The Potential of Porang (Amorphophallus muelleri Blume) Flour and Porang Flour Formulation as an Anti-Diabetes Type-2 Agent

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1. Introduction

Diabetes mellitus is a heterogeneous-metabolic disorder distinguished by hyperglycemia due to impaired insulin secretion, defective insulin action, or both (Punthakee et al. 2018). The World Health Organization (WHO) has estimated that 439 million people will have diabetes in 2030 (Reagan-Shaw et al. 2008). There are two types of DM; in type-1 diabetes mellitus (T1DM), the pathogenesis is pancreas secretion of damaged β-cells to prevent it from lowering the blood glucose level in time, while insulin resistance and insulin secretion insufficiency are the pathogeneses of type-2 diabetes mellitus (T2DM) which is also known as non-insulin dependent DM (Wu et al. 2018). Diabetes can be a complication and is affiliated with fundamental changes in the serum lipids profile (Ani and Aginam 2018).

One of the ingredients used for diabetes mellitus treatment is glucomannan, a fermentable dietary fiber extracted from konjac (Amorphophallus konjac or Amorphophallus rivieri), which consists of a polysaccharide chain of β-D-glucose and β-D-mannose with attached acetyl groups in a molar ratio of 1:1.6 with β-1–4 linkages (Keithley et al. 2013). Soluble dietary fibers, especially konjac fiber, have beneficial effects on serum glucose levels, which might be caused by delayed stomach emptying and glucose diffusion in the intestinal lumen. Supplement of konjac glucomannan has been reported to have an...
effect in decreasing blood glucose and cholesterol levels in healthy diabetic and hypercholesterolemic patients with daily ingestion of (0.7 g KGM/100 kcal intake) KGM-rich diet (Shah et al. 2015). Having a high level of glucomannan content, *Amorphophallus muelleri* Blume, locally called porang, may be considered a promising glucomannan source in Indonesia (Yanuriati et al. 2017). Porang flour has high glucomannan levels of 60–80% (Widjanarko et al. 2015; Witoyo et al. 2020, 2021, 2022).

Besides glucomannan, many studies have shown that inulin and carrageenan can potentially treat diabetes mellitus. Inulin is a water-soluble polysaccharide with an anti-diabetic ability with increased insulin resistance and acts as a prebiotic to improve intestinal health. It is a new therapeutic agent for diabetes prevention and control (Roshanravan et al. 2018; Valenlia et al. 2018). Carrageenan is a hydrocolloid extracted from red seaweed that acts as a prebiotic (Hamed et al. 2015).

Optimizing porang flour, inulin, carrageenan, and modified cassava flour (MOCAF) fillers proportion uses Design Expert 7.1.5 software. The Response Surface Methodology (RSM) method was designed by Box Behnkken Design (BBD) to produce the optimum porang supplement as DM therapy material (Setiabudi 2018). MOCAF has functioned as a filler and binder in the pharmaceutical industry, especially in making capsules (Tonukari et al. 2015). Research on porang flour formulation supplement resulting from optimization as DM therapy agents have never been done, so it is necessary to conduct in vivo studies of porang flour supplements, inulin, carrageenan, and MOCAF as DM therapy ingredients, especially type-2 DM.

2. Materials and Methods

2.1. Materials

Porang flour (PF) 80 mesh was donated by Porang Research Centre, Institute of Research and Community Service, Brawijaya University, Malang, Indonesia. Inulin 100 mesh was bought from Xi’an Lyphar Co., Ltd, China. κ-Kappa Carrageenan Nusantara Ltd., Pasuruan, Indonesia, was donated carrageenan. Modified Cassava Flour (MOCAF) 80 mesh Greenstar Brand was purchased from the local market. Lipid peroxidation malondialdehyde (MDA) assay kit (Sigma Aldrich, Germany), superoxide dismutase (SOD) assay kit (Sigma Aldrich, Germany), antibody anti-insulin (ScyTek Laboratories, Inc., USA) and the other chemicals used were analytical grade.

2.2. Preparation of Porang Flour Formulation (PFF)

The formulation Porang Flour Formulation (PFF) was followed by the previous study reported by Setiabudi (2018). PFF consists of 85% porang flour (PF), 1.03% κ-carrageenan flour, 12% inulin flour, and 1.97% modified cassava flour (MOCAF) were weighed accurately using an analytical balance (Denver digital balance, Germany) and mixed homogeneously by a ball mill. PFF was then packed into a PET bottle.

