PSP Yogurt Lowers Blood Glucose Levels, Improves Lipid Profile and Antioxidant Status In Diabetic Conditions

1st Muji Rahayu
Department of Medical Laboratory
Poltekkes Kemenkes Yogyakarta Indonesia
Hayuningpuji@gmail.com

2nd M. Atik Martsiningsih *Department of Medical Laboratory*
Poltekkes Kemenkes Yogyakarta Indonesia
atikmartsiningsih@gmail.com

Abstract— Probiotic can improve the condition of diabetes. Anthocyanins are potent antioxidant compounds. This study aims to analyze the effect of probiotic combination of *Lactobacillus bulgaricus* and *Streptococcus thermophyllus* in the form of yogurt with purple sweet potato (PSP) on blood glucose levels and lipid profiles (triglycerides, total cholesterol, LDL and HDL) ang MDA in streptozotocin-induced rats. This research with the pre-post test with control group design, using male white rats, Rattus norvegicus, Wistar strain. A total of 30 rats divided into 6 groups each consisting of 5 rats namely the negative control group K(-), diabetic control K(+), YP group was given plain yogurt 3 ml/200 grams of body weight, YU1 and YU2 groups were given purple sweet potato yogurt 3 ml/200 grams of body weight and 2 x 3 ml/200 grams of body weight. The results showed that the administration of purple sweet potato yogurt with *Lactobacillus bulgaricus* and *Streptococcus thermophyllus* isolates reduced blood glucose levels, triglycerides, total cholesterol, LDL and raised HDL levels, and decreased levels of malondialdehyde (MDA) in diabetic rats ($p<0.05$). Decreased blood glucose levels, triglycerides, total LDL cholesterol, and elevated HDL levels and decreased levels of malondialdehyde (MDA) were better in purple sweet potato yogurt than plain yogurt.

Keywords: yogurt, purple sweet potato, diabetes mellitus, lipid profile, MDA.

1. INTRODUCTION

The optimal management of DM is done by combining three things, namely physical activity, nutrition and treatment. Nutritional interventions given to DM therapy are by counting carbohydrates, decreasing energy and fat intake, and choosing functional foods that can have a positive effect.[1] Probiotics are one of the functional foods that can change intestinal flora, improve total cholesterol levels and lipoprotein cholesterol levels. low density, and reduce blood glucose levels and insulin resistance in diabetes mellitus.[2]

Purple sweet potato (PSP) is one of the foods with high anthocyanins content. Anthocyanins are antioxidant phenol compounds. It has been proven from previous research that anthocyanins have free radicals scavenging activities.

Purple sweet potato (Ipomoea batatas (L.) Lam) (PSP) a higher anthocyanin content than sweet potatoes of other colors, which differ depending on the variety, are mono- or di-acylated forms of cyanidin and peonidin.[3] Many reports have demonstrated that the anthocyanins from PSP possess a stronger free radical scavenging activity than other fruits and vegetables, such as red cabbage, grape skin, elderberry, and purple corn.[4]

2. MATERIALS AND METHOD

Purple sweet potatos tubers are obtained from the Karangkajen market in Yogyakarta, and plant determination is carried out in the Biology laboratory of the Faculty of Biology as *Ipomea batatas* (L) Lam, Gadjahmada University Yogyakarta. Yogurt is made in the home industry of Firani, Wates, Kulon Progo, Yogyakarta using starter cultures of *Lactobacillus bulgaricus* FNCC 0041 and *Streptococcus thermophyllus* FNCC 0040 obtained and verified in the Microbiology laboratory of PSPG UGM Yogyakarta. The male white rat *Rattus norvegicus* Wistar strain was obtained from the Center for Food and Nutrition Studies, UGM Yogyakarta. Glucose, triglycerides, total cholesterol and LDL reagent kits from Dyasis.

The study was proved and allowed by ethical commission of Polytechnic of Health Yogyakarta on No.

LB.01.01/KE-02/XXIII/588/2018, 26 Juni 2018. Determination of biochemical markers and experimental animal maintenance in the Food and Nutrition laboratory of the UGM PSPG Yogyakarta. Treatment of experimental animals is carried out in the Central Food and Nutrition Laboratory of the UGM University, as follows: As many as 30 rats, acclimatized using individual cages for 3 days at room temperatures ranging from 25-28°C and lighting cycles 12 hours each day. During acclimatization and during the study rats were fed standard AD II pellets and drinking water *ad libitum*. All rats are drawn from the retroorbital vein, then their blood glucose levels are determined. Rats are divided into 6 groups, each consisting of 5 rats recpectively K (-), K (+), PY, PSPY1, PSPY2 and GLB group. Group K (-) is a normal control group, K (+), PY, PSPY1, PSPY2 and GLB groups were diabetic group induced by streptozotocin (STZ) 0.45 mg / kg bwt. On third day, all rats were determined by their blood glucose levels, triglycerides, total cholesterol, LDL, HDL and MDA levels (*pre test*). PY group treated with plain yogurt for 3 ml / 200 gram body weight, PSPY1 and PSPY2 groups treated with PSP yogurt for 3 ml and 2 x 3 ml/200 gram bwt each day for 28 days through sonde, and GLB group was given glibenclamide 0.45 mg/kg bwt. Twenty four hours after the last treatment, rat blood was taken for determination of blood glucose levels, triglycerides, total cholesterol, LDL, HDL and MDA (*post test*).

