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Supplementation of Glucomannan Derived from Konjac Flour Improve Glucose Homeostasis and Reduce Insulin Resistance in Diabetes Rat Models

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Abstract: Dietary fiber from glucomannan has been studied to decrease blood glucose concentration, but its mechanism in diabetes is still unclear. The aim of our research is to study the effect of glucomannan, derived from konjac flour, in rat models of diabetes including gastrointestinal function, inhibition of DPP-IV enzyme and reducing in insulin resistance. A total of 25 male wistar rats were divided into 5 groups; normal group (Normal), diabetes group (DM), diabetes group administered with 100 mg/kg BW konjac flour (DM+KF1), 200 mg/kg BW konjac flour (DM+KF2) and 400 mg/kg BW konjac flour (DM+KF3). Diabetes was induced by a combination of 60% high fructose diet and twice intraperitoneal injection of streptozotocin (25 and 30 mg/kg BW) at one week interval. Konjac flour was given according to each dose for 4 weeks. At the end of the study, blood and tissue sample were collected for subsequent analysis, while isolated intestine used to measure jejunal serous glucose concentration using everted sac technique. The results indicate that glucomannan reduced fasting blood glucose levels, improved glucose tolerance, increased jejunal serous glucose concentration at *in vitro* technique and decreased insulin resistance as evidenced by a decreased in HOMA-IR index and increased in PI3K levels. However, glucomannan not decreased DPP-4 levels in any dose. This results indicate that glucomannan derived from Konjac flour had antidiabetic effects through improving in glucose homeostasis and reducing in insulin resistance in rat models of diabetes.

Key words: Konjac flour, diabetes, glucose homeostasis, insulin resistance

INTRODUCTION

The global prevalence of diabetes worldwide continue to increase with the mortality rate is very high. It was estimated that 382 million peoples have diabetes and 5.1 million of them died in 2013 (IDF, 2013). Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in pancreatic β-cells so that unable to produce insulin adequately, insulin resistance in peripheral tissue, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of different organs (ADA, 2013).

Recently, nutraceuticals are used throughout the world for a range of diabetic presentations because of their effectiveness, less side effects and relatively low cost. Research is supportive of the benefits of diets high in certain types of fiber for promoting improved post-prandial glucose and insulin responses in individuals with diabetes, dyslipidemia and insulin resistance (Pandey and Vijayakumar, 2011).

Glucomannan is one of a soluble and fermentable dietary fiber extracted from the tuber of konjac (*Amorphophallus muelleri* Blume). Several studies have shown the effects of Konjac flour to decrease blood glucose levels in diabetes (Li *et al*., 2004; Chearskul *et al*., 2007), but its mechanism in diabetes is still unclear. The aim of our research is to study the effect of Konjac flour in rat models of diabetes including gastrointestinal function, inhibition of DPP-IV enzyme and reducing in insulin resistance.

MATERIALS AND METHODS

Animals and treatments: Male *Rattus norvegicus Wistar strain* (150-200 g) were obtained from the Laboratory of Pharmacology, Brawijaya University of Malang, Indonesia. The animals were housed in cages in a

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room maintained at±28°C on 12:12 h light-dark-cycle. They were fed standard laboratory chow with water *ad libitum* and fasted overnight before the experiments. Body weights were measured every week. All animal experiments were approve by the Research Ethics Committee of Brawijaya University with official statement No.025/EC/IEC/01/2015.

Rats were randomly divided into five groups of five animals; normal rats controls (N), diabetic rats controls (DM), diabetic rats supplemented with Konjac flour at dose 100 mg/kg BW (DM+KF1), dose 200 mg/kg BW (DM+KF2) and dose 400 mg/kg BW (DM+KF3). Diabetes was induced by 60% diet in high fructose for 6 weeks and twice intraperitoneal injection of streptozotocin at dose 25 and 30 mg/kg BW at 1 week intervals. Animals whose fasting blood glucose level exceeded 150 mg/dl were considered diabetic (Butler, 1995). Konjac flour given orally according to each dose for 4 weeks. Konjac flour was obtained from Porang Research Center of Brawijaya University.

Oral glucose tolerance test: After an overnight fast, konjac flour was given 30 minutes before administration of an oral glucose load (2 g/kg BW). Blood samples were collected from the tail vein at 0 (before glucose administration), 30, 60 and 120 min for measurement of glucose. The curves were plotted as the change in plasma glucose over time and the integrated area under the curves (AUCs) was calculated.

