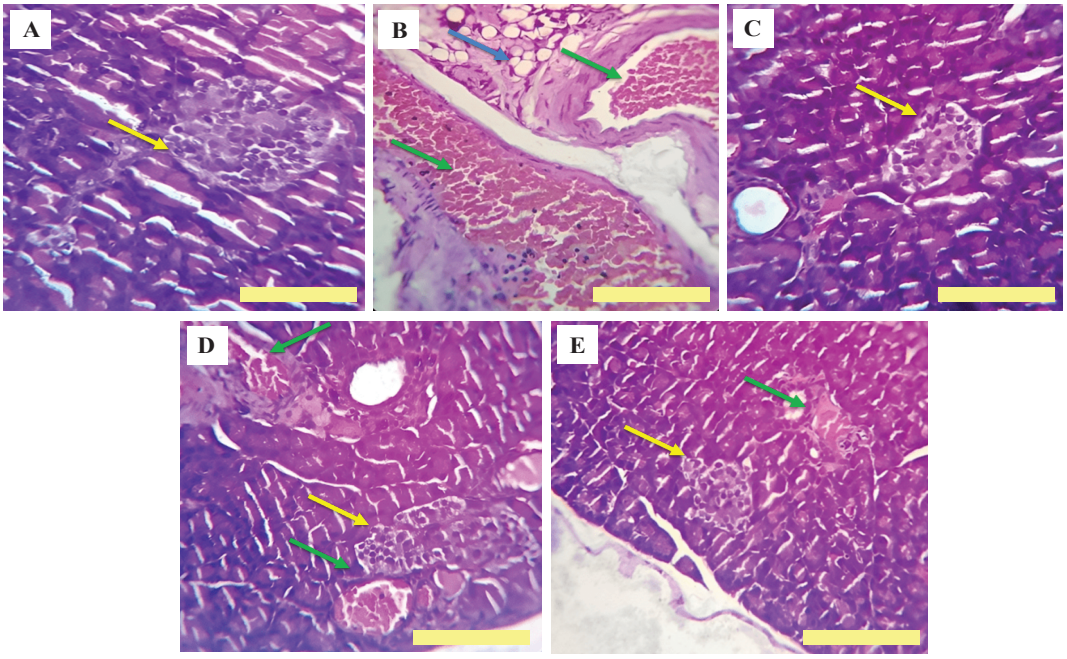

Figure 1. Histopathological analysis of the pancreas. A: Histopathological picture of the pancreas of NC group: islets of Langerhans (yellow arrow) are intact and well concentrated, β cells are located in the center of the islets, and there is no inflammation of lymphocytes and blood vessel congestion. B: In the histopathologic picture of the pancreas of the T2DM group (DM model), there are large amounts of congested blood vessels (green arrow) and inflammation lymphocytes (blue arrow). C: Histopathology of the pancreas of the PC group (acarbose treatment): There was inflammation of lymphocytes, and islets of Langerhans (yellow arrow) were intact and well concentrated. D: Histopathology of the pancreas of the PADNH16 group (treated with DNH16 isolate) showed that there were smaller-scale congested blood vessels (green arrow), and islets of Langerhans were intact (yellow arrow). E: Histopathology of the pancreas of LC group, showed intact Langerhans islets (yellow arrow) and small amounts of congested blood vessels (green arrow). Yellow bar: 50µm

interstitial hemorrhage between pancreatic cells, and no eosinophilic cytoplasm (Figure 1D). While in LC group (as comparison), there was intact Langerhans islets and small amounts of congested blood vessels (Figure 1E).

Histopathological analysis of the pancreas was carried out based on the observation of islets of Langerhans, congested blood vessels, interstitial vessels, and lymphocyte inflammatory cells. The scale given was from (+) to (+++). Data in Table 3 indicated that the NC group exhibited superior pancreatic histology compared to the other groups. The acarbose treatment group, consisting of diabetic rats given control drugs, exhibited superior histology compared to the diabetic group and was nearly identical to the normal group. Furthermore, the PADNH16 and LC groups demonstrated noteworthy improvements in the histology of the pancreas of diabetic rats, which were comparable to those observed in the acarbose treatment group. These results underscored the potential of the two probiotic groups to inhibit and enhance the histological characteristics of the pancreas in rats with diabetes.