Determination of blood glucose levels using the enzymatic GOD-PAP method, triglycerides by the POD method, cholesterol by the CHOD PAP method, LDL by precipitation method, and MDA level determined by TBARS method. The pancreatic organs are taken, histological preparations are made and stained by the haematoxylin eosin (HE) method.

3. RESULTS AND DISCUSSION

The results of this study showed that the administration of yogurt with probiotic *Lactobacillus bulgaricus* and *Streptococcus acidophyllus* significantly lowered blood glucose levels in streptozotocin-induced rat, both yoghurt plain and PSP yogurt. Decreased blood glucose levels were higher in rat treated with PSP yogurt than plain yogurt ($p<0.05$). This proves that purple sweet potato has an activity to lower blood glucose level. However, when compared with glibenclamide, the decrease in blood glucose levels in rats given plain yogurt and PSP yogurt was still lower ($p<0.05$) (Table 1).

Table 1. Results of measurements of blood glucose levels, lipid profiles and mda post test (mean± SD).

Group	K(-)	K(+)	PY	PSPY1	PSPY2	GLB	p
Glu (mg/dL)	71.21 ± 1.16	255.26 ± 2.39	190.54 ± 1.69	122.93 ± 1.81	115.56 ± 1.88	107.78 ± 2.44	<0.05
TG (mg/dL)	67.00 ± 2.20	119.01 ± 1.91	98.37 ± 2.20	87.89 ± 1.58	79.01 ±3.14	73.50 ± 2.50	<0.05
CT (mg/dL)	79.41 ± 2.48	182.29 ± 4.52	130.89 ± 1.91	109.23 ± 4.27	**96.23 ± 2.37***	**89.00 ± 1.78***	<0.05
LDL(mg/dL)	26.80 ± 3.51	93.20 ± 2.86	56.91 ± 2.19	42.61 ± 1.75	**35.74 ± 1.75***	**31.20 ± 1.43***	<0.05
HDL (mg/dL)	83.04 ± 2.64	22.42 ± 1.44	42.35 ± 2.91	50.66 ± 2.10	59.93 ± 3.04	69.07 ±2.21	<0.05
MDA ug/mL	1.38 ± 0.24	4.71 ± 0.66	7.42 ± 0.43	5.65 ± 0.59	3.50 ± 2.20	2.52 ± 0.50	<0.05

