

EFFECT OF PUMPKIN SOYGURT ON MICROBIOTA BALANCE IN DIABETES MELLITUS MODEL RATS

Annisa Avelia^{1*}, Didik Gunawan Tamtomo², Yulia Sari³

¹Postgraduate Program of Clinical Nutrition, Universitas Sebelas Maret, Surakarta, Jl. Ir Sutami No. 36, Kentingan, Kec. Jebres, Surakarta City, Central Java 57126, Indonesia

²Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Jl. Ir Sutami No. 36, Kentingan, Kec. Jebres, Surakarta City, Central Java 57126, Indonesia

³Department of Parasitology, Faculty of Medicine, Universitas Sebelas Maret, Jl. Ir Sutami No. 36, Kentingan, Kec. Jebres, Surakarta City, Central Java 57126, Indonesia

*annisa.avelia@poltekkes-malang.ac.id

ABSTRACT

Microbiota imbalance in the gastrointestinal tract causes low-grade systemic inflammation, which triggers metabolic changes and insulin resistance to become diabetes mellitus. This condition can be controlled through synbiotic supplementation through pumpkin soygurt containing lactic acid bacteria and polysaccharides. This study aimed to analyze the effect of complementary therapy using pumpkin soygurt and metformin on the abundance of lactic acid bacteria and *Escherichia coli* in a rat model of diabetes mellitus. The research design is experimental with a post-test only. Male Wistar rats were divided into six groups; healthy rats (KS), DM rats (K-), DM+metformin rats (K+), DM+metformin+1 mL pumpkin soygurt rats (P1), DM+metformin+ 1.5 mL pumpkin soygurt rats (P2), DM + metformin + 2 mL of pumpkin soygurt (P3) rats. The test material was given for four weeks. The abundance of LAB and *Escherichia coli* was analyzed using a sample of the contents of the cecum with the method total plate count. Data were analyzed using the One way ANOVA test. This study showed a dysbiosis condition on K- (BAL 6.85 CFU/mL; *Escherichia coli* 8.80 CFU/mL) dan K+ (BAL 4,99 CFU/ml; *Escherichia coli* 7.67 CFU/mL). In the treatment group, eubiotics were maintained, with the best conditions in group P2 with a BAL of 7.46 CFU/mL and *Escherichia coli* 4,62 CFU/mL. The combination of pumpkin soygurt and metformin can maintain the balance of the gastrointestinal microbiota in DM model rats, especially lactic acid bacteria and *Escherichia coli*.

Keywords: diabetes mellitus; *escherichia coli*; lactic acid bacteria; synbiotic

INTRODUCTION

Microbiota plays a role in maintaining health through the breakdown of polysaccharides, modification of bile acids, assimilation of nutrients, intestinal permeability, and inflammatory reactions (Gulnaz *et al.*, 2021). In conditions of dysbiosis (a condition of imbalance between microflora populations in the gastrointestinal tract, conditions of dysfunction of the gastrointestinal microflora), this microflora causes various health problems, including type 2 diabetes mellitus (Hosainzadegan, 2019). The condition of dysbiosis in the gastrointestinal tract results in impaired intestinal permeability, so that lipopolysaccharide (LPS), a constituent of the bacterial cell wall that should be in the gastrointestinal tract, enters the circulation and triggers *low-grade systemic inflammation*, thus causing insulin resistance (Souza, Alves and Fusco, 2022).

In type 2 diabetes mellitus, there is a decrease in the number of classes *Clostridia*, phylum *Firmicutes*, and *Actinobacteria*, as well as an increase in class *Betaproteobacteria* and *Bacteroidetes* which has a positive correlation increased plasma blood glucose (Larsen *et al.*, 2010; Que *et al.*, 2021). *Escherichia coli* is a gram-negative bacteria that shows increased diabetes mellitus (Kostic *et al.*, 2015). Conditions of insulin resistance can lead to an increased abundance of *Escherichia coli* in the intestine due to increased metabolism by forming a *biofilm*. Insulin acts

as a virulent enzymatic expression factor of *Escherichia coli* (Madacki-Todorović *et al.*, 2018). One form of management of diabetes mellitus is complementary therapy using synbiotics as a functional food. Pumpkin soygurt is a synbiotic formula containing probiotics in the form of lactic acid bacteria (LAB), isoflavones, and polysaccharides, which act as prebiotics. *Bifidobacterium* is one of the LAB groups helpful in improving the intestinal environment and maintaining the intestinal barrier. *Bifidobacterium* cannot degrade intestinal mucus glycoproteins to maintain healthy microphiles by preventing increased bacterial permeability and translocation. A good condition of the intestinal mucosa can control the entry of LPS into the blood circulation; *low-grade systemic inflammation* can be avoided (Wang *et al.*, 2012).