Discussion

"STZ diabetes" occurs when pancreatic cells undergo necrosis; this agent is the primary choice for causing diabetes in animals. Depending on the type of animal, the dose of the drug, how it is administered, and the duration

Table 3. The results of pancreas histopathology analysis of each treatment group.

Parameters	NC	T2DM	PC	PADNH16	LC
Langerhans islets	++ (round)	++ (oval)	++ (round)	++ (oval)	++ (oval)
Congested blood vessel	+	+++	++	++	++
Interstitial vessel	+	+++	++	++	++
Lympochyte inflammatory cells	+	+++	++	++	++

of STZ use in rats, diabetes can be severe (blood glucose above 200/300mg/dL) or mild (glycemia between 120 and 200/300mg/dL). To induce severe diabetes, STZ is administered to adult rats at a dose of 40-50mg/kg body weight via intravenous or intraperitoneal injection.(17)

On day 3, there was a significant increase in glucose levels (p<0.001) in the T2DM group compared to groups NC, PC, PADNH16, and LC. However, glucose levels in groups PC, PADNH16, and LC did not differ significantly. Glucose levels that were not significantly different between groups PC, PADNH16, and LC continued until the end of the experiment. This indicates that PADNH16, acarbose, and *L. casei* showed almost equivalent hypoglycemic activity. Through competitive inhibition of the α-glucosidase enzyme in the lumen of the small intestine, acarbose interferes with carbohydrate digestion. Consequently, it enhances insulin sensitivity, inhibits postprandial hyperglycemia and hyperinsulinemia, and decreases glucose absorption.(18) These result was in line with previous study which explains that administering *Lactobacillus fermentum* to diabetic rats can reduce blood glucose levels. It was reported that there was a significant decrease in blood glucose levels after two weeks of treatment compared to the negative control group.(19)

The administration of "Shubat," a traditional food from camel milk containing probiotics, showed hypoglycemic activity in experimental rats with T2DM as indicated by a decrease in fasting blood glucose (p<0.01) and HbA1c (p<0.05), C-peptide and glucagon like peptide 1 (GLP-1) levels.(20) A prior investigation documented that diabetic experimental rats fed milk fermented with *P. acidilactici* strain BE experienced a reduction in blood glucose levels from 410.27±51.60 to 304.07±9.88 mg/dL (p<0.05). This effect was comparable to that of metformin, which decreased blood glucose levels from 382.30±13.39 mg/dL to 253.33±40.66 mg/dL (p<0.05).(21) In another study, it was explained that supplementation of *P. acidilactici* CECT9879 (pA1c) in nematodes with high glucose conditions was able to counteract the effects of glucose levels by reducing the effects of reactive oxygen species (ROS) by as much as 20%.(22) In another study, it was reported that *P. acidilactici* 004 and *Lactobacillus plantarum* 152 isolated from yogurt and pickles, given to T2DM rats, had a better glucose-lowering effect than the commercial probiotic *Lactobacillus rhamnosus* CCFM0528.(23)

By delaying the digestion and assimilation of carbohydrates, inhibition of the α-glucosidase enzyme can reduce fasting glucose levels. In the small intestine, α-glucosidase has the capability to hydrolyze oligosaccharides and disaccharides into glucose/monosaccharides, which are subsequently prepared for absorption. Probiotics' capacity to inhibit α-glucosidase may potentially aid in the reduction of fasting glucose levels, thereby offering prospects for the prevention and management of diabetes. It was reported that administering L. rhamnosus as much as 10^9 CFU/mL reduced blood glucose levels in experimental rats induced with STZ. Oral administration of *L. rhamnosus* BSL and *L. rhamnosus* R23 reduced fasting blood glucose (FBG) and improved glucose tolerance by regulating glucose-6-phosphatase (G6pc) expression.(24) An administration of probiotic products WBF-010 and WBF-011 to human patients with T2DM improved postprandial glucose control. However, there was no change in fasting glucose levels.(25)