Everted sac technique: Rats were fasted overnight before sacrificed then isolated intestine and soaked in tyrode solution. Everted sac technique was performed according to Wilson and Wiseman (1954) and Adeniyi and Oloowookurun (1987) with modifications. Each jejunum cut along 4 cm. Sac created by everting a pieces of jejunum with needle and thread. After the upside, tie one end of a sac with the dry thread. Sac was filled with 1 ml tyrode solution (as serous fluid) and tie the other end with thread.

40 ml tyrode solution put into a tube that must be kept warm in 36-37°C using tap water connected to lamp in the outer tube. Tube also must be connected to oxygen hose for aeration. Sac inserted into the tube and adapted for 30 min with replacing tyrode solution every 15 min. Sac incubate in new tyrode solution for 30 min and konjac flour was added according to each dose. After incubation, tyrode that loaded into the sac was taken and glucose concentration was measured by using spectrophotometry at 520 nm.

Biochemical analysis: Rats were fasted overnight, blood was taken from tail vein to measure fasting blood glucose concentration by glucometer. Then after sacrificed, blood sample was collected through cardiac puncture and centrifuged 3000 rpm for 15 min at 4°C. Serum was separated and stored at -20°C until further analysis. DPP-IV and insulin level was measured by using DPP-IV ELISA kit (Elabscience, E-EL-R0337) and Insulin ELISA kit (Elabscience, E-EL-R0023). Femoral muscle were collected immediately to estimate PI3K level by using Rat PI3K ELISA Kit (Elabscience E-EL-R0739) as a method according to the instructions.. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from the fasting blood glucose (mg/dL) and fasting serum insulin (mU/mL) divided by 450.

Statistical analysis: Data were expressed as means±SD. Statistical analysis was performed by One Way ANOVA and Kruskal-Wallis followed by Post Hoc Tuckey or Mann-Whitney U test. Statistic was significantly different if the p-value less than 0.05.

RESULTS

Characteristics of samples: The characteristics of animals were used in this study can be seen in Table 1. The diabetic rats showed no statistically different in body weight after induction of diabetes, as compared to the normal group. It was found that body weight was a slight decrease after supplementation of konjac flour at various doses, as compared to DM group. All group that induced diabetes has significantly higher in fasting blood glucose than normal group.

Effect of konjac flour on fasting blood glucose levels: After 10 weeks of the experiment, the DM group had up to a 2.7-fold increase in fasting blood glucose levels. The plasma glucose of DM group supplemented with all doses of konjac flour were significantly lower than the diabetes group by 56, 55 and 50%, respectively (Fig. 1).

Effect of konjac flour on jejunal serous glucose concentrations *in vitro*: The jejunal serous glucose concentration in DM group was significantly higher than normal group. Konjac flour administered *in vitro* cause slightly increase in jejunal serous glucose concentration by 0.7, 31.7 and 51.6%, as compared to DM group (Fig. 2).

Effect of konjac flour on DPP-4 levels: DPP-4 level in DM group slightly increase, as compared to normal group. While, DM group administered with konjac flour at doses 100 and 200 mg/kg BW had up to 2.2 and 2.3-fold increase in DPP-IV levels (Fig. 3).

Effect of konjac flour on the oral glucose tolerance test: Figure 4 shows the incremental changes in plasma glucose concentration of rats following an oral glucose load. The incremental glucose concentrations and AUC during OGTT of diabetes group was significantly higher than those of normal group. But, in all

Table 1: Characteristics of samples

	Normal	DM	DM+KF1	DM+KF2	DM+KF3
BW1 (g)	166.8±10.3	179±15.7	167±7.1	167.2±14	171.8±14.9
BW2 (g)	252.4±25.7	224.6±20	218±15.5	228.4±33.2	205±28.8
BW3 (g)	302.8±26	223±36.7#	206.6±38.2	217.8±52.8	198±7.52
FBG1 (mg/dl)	90.4±10.1	347.2±9#	289.2±44.8	305±71.8	297±97.9

Data is mean±SD. #p<0.05 compared with normal group. *p<0.05 compared with DM group. BW1: Body weight at the initial experiment, BW2: Body weight after diabetes induction, BW3: Body weight at the end of study, FBG1: Fasting blood glucose after diabetes induction

Table 2: Effect of konjac flour on insulin resistance

	HOMA-IR	PI3K (pg/ml)
Normal	0.81±0.52	976.92±130.93
DM	6.6±2.75#	548.38±116.76
DM+KF1	2.52±1.11*	766.49±88.92
DM+KF2	1.59±0.5*	720.12±56
DM+KF3	1.56±0.78*	684.22±124.67

Data is mean±SD. #p<0.05 compared with normal group. *p<0.05 compared with DM group

of DM groups supplemented by konjac flour showed that the incremental glucose concentration and AUC were lower than DM group by 37, 31 and 19%.