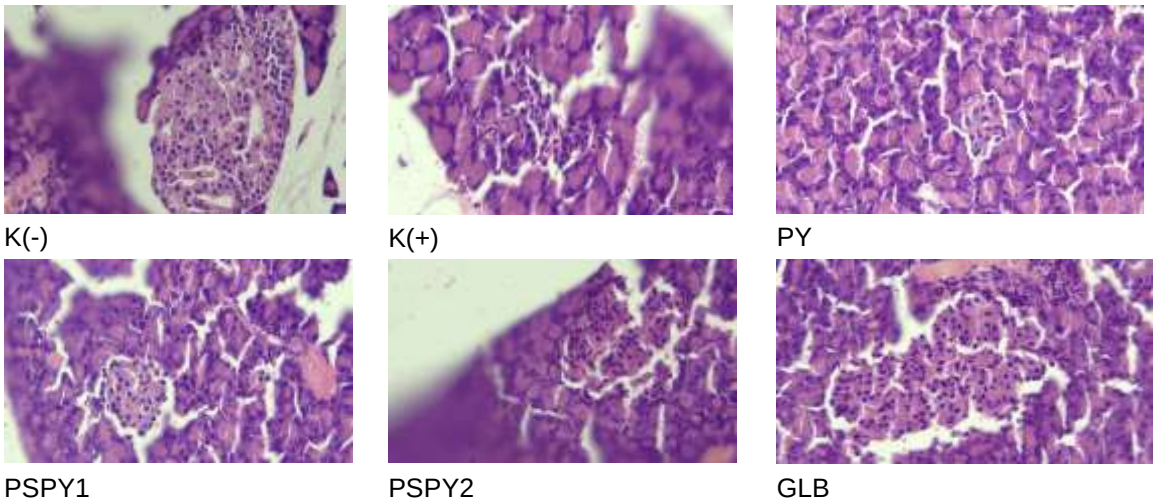
*not significance different

In accordance with previous research, a possible explanation for the hypoglycemic effect is that probiotics affect intestinal bacteria to produce insulinotropic polypeptides and glucagon-like peptide (GLP) so that it induces glucose uptake by muscles, and stimulated liver absorbs more blood glucose to form glycogen. Probiotics could be effective for reducing glucose absorption from intestine tract and could be alter the metabolic use of glucose[5]. In previous studies it has also been shown that anthocyanins have a hypoglycemic effect. The mechanism is to increase phosphorylation of AMP-activated protein kinase (AMPK) on AMPK signaling pathway, not only increase glucose uptake but also inhibit gluconeogenesis and increase glycogen synthesis [6]. The activation of AMPK

causes upregulation of glucose transporter-4 (GLUT4) in the skeletal muscle and white adipose tissue while inhibiting glucose production in the liver. AMPK activation in the liver also results in a significant reduction in liver and serum lipid content via the phosphorylation of acetyl-CoA carboxylase (ACC), upregulation of peroxisome proliferator-activated receptor alpha (PPARα), acyl-CoA oxidase, and carnitine palmitoyl- transferase-1A gene expressions.[7]

The treatment with plain yogurt and PSP yogurt in this study was also shown to improve lipid profiles, that is lowering triglyceride levels, total cholesterol and LDL and increasing HDL levels. Improvement of lipid profile in the treatment with PSP yogurt is better than plain yogurt ($p < 0.05$). Decreasing total cholesterol and LDL levels in the administration of PSP yogurt with 2 times 3 ml doses per day for 28 days is equivalent to a decrease in glibenclamide administration ($p > 0.05$).

The results of this study also show that the administration of PSP yogurt can improve pancreatic β cells (picture 1).



Picture 1. Pancreatic tissue with haematoxylin eosin stain

Anthocyanin like cyanidin, delphidin, pionidin, pelargonidin effectively treat the hyperglycemia by decreasing the tumor necrosis factor (TNF)-α mRNA levels and decreasing oxidative stress protect pancreatic β cells and also it decreases the glucose production by increases the AMPK, increase the gucose absorption and transport then lower risk of diabetes.[8]

Free radicals are produced from vital metabolic activity in body and some of the external source also responsible for the free radical formation X-rays, ozone, cigarette smoking, air pollutants, industrial chemicals, mitochondria, xanthine oxidase, peroxiome, inflammation, phagocytosis, exercise, ischemic injury, certain drug and pesticide. These free radicals may cause different diseases in body like cancer, ischemia disease. Anthocyanins rich food contains a cyanidin, delphinidin, malvidin, peonidin these all are pays a crucial role in scavenge free radicals and reduces the lipid peroxidation and DNA damage.[8]

Probiotics settle in the gut and increase the production of short chain fatty acids (SCFAs) following the fermentation of indigestible carbohydrates from food, and finally SCFAs produced in the gut enter the liver. SCFAs might inhibit the synthesis of fatty acids in the liver, thereby decreasing the triacylglycerol secretion rate and serum triglyceride levels. [9]

Decreasing cholesterol due to prebiotic influences, according to Yoo and Kim (2016) is possible through two different mechanisms. First, it reduces cholesterol absorption caused by increased cholesterol excretion through feces. The second mechanism is the production of short chains fatty acid (SCFA) in selective fermentation by intestinal bacterial microflora.[10]

There are several hypotheses regarding the mechanisms through which probiotics can lower cholesterol. Laboratory studies have shown that intestinal lactic acid bacteria can not only bind to bile acids, but also to cholesterol, and thereby decrease the dietary cholesterol available for absorption in the intestine, and consequently, the total cholesterol. Deconjugation of bile acids with hydrolase inhibits the enterohepatic circulation of bile salts. Bile is a water-soluble composition made in the liver by cholesterol and stored in the gallbladder, and it is secreted when food enters the duodenum. Bile is made of cholesterol, phospholipids, conjugated bile acids, bile pigments, and electrolytes. Deconjugated bile acids have a low solubility and

absorbability in the intestine and are more likely to be excreted in the feces. As a result, the body may use cholesterol to make new bile acids, which may lead to lower serum concentration of cholesterol. The other hypothesized mechanisms are the binding of cholesterol to cell walls of probiotics and assimilation of cholesterol with cellular membrane of bacteria, all leading to lower absorption of dietary cholesterol [11].

The most relevant mechanisms postulated to date include, increasing GLUT-4 translocation, activation of AMPK enzymes and lipolytics, decreasing serine IRS-1 phosphorylation (insulin receptor substrate-1), and inhibition of fatty acids and triglycerol synthesis enzymes and lypogenic activity [6].