Probiotics facilitate the conversion of lipopolysaccharides (LPS) and oligosaccharides to short-chain fatty acids (SCFA), including lactate and acetate, which are subsequently converted back to butyrate to provide energy to the intestinal mucosa. Moreover, probiotics can reduce the permeability of intestinal mucosa to LPS in a direct manner. T2DM, lipopolysaccharide reduction decreases the systemic inflammatory response and oxidative stress linked to insulin resistance. Through cross-kingdom cell-to-cell transmission, probiotics can affect antioxidant enzyme levels and activity and reduce oxidative stress that can destroy pancreatic beta cells and cause insulin resistance by lowering LPS.(26)

In the measurement of liver function enzymes, there was a non-significant increase in SGOT and SGPT levels in the T2DM group compared to the NC group. In addition, there were no significant differences between groups for these liver function enzymes. Liver function biomarkers, including SGOT and SGPT, are valid toxicological parameters.(24) This indicates that PADNH16 isolate is not toxic to the liver. One study reported that probiotic *Escherichia coli* 16 could even provide a protective effect on the liver, as shown by a decrease in SGOT and SGPT enzymes to approach average numbers in experimental rats. (27) The blood test for blood urea nitrogen (BUN) measures the major nitrogenous end-products of the breakdown of proteins and amino acids, as well as creatinine, which comes from the breakdown of creatine phosphate in muscles and is excreted by the kidneys. BUN is a crude measurement of kidney function as it measures the amount of urea nitrogen in the blood, which is directly related to kidney excretion. On the other hand, the creatinine test helps diagnose impaired kidney function by measuring the amount of creatinine phosphate in the blood. Elevated levels of BUN and creatinine in the blood indicate kidney dysfunction.

These tests are prevalent and widely accepted to assess kidney function.(28) This indicates that the administration of PADNH16 is not toxic to kidney function.

ANOVA results showed significant differences between groups in triglycerides, HDL, and LDL testing parameters. There was a significant increase in triglycerides levels in the T2DM group compared to groups NC, PC, PADNH16, and LC. While for HDL and LDL parameters, there were no significant differences between groups. There was an increase in LDL levels in T2DM group rats, but it was insignificant. When compared between patients with diabetes, individuals with diabetes have a 2 to 4x more significant risk of suffering from stroke and death from heart disease. This is because elevated triglycerides levels are widespread in patients with T2DM.(29) Triglycerides levels in diabetic rats were also found to be higher than diabetic rats treated with *Celastrus pamiculatus* methanolic seed extract which is known to have hypoglycemic, hypolipidaemic and antioxidant activities.(30)