Effect of konjac flour on insulin resistance: The HOMA-IR index was found to be higher in DM group. Supplementation of konjac flour can significantly lower HOMA-IR index as compared to DM group. Whereas the PI3K level in DM group was lower than normal group, but in all of DM groups supplemented by all doses of konjac flour showed a slight increase as compared to DM group by 40, 31 and 25% (Tab. 2).

DISCUSSION

The present study was designed to investigate the possible mechanisms of konjac flour in rat models of diabetes including gastrointestinal function, inhibition of DPP-IV enzyme and reducing in insulin resistance. Induction of diabetes was done by 60% of high fructose diet and twice intraperitoneal injection of Streptozotocin (STZ) in doses of 25 and 30 mg/kg BW at one week interval. The results showed that in this rat models, plasma glucose concentration was significantly elevated at week 6. The development of hyperglycemia in this models is preceded by the partial destruction of the pancreatic β cell mass by multiple low dose of STZ resulting in relative insulin deficiency (Zhang *et al*., 2008) and increasing in visceral adiposity by high fructose diet that decrease insulin sensitivity (DeBosch *et al*., 2013). Our results showed that konjac flour can decrease fasting blood glucose concentration. This hypoglycemic effect was evidenced in previous researches by Li *et al*. (2004) and Chearskul *et al*. (2007). This effect is thought to be caused by glucomannan that content in konjac flour. Glukomanan is soluble dietary fiber which is fermented by anaerobic bacteria in the colon (Keithley and Swanson, 2005). Several studies have investigated the effects of soluble fiber in diabetes. Hannan *et al*. (2007) shown that soluble fibre from fenugreek

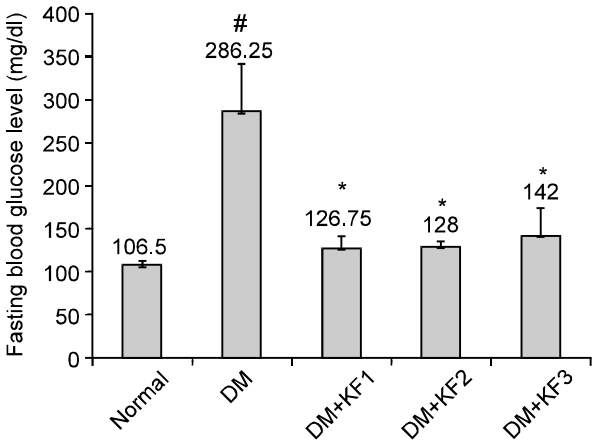

Fig. 1: Effect of konjac flour on fasting blood glucose levels. The data is mean±SD. #p<0.05 compared with normal group. *p<0.05 compared with DM group

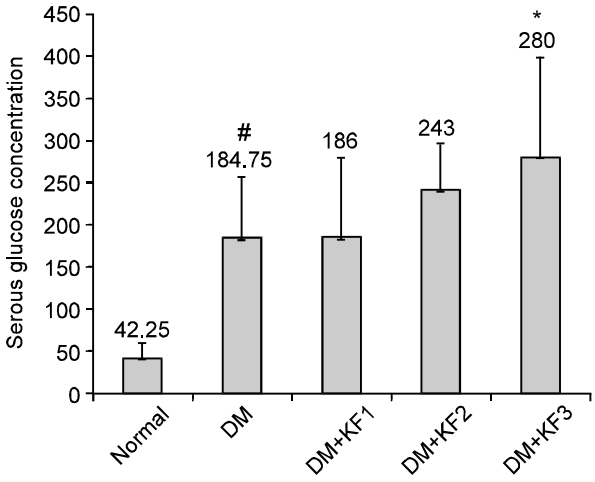

Fig. 2: Effect of konjac flour on jejunal serous glucose concentration *in vitro*. The data is mean±SD. #p<0.05 compared with normal group. *p<0.05 compared with DM group

seed improves glucose homeostasis in animal models of 1 and type 2 diabetes by delaying carbohydrate digestion and absorption and enhancing insulin action. While Cameron-Smith *et al*. (1997) compared the effect of soluble and insoluble fiber and suggested that soluble fiber could improve insulin sensitivity in diabetic rat.