The insulin secreting activity of cyanidin has been demonstrated in pancreatic β-cells. Suantawee at al (2017) findings revealed that its mechanism of action is linked to intracellular Ca^{2+} signaling via L-type voltage-dependent Ca2+ channels.[12]

Moreover, anthocyanin protected pancreatic tissue from STZ-induced apoptosis through regulation of caspase-3, Bax, and Bcl-2 proteins. Furthermore, ANT significantly suppressed malondialdehyde levels and restored superoxide dismutase and catalase activities in diabetic rats (Nizamutdinova in Belwal et al.)[6]

It was observed that anthocyanins significantly reduced the blood glucose level in diabeticrats. Anthocyanins also increased the expression and translocation of GLUT4 as well as enhanced the activation of the insulin receptor phosphorylation, thereby increasing the up-take and utilization of glucose by cells. It was also found that anthocyanins may prevent streptozotocin-induced apoptosis in pancreatic cells.[13]

4. CONCLUSION

The administration of purple sweet potato yogurt with *Lactobacillus bulgaricus* and *Streptococcus thermophyllus* isolates in diabetic mice, was able to reduce blood glucose levels, improve lipid profiles, namely lower triglyceride levels, total cholesterol and LDL and increase HDL levels and repair damaged pancreatic beta cells.

5. ACKNOWLEDGEMENT

This research is supported by the BOPTN Health Polytechnic of Yogyakarta, 2018

REFERENCE

1. Proceeding Annual Scientific Meeting: Riset Nutrigenomik terkait Diabetes-Mellitus. 2004.
2. Li C, Li X, Han H, et al. Effect of probiotics on metabolic profiles in type 2 diabetes mellitus. *Med (*United *States)*. 2016;95(26). doi:10.1097/MD.0000000000004088
3. Zhang X, Yang Y, Wu Z, Weng P. The Modulatory Effect of Anthocyanins from Purple Sweet Potato on Human Intestinal Microbiota in Vitro. *J Agric Food Chem*. 2016;64(12):2582-2590. doi:10.1021/acs.jafc.6b00586
4. Wu TY, Tsai CC, Hwang YT, Chiu TH. Effect of antioxidant activity and functional properties of Chingshey purple sweet potato fermented milk by Lactobacillus acidophilus, L. delbrueckii subsp. lactis, and L. gasseri strains. *J Food Sci*. 2012;77(1):2-8. doi:10.1111/j.1750-3841.2011.02507.x
5. Ostadrahimi A, Taghizadeh A, Mobasseri M, et al. Effect of Probiotic Fermented Milk (Kefir) on Glycemic Control and Lipid Profile In Type 2 Diabetic Patients: A Randomized Double-Blind Placebo-Controlled Clinical Trial. *Iran J Public Heal*. 2015;44(2):228-237. http://ijph.tums.ac.ir.
6. Belwal T, Nabavi SF, Nabavi SM, Habtemariam S. Dietary anthocyanins and insulin resistance: When food becomes a medicine. *Nutrients*. 2017;9(10). doi:10.3390/nu9101111
7. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food Nutr Res*. 2017;61(1):1361779. doi:10.1080/16546628.2017.1361779
8. Shaik A, Naidu KK, Panda J. A Review on Anthocyanins: a Promising Role on Phytochemistry and Pharmacology. *Int Res J Pharm*. 2018;9(1):1-9. doi:10.7897/2230-8407.0911
9. Seo BK, Kim NE, Park KM, Park KY, Park HK, Hwang HS. Serum lipid levels in relation to consumption of yogurt: The 2012 Korea National Health and Nutrition Examination Survey. *Korean J Fam Med*. 2017;38(5):249-255. doi:10.4082/kjfm.2017.38.5.249

10. Yoo JY, Kim SS. Probiotics and prebiotics: Present status and future perspectives on metabolic disorders. *Nutrients*. 2016;8(3):1-20. doi:10.3390/nu8030173
11. Rezaei M, Sanagoo A, Jouybari L, Behnampoo N, Kavosi A. The effect of probiotic yogurt on blood glucose and cardiovascular biomarkers in patients with type II diabetes: A randomized controlled trial. *Evid Based Care J*. 2017;6(4):26-35. doi:10.22038/ebcj.2016.7984
12. Suantawee T, Elazab ST, Hsu WH, Yao S, Cheng H, Adisakwattana S. Cyanidin stimulates insulin secretion and pancreatic β-cell gene expression through activation of L-type voltage-dependent ca2+channels. *Nutrients*. 2017;9(8):1-14. doi:10.3390/nu9080814
13. Różańska D, Regulska-Ilow B. The significance of anthocyanins in the prevention and treatment of type 2 diabetes. *Adv Clin Exp Med*. 2018;27(1):135-142. doi:10.17219/acem/64983