One study reported that the ratio of triglycerides to cholesterol (TG/HDL-c) was positively and significantly correlated with glycated hemoglobin and fasting glucose levels.(33) One of the prevalent conditions linked to inadequate glycemic control in T2DM is dyslipidemia. Hypoglycemia or resistance to insulin leads to a reduction in lipoprotein lipase activity, which is the pathogenesis of dyslipidemia in T2DM. Lipids are metabolized by the enzyme lipoprotein lipase in healthy individuals in response to insulin. Reduced adiponectin and relative insulin deficiency result in impaired lipoprotein lipase activity in T2DM, which is characterized by elevated levels of LDL, triglycerides, and reduced levels of HDL.(31) Based on Figure 1, there were significant morphological changes between the pancreas of the control group and the diabetic group. Prior research documented substantial morphological impairment in the liver and pancreas of rats induced with diabetes using STZ. Specifically, the pancreatic tissue exhibited β-cell damage and acinar cell enlargement, while the liver tissue demonstrated hepatocyte degeneration.(32) Figure 1 shows an improvement in pancreatic morphology in the pancreas of PC group rats, characterized by islets of Langerhans that are still intact. However, there is still a small scale of blood vessel congestion. Previous research found that probiotic *L. acidophilus* improved the shape of the islets of Langerhans in rats, resulting in a decrease in the degree of insulitis to 0. This is most likely owing to *L. acidophilus*' ability to lower TNF-α levels, which inhibits Nuclear Factor-Kappa B (NF-κB) activation. The activation of NF-κB is caused by the stimulation of reactive oxygen species (ROS), which leads to endothelial dysfunction.(33) Probiotic bacteria can help promote cytokine release from intestinal epithelial cells in a strain- and dose-dependent way. In the colon, probiotics have been shown to interact with enterocytes and dendritic cells, as well as Th1, Th2, and regulatory T cells (Treg). These probiotics must come into contact with macrophages and dendritic cells in order to promote the production of anti- or pro-inflammatory cytokines, which can then activate immunological signaling pathways. It has been proposed that probiotics can reduce inflammation by boosting anti-inflammatory cytokines and reducing pro-inflammatory cytokines, which can then alter the activity of NK cells, block toll-like receptors (TLR), and, ultimately, the NF-κB pathway.(34) It is recommended to conduct further genomic mapping through whole genomic sequencing and annotation to determine whether the probiotics do not have the presence of virulence genes and antimicrobial resistance genes so that probiotics can be used as candidates for supplements that are safe for humans.

Conclusion

From the study results, it can be concluded that PADNH16 can provide hypoglycemic effects in T2DM rats. The hypoglycemic effect shown by PADNH16 is almost equivalent to the effect given by acarbose and *L. casei*. PADNH16 isolate was also safe for liver and kidney function, as indicated by no increase in the observed liver and kidney function parameters, namely SGPT, SGOT, urea, and creatinine.

Acknowledgments

The research team would like to thank the Directorate of Technology Research and Community Service for the advanced decentralized research grant for fiscal year 2023. The research team would also like to thank the integrated laboratory analyst team, Lisa, Edi, and Finna Piska who have assisted in the completion of this research.

Authors Contribution

EF was involved in concepting, planning the research, performed the data acquisition/collection and interpreting the results. SA calculated the experimental data and performed the analysis, H drafted the manuscript and

designed the figures. WYH conducted statistical analysis and contributed in discussion. JL analyzed the histopathology and contributed in discussion. All authors took parts in giving critical revision of the manuscript.