Genistein is an isoflavone found in soybeans. Genistein has a role in carbohydrate metabolism by interacting with the gut microbiota (Guevara-Cruz *et al.*, 2020). Consumption of genistein can increase the abundance of the phyla *Verrucromicrobia* associated with genus increase *Akkermansia*. Abundance *Akkermansia muciniphila* associated with decreased blood glucose levels and HOMA-IR (Dao *et al.*, 2015; Schneeberger *et al.*, 2015; Depommier *et al.*, 2019). The role of polysaccharides as probiotics through increasing short-chain fatty acid-producing bacteria such as *Bacteroidetes*, *Prevotella*, *Deltaproteobacteria*, *Oscillospira*, *Veillonellaceae*, *Phascolarctobacterium*, *Sutterella*, and *Bilophila* (Liu *et al.*, 2018). Polysaccharides can increase the proliferation of *Akkermansia muciniphila*, increasing insulin sensitivity by decreasing mucin and acetic acid production (Dao *et al.*, 2016).

METHOD

This research design is a post-test with a control group carried out at Microbiology Laboratory, Faculty of Medicine, Andalas University from January until Maret 2023. This research protocol has received ethical approval from the Research Ethics Commission of the Faculty of Medicine, Andalas University, with the number 1063/UN.16.2/KEP-KP/2022. This research design is post-test with control group, with male Wistar rats aged 8 – 12 weeks and weighing \pm 200 grams. Rats were divided into six groups; healthy control, negative control, DM rats were given metformin, DM rats were given metformin and pumpkin soygurt at a dose of 1 mL, DM rats were given metformin and pumpkin soygurt at a dose of 1.5 mL, DM rats were given metformin and pumpkin soygurt at a dose of 2 mL. Then the samples were given the test material for four weeks, once a day at 10.00 WIB.

Rats were conditioned with diabetes mellitus by induction of 230 mg/kgBB nicotinamide and 65 mg/kgBB STZ (bioWORLD, Dublin, Ohio, USA) intraperitoneally. On the third day after the injection, blood glucose levels were measured, and only rats with blood glucose levels above 200 mg/dL were used as research subjects. On day 29, the rats were terminated to take cecum samples. Samples of the contents of the rat cecum were taken, accommodated in a sterile test tube, and diluted. Calculation of the number of bacteria was carried out by the total plate count method. Contents of the cecum were then diluted using sterile peptone water to a dilution of 10^{-6} . Calculation of total lactic acid bacteria was carried out by the method *Total Plate Count* using *de Man Rogosa Sharpe Agar* media and incubated at 36°C for 24 to 48 hours (Badan Standardisasi Nasional Indonesia, 2012a). *Escherichia coli* was measured at the end of the study by taking samples of the contents of the rat cecum. Samples were diluted using 0.86% physiological NaCl up to dilution of 10^{-8} . Samples were grown on selective media *Eosin Methylene Blue Agar* (EMBA)

with the scatter method. Samples planted on the media were incubated at 37°C for 24 hours (Badan Standardisasi Nasional Indonesia, 2012b). Colony *Escherichia coli*, which grows dark blue to violet. The results of the research data were processed using IBM SPSS Statistics 26. Analysis of the differences in each treatment group used Test *One Way ANOVA* ($p \leq 0.05$), carried out by test *LSD* when significant differences are found between groups.

RESULTS AND DISCUSSION

Microbiota has an essential role in the body's homeostasis; changes in composition and diversity can cause health problems. Based on the study's results, there was a dysbiosis condition, namely, an increase in *Escherichia coli* and a decrease in the amount of LAB, which triggers glucose homeostatic disturbances in the rat model of diabetes mellitus. Dysbiotic conditions can cause insulin resistance due to impaired intestinal permeability, so LPS, which should be in the gastrointestinal tract, enters the blood circulation and triggers low-grade systemic inflammation (Liang *et al.*, 2013; Kuwabara *et al.*, 2018). The results showed differences in the number of lactic acid bacteria in each group. Mice with DM conditions experienced a 16% decrease in BAL compared to healthy mice. In diabetes mellitus, there is a decrease in the number of *Lactobacillus* and *Bifidobacterium*; reduced numbers of these two bacteria can trigger low-grade inflammation, thus causing insulin resistance. Low-grade inflammation can be triggered by components of pathogenic bacteria, namely lipopolysaccharide, flagella, and peptidoglycan, which cause an inflammatory response (Huda, Kim and Bennett, 2021).