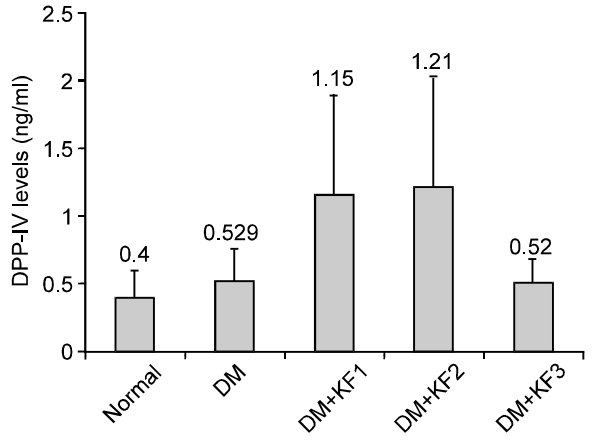

Fig. 3: Effect of konjac flour on DPP-4 levels. The data is mean±SD. #p<0.05 compared with normal group. *p<0.05 compared with DM group

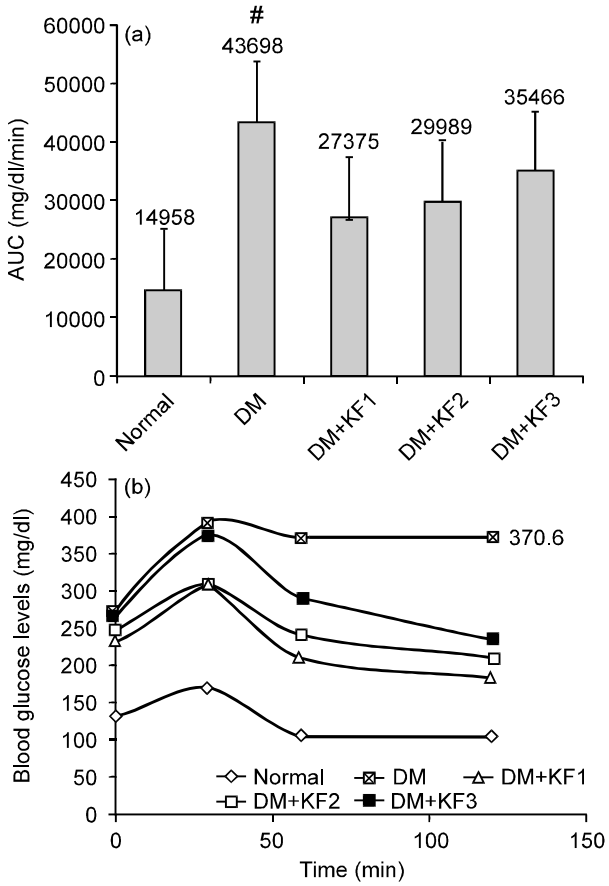

Fig. 4: Effect of Konjac Flour on the oral glucose tolerance test: (A) Curve of incremental change in blood glucose concentration at selected time intervals (0, 30, 60 and 120 min), (B) Histogram of AUC. The data is mean±SD. #p<0.05 compared with normal group. *p<0.05 compared with DM group

Glucomannan increases viscosity by forming an impermeable gel layer in the gastrointestinal tract and inhibits contact of enzymes, alpha-glucosidases and alpha-amylase, with carbohydrates complex so that can not be digested into glucose (Leclere *et al*., 1994). Low glucose levels in the gut mucosa may cause downregulation of expression intestinal glucose transporters, SGLT-1 and GLUT-2, thus decrease uptake of glucose along the membrane and cause decrease in blood glucose levels in circulation (Nasir *et al*., 2010).

Although, the present study did not examine the inhibition to that enzymes, this has been further confirmed by *in vitro* studies. Administration of konjac flour to isolated intestine models increase jejunal serous glucose concentration. It shown that no inhibition mechanism to alpha-glucosidase and alpha-amylase by glucomannan due to pure glucose that is given and also the characteristic of glucomannan as a water absorbent that is able to absorb water which dissolves the free glucose so that glucose transported into basolateral membrane more numerous. According to Koroskenyi and Carthy (2001) and Holford (2010), glucomannan can absorb water properties in which this trait is 50 times higher than wheat bran.