References

1. Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: A review of current trends. Oman Med J. 2012; 27(4): 269–73.
2. Hussain H, Nazir M, Saleem M, Al-Harrasi A, Elizbit, Green IR. Fruitful decade of fungal metabolites as anti-diabetic agents from 2010 to 2019: Emphasis on α-glucosidase inhibitors. Phytochem Rev. 2021; 20: 145–79.
3. Tanoey J, Becher H. Diabetes prevalence and risk factors of early-onset adult diabetes: Results from the Indonesian family life survey. Glob Health Action. 2021; 14(1): 2001144. doi: 10.1080/16549716.2021.2001144.
4. Derosa G, Maffioli P. α-Glucosidase inhibitors and their use in clinical practice. Arch Med Sci. 2012; 8(5): 899–906.
5. Ren F, Ji N, Zhu Y. Research progress of α-glucosidase inhibitors produced by microorganisms and their applications. Foods. 2023; 12(18): 3344. doi: 10.3390/foods12183344.
6. Van De Laar FA, Lucassen PLBJ, Akkermans RP, Van De Lisdonk EH, Rutten GEHM, Van Weel C. Alpha-glucosidase inhibitors for type 2 diabetes mellitus. Cochrane Database Syst Rev. 2005; 2005(2): CD003639.
7. Fijan S. Microorganisms with claimed probiotic properties: An overview of recent literature. Int J Environ Res Public Health. 2014; 11(5): 4745–67.
8. Muganga L, Liu X, Tian F, Zhao J, Zhang H, Chen W. Screening for lactic acid bacteria based on antihyperglycaemic and probiotic potential and application in synbiotic set yoghurt. J Funct Foods. 2015; 16: 125–36.
9. Frediansyah A, Suryani, Nurhayati R, Miftakhussolikhah, Sholihah J. Lactobacillus pentosus isolated from Muntingia calabura shows inhibition activity toward alpha-glucosidase and alpha-amylase in intra and extracellular level. IOP Conf Ser Earth Environ Sci. 2019; 251(1): 012045. doi: 10.1088/1755-1315/251/1/012045.
10. Nurhayati R, Miftakhussolikhah, Frediansyah A, Rachmah DL. Lactic acid bacteria producing inhibitor of alpha glucosidase isolated from ganyong (Canna edulis) and kimpul (Xanthosoma sagittifolium). IOP Conf Ser Earth Environ Sci. 2017; 101(1): 012009. doi: 10.1088/1755-1315/101/1/012009.
11. Hutahaean AJN, Silalahi J, Suryanto D, Satria D. Characterisation of lactic acid bacteria from Dengke Naniura of common carp (Cyprinus carpio) with α-glucosidase inhibitory activity. Open Access Maced J Med Sci. 2019; 7(22): 3794–8.
12. Fachrial E, Anggraini S, Harmileni, Saryono, Nugroho TT. Inhibitor α-glucosidase activity of Pediococcus acidilactici DNH16 isolated from Dali ni Horbo, a traditional food from North Sumatra, Indonesia. Biodiversitas. 2023; 24(2): 958–65.
13. Bamgbose T, Sinha S, Abdullahi IO, Inabo HI, Bello M, Kori LD, *et al.* Identification of predominant lactic acid bacteria associated with kunun-zaki and kindirmo a traditional fermented food of Nigeria. Curr Top Lact Acid Bact Probiotics. 2022; 8(1): 17–31.
14. Zhang M, Lv XY, Li J, Xu ZG, Chen L. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. Exp Diabetes Res. 2008; 2008: 704045. doi: 10.1155/2008/704045.
15. Kumar M, Dandapat S, Prasad Sinha M, Kumar A, Raipat BS. Different blood collection methods from rats: A review. Balneo Res J. 2017; 8(2): 46–50.
16. Widyatmaka MN, Ismail A. The effect of antlion (Myrmeleon sp.) extract towards histopathology image of pancreas in diabetic mice. Sains Med. 2021; 11(2): 48. doi: 10.30659/sainsmed.v11i2.7656.
17. Damasceno DC, Netto AO, Iessi IL, Gallego FQ, Corvino SB, Dallaqua B, *et al.* Streptozotocin-induced diabetes models: Pathophysiological mechanisms and fetal outcomes. Biomed Res Int. 2014; 2014: 819065. doi: 10.1155/2014/819065.
18. Altay M. Acarbose is again on the stage. World J Diabetes. 2022; 13(1): 1–4. doi: 10.4239/wjd.v13.i1.1.
19. Bobga PT, Fossi BT, Taiwe GS, Nkanpira KT, Yolande NE, Ngwa FA, *et al.* Evaluation of the anti-diabetic potential of probiotic Lactobacillus fermentum (PRI 29) isolated from cameroonian fermented cow milk in alloxan induced diabetes type-1 mice model. Saudi J Pathol Microbiol. 2022; 7(10): 381–93.
20. Manaer T, Yu L, Zhang Y, Xiao XJ, Nabi XH. Anti-diabetic effects of shubat in type 2 diabetic rats induced by combination of high-glucose-fat diet and low-dose streptozotocin. J Ethnopharmacol. 2015; 169: 269–74. doi: 10.1016/j.jep.2015.04.032.
21. Widodo W, Kusumaningrum HRP, Wihadmadyatami H, Wicaksana AL. Milk fermented with Pediococcus acidilactici strain BE improves high blood glucose levels and pancreatic beta-cell function in diabetic rats. Food Sci Anim Resour. 2023; 43(1): 170–83.
22. Yavorov-dayliev D, Milagro FI, Ayo J, Oneca M, Aranaz P. Pediococcus acidilactici CECT9879 (pA1c) counteracts the effect of a high-glucose exposure in C. elegans by affecting the insulin signaling pathway (IIS). Int J Mol Sci. 2022; 23(5): 2689. doi: 10.3390/ijms23052689.
23. Cai T, Wu H, Qin J, Qiao J, Yang Y, Wu Y, *et al.* In vitro evaluation by PCA and AHP of potential antidiabetic properties of lactic acid bacteria isolated from traditional fermented food. LWT - Food Sci Technol. 2019; 115(2): 108455. doi:10.1016/j.lwt.2019.108455.
24. Farida E, Nuraida L, Giriwono PE, Jenie BSL. Lactobacillus rhamnosus reduces blood glucose level through downregulation of gluconeogenesis gene expression in streptozotocin-induced diabetic rats. Int J Food Sci. 2020; 2020: 6108575. doi: 10.1155/2020/6108575.
25. Perraudeau F, McMurdie P, Bullard J, Cheng A, Cutcliffe C, Deo A, *et al.* Improvements to postprandial glucose control in subjects with type 2 diabetes: A multicenter, double blind, randomized placebo-controlled trial of a novel probiotic formulation. BMJ Open Diabetes Res Care. 2020; 8(1): e001319. doi: 10.1136/bmjdrc-2020-001319.
26. Murti LNW, Ayumuyas NP, Suharjono. Probiotics usage as therapy on diabetes mellitus type II: A literature review. Pharm J Indones. 2019; 4(2): 73–7.
27. Kumar P, Ranawade AV, Kumar NG. Potential probiotic Escherichia coli 16 harboring the Vitreoscilla hemoglobin gene improves gastrointestinal tract colonization and ameliorates carbon tetrachloride induced hepatotoxicity in rats. Biomed Res Int. 2014: 2014: 213574. doi: 10.1155/2014/213574.
28. Pandya D, Nagrajappa AK, Ravi KS. Assessment and correlation of urea and creatinine levels in saliva and serum of patients with chronic kidney disease, diabetes and hypertension: A research study. J Clin Diagnostic Res. 2016; 10(10): ZC58–62.
29. Alexopulos AS, Qamar A, Hutchins K, Crowley MJ, Batch BC, Guyton JR. Triglycerides: Emerging targets in diabetes care? Review of moderate hypertriglyceridemia in diabetes. Curr Diab Rep. 2019; 19(4): 13. doi: 10.1007/s11892-019-1136-3.
30. Ahmad MF, Haidar MA, Naseem N, Ahsan H, Siddiqui WA.