The K+ group showed the most decrease in LAB, a reduction of 39% compared to the healthy rat group (table 1). The combination intervention of pumpkin soygurt and metformin showed that the amount of LAB tended to be close to that of healthy controls. The dose and duration of metformin use can cause this. Metformin intervention showed inconsistent results against good bacteria in the digestive tract (Gao *et al.*, 2018). A combination of metformin and pumpkin soygurt can maintain the amount of LAB under normal conditions because this product contains 19.5×10^7 BAL (Avelia, Tamtomo and Sari, 2023). Polysaccharides' role in pectin as a prebiotic is an indigestible fiber. Still, it can increase the amount and activity of LAB, especially the genus *Lactobacilli* and *Bifidobacteria*. It can lower the gastrointestinal tract's pH to prevent pathogenic bacteria's growth, stimulating the secretion of incretin hormones and reducing endotoxemia (Pandey, Naik and Vakil, 2015; Zhang *et al.*, 2019, 2021). Besides that, pectin fermentation by the microbiota in the digestive tract produces short-chain fatty acids such as butyrate, acetate, and propionate, which can control blood glucose by increasing the production of GLP-1 and PPY in the intestine (Kim, 2018; Huda, Kim and Bennett, 2021; Zhang *et al.*, 2021). SCFA, through FFAR2 activation, can suppress insulin signaling in adipose tissue and prevent fat accumulation in adipocyte tissue. Butyrate can increase plasma insulin levels and sensitivity by stimulating the hormone incretin in pancreatic β cells (Puddu *et al.*, 2014).

Table 1
Mean Abundance Of Lactic Acid Bacteria and *Escherichia coli* For Each Group

Group	f	Mean±SD (log CFU/mL)	
		Lactic Acid Bacteria	<i>Escherichia coli</i>
KS	5	8,16±0,20	4,22±1,36
K-	5	6,85±0,38	8,80±0,26
K+	4	4,99±1,02	7,67±0,76
P1	4	7,38±0,11	4,17±0,54
P2	4	7,46±0,74	4,67±1,20
P3	5	7,96±0,74	5,73±0,60
<i>p-value</i>		0,000*	0,000*

Description: KS: healthy rats, K-: DM rats, K+: DM+metformin rats, P1: DM+metformin mice+ 1 mL pumpkin soygurt, P2: DM+metformin mice+ 1.5 mL pumpkin soygurt, P3: DM rats + metformin + 2 mL pumpkin soygurt, *: significant $p < 0,05$

Escherichia coli is a gram-negative bacterium that has a role in maintaining intestinal microbiota. Still, in large quantities, it can be one of the triggers of the etiology of diabetes mellitus (Kostic *et al.*, 2015). Table 1 shows a significant difference in the abundance of *Escherichia coli* in each treatment group. In the DM model rat condition, the number of *Escherichia coli* was 52% more when compared to healthy mice. *Escherichia coli* O157:H7 can affect glucose metabolism, causing hyperglycemia (Suri *et al.*, 2009). Low insulin levels can increase the metabolism of *Escherichia coli* and play an autoinducer on biofilm formation, which later develops into insulin resistance (Tetz *et al.*, 2019). Additionally, insulin is a virulent enzymatic expression factor for *Escherichia coli* (Madacki-Todorović *et al.*, 2018).

In the DM rat group that was given the metformin intervention, it was found 7.67 ± 0.76 logs CFU/mL (table 1). Metformin can create a competitive environment for *Escherichia coli*, which can cause changes in the abundance of microbiota in the digestive tract (Winter *et al.*, 2014). The group given the combination of metformin and pumpkin soygurt intervention was total *Escherichia coli* close to that in the healthy control group. This shows that synbiotics can suppress the growth of *Escherichia coli*. Prebiotics can inhibit the growth of *Escherichia coli* in the mucous layer, thus preventing infection in the host (Zou *et al.*, 2020). In addition, the prebiotic fermentation process by probiotic bacteria will cause the intestinal environment to become acidic, thereby inhibiting the growth of *Escherichia coli* (Orr *et al.*, 2019; Fuhrmann *et al.*, 2022).

CONCLUSION

The combination of pumpkin soygurt and metformin can maintain the balance of the gastrointestinal microbiota in DM rat models, especially lactic acid bacteria and *Escherichia coli*. Administration of 1.5 mL of pumpkin soygurt was more effective in maintaining the balance of the gastrointestinal microbiota of the DM rat model.

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