As dietary fiber, glucomannan fermented by anaerobic bacteria in the colon and produce Short Chain Fatty Acid (SCFA), an organic fatty acids with 1 to 6 carbon atoms existing in straight- and branched-chain conformations (Chen *et al*., 2008). About 95% of the produced SCFAs are rapidly absorbed by the colonocytes and transported across the membrane via different transporter (Besten *et al*., 2013). Beside acting as a local nutrient source, SCFA can also triggers cell-specific signaling cascades by activation of Free Fatty Acid Receptor (FFAR) 2 and 3 in a variety of cells, including colonic enteroendocrine L cells, mucosal mast cells, adipose tissue, neutrophils and monocytes. Activation of the receptors affects distinct function depending on their tissue distribution (Puddu *et al*., 2014).

Previous study proved that SCFA stimulate secretion of Glucagon Like Peptide (GLP)-1 via activation of FFAR2 (Psichas *et al*., 2014; Tolhurst *et al*., 2012). GLP-1 is one of in cretin hormones that released from colonic enteroendocrine L cells and modulates insulin secretion (Augustyns *et al*., 2005). This hormone is substrate for DPP-4 enzyme, so inhibiting this enzyme will increase circulating active GLP-1 levels (Thornberry and Gallwitz, 2009). This present study examine the effect of glucomanan from konjac flour to dipeptidyl peptidase (DPP)-4 enzyme. Since our results did not reveal inhibition to DPP-4 enzyme, even at doses of 100 mg/kg BW and 200 mg/kg BW DPP-4 enzyme levels actually increased. It was not be ascertained, but it might be explained by study of Makdissi *et al*. (2012) that sitagliptin, one of specific inhibitor for DPP-IV enzyme,

increased DPP-4 enzyme level at week-12 but decreased 80% of its activity. Thus, further research is expected not only to measure enzyme level but also to examine enzyme activity.

Our results also showed that glucose intolerance and insulin resistance decreased after administration of konjac flour. This is may be due to the action of SCFA to reduce the accumulation of free fatty acid in insulin target tissue that cause insulin resistance. In liver and muscle tissue, SCFA have been shown to increase the AMPK activity triggers PGC-1 α expression, which is known to control the transcriptional activity of several transcription factor that is important in regulation of cholesterol, lipid and glucose metabolism. As consequence, fatty acid oxidation is enhanced in both tissues and de novo fatty acid synthesis in the liver is decreased (Besten *et al*., 2013). Study by Higgins *et al*. (2004) showed that consumption of fiber associated with SCFA production, can significantly enhance fat oxidation so that reduce fat accumulation.

SCFA strongly reduce lipolysis in adipose tissue through inactivation of hormone sensitive lipase (HSL). Binding of SCFA to FFAR2 lead to the dissociation and thereby the activation protein Gi/o protein which inhibit adenylate cyclase and reduce the production of cAMP from ATP, which subsequently decreases the activity of Protein Kinase A (PKA). Decrease of PKA activity lead to dephosphorylation and deactivation of HSL in adipose tissue (Besten *et al*., 2013). Robertson *et al*. (2005) studied that supplementation of Resistant Starch associated with production of SCFA, reduce lipolysis in adipose tissue, evidenced by significantly decrease in post prandial output NEFA and glycerol from adipose tissue reflecting a decrease in HSL activity. SCFA also plays an important role in adipogenesis through suppress insulin signaling by inhibition of Akt phosphorylation. Decrease storage in adipose tissue would promote the metabolism of lipid and glucose in other tissues (Besten *et al*., 2013). Research by Kimura *et al*. (2013) proved that the intestinal microbiota could suppress insulin mediated fat accumulation in adipose tissue through activation FFAR2.

Conclusion: In conclusion, dietary supplementation of glucomannan derived from konjac flour can improve glucose homeostasis and reduces insulin resistance in diabetes. One of the mechanism of glucomannan action is related to physical characteristic of soluble fiber which form impermeable layer in the gastrointestinal tract so that inhibit contact of enzyme with carbohydrate complexes. Stimulation of PGC-1 α activity in liver and muscle, inactivation of HSL and inhibition of Akt phosphorylation in adipose tissue may be a molecular mechanism of glucomannan activity. Konjac flour may have potential application in the treatment of diabetes in humans.

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