Hypoglycaemic, hypolipidaemic and antioxidant properties of Celastrus paniculatus seed extract in STZ-induced diabetic rats. Mol Cell Biomed Sci. 2023; 7(1): 10–7.

31. Naqvi S, Naveed S, Ali Z, Ahmad SM, Asadullah Khan R, Raj H, *et al.* Correlation between glycated hemoglobin and triglyceride level in type 2 diabetes mellitus. Cureus. 2017; 9(6): e1347. doi: 10.7759/cureus.1347.
32. Khan NU, Qazi NG, Khan AU, Ali F, Hassan SSU, Bungau S. Anti-diabetic activity of Brucine in streptozotocin-induced rats: In silico, in vitro, and in vivo studies. ACS Omega. 2022; 7(50): 46358–70.
33. Sariyanti M, Andita TA, Erlinawati ND, Yunita E, Nasution AA, Sari K, et al. Probiotic Lactobacillus acidophilus FNCC 0051 improves pancreatic histopathology in streptozotocin-induced type-1 diabetes mellitus rats. Indones Biomed J. 2022; 14(4): 329–441.
34. Kwok KO, Fries LR, Silva-Zolezzi I, Thakkar SK, Iroz A, Blanchard C. Effects of probiotic intervention on markers of inflammation and health outcomes in women of reproductive age and their children. Front Nutr. 2022; 9: 889040. doi: 10.3389/fnut.2022.889040.