2.3. Characteristic Analysis of Porang Flour (PF) and Porang Flour Formulation (PFF)

Porang flour (PF) and porang flour formulation (PFF) were analyzed for soluble fiber, water absorption capacity (Geng et al. 2009), disintegration time (Pharmacopoeia 2004), and viscosity (Peiying et al. 2002).

2.4. Animal Experiments and Design

The rats were obtained from D’wistar Animal Laboratory, Bandung, Indonesia. The protocols and procedures of care and use of animals in the present experiment were approved by the Ethics Committee of Biosciences Institute, Brawijaya University, Malang, Indonesia, with ethical clearance number: 117–KEP-UB-2018.

Twenty-eight (28) male Rattus Nobergicus Strain Wistar weighing 150–200 g were assigned into seven groups. Each group of treatments contained four (4) rats. The rats were kept in individual stainless-steel cages with elevated wire-mesh floors. The calculation of the sample size of animals per group was described by Charan and Kantharia (2013), where $E = \text{Total number of animals} – \text{total number of groups}$. In this case, $E = (4 \times 7) – 7 = 28 – 7 = 21$. The total number of animals is considered to be an adequate sample size. The rats were divided into non-diabetic and diabetic groups. To condition the rats to become type-2 diabetic rats, streptozotocin (STZ, Bioworld-Dublin, USA; 40 mg/kg bw) in freshly prepared 0.1 M citrate buffer (pH 4.4) in 5 days consecutively was injected into 9-week-old Wistar rats intraperitoneally. STZ-induced rats can be classified as type 2-diabetic due to increased activity of CD-4, CD-8, T and B lymphocytes, monocytes, and macrophages. As a result, there was an increase in the production of nitric oxide (NO),
TNF-α, interferon, interleukin, and PG (Gundala et al. 2018). However, this is not the focus of this research. Another characteristic of type 2 diabetic rats was indicated by increased LDL and triglyceride levels in the circulating blood (Srinivasan and Ramarao 2007). We used these criteria to make sure that our treated samples were in the phase of type 2 diabetic rats. In group 1, called a normal group, the rats were injected with the same volume of citrate buffer. Group two (2), called the DM control group, diabetic rats were with no additional treatment. In group three (3), diabetic rats were administrated orally with PF (300 mg/kg bw). Group 4 diabetic rats were orally administered PFF (100 mg/kg bw). Group 5 diabetic rats were orally given PFF (300 mg/kg bw). Group six (6) diabetic rats were given orally with PFF (500 mg/kg bw). Group seven (7) diabetic rats were given metformin (51.38 mg/kg bw) orally. The rats had access to a standard diet (Comfeed II; Japfa Comfeed Indonesia Tbk), and water was provided ad libitum. PF, PFF, and metformin were orally administered to the rats daily during the 28-day experiment. Metformin was used as a controlled drug by type-2 diabetic patients. Fasting plasma glucose (FPG) levels were measured on the initial and final. The rats were fasted overnight and sacrificed by cervical dislocation at the end of the experimental period.

2.5. Biochemical Analysis

Blood samples were obtained from the heart organ using a 3 ml syringe, and then the blood was separated using a vacutainer without coagulants to obtain blood serum. Then, the blood serum was used to be analyzed for the MDA, level of total cholesterol (TC), and high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol, triglyceride (TG), while AST and ALT were measured by enzymatic method using absorbance spectrophotometers (Labomed Inc, California, USA) (Adeyemi et al. 2015; Zubaidah et al. 2018).

2.6. Pancreas Immunohistochemical (IHC) study

The rat pancreases were settled in 10% buffered formalin for 24 hours and installed in paraffin. Immunohistochemistry (IHC) staining was done according to the Beesley procedure (Beesley 1995) and visualization by using diaminobenzidine (DAB) for 3 minutes and counterstaining with hematoxylin for 3 minutes. Pancreatic β-cells producing insulin would be brown-coloured. The observation of pancreatic β-cells was performed with a binocular microscope at 400x magnification using an immunohistochemical staining sample. Pancreatic β-cells quantification was done by calculating the average number of pancreatic β-cells from 10 Langerhans islets using three prepared samples for each treatment group (Suarsana et al. 2010).

2.7. Statistical Analysis

The statistics of the characteristic data of porang flour (PF) and porang flour formulation (PFF) were assessed using paired t-tests, and the statistical value was significant at p<0.05. Moreover, biochemical analysis and IHC data were expressed as mean ± standard deviation for the four rats in each group (n = 4), and the statistical significance was assessed by general linear model (GLM) analysis of variance (ANOVA). The following step employed honest significance difference (HSD) or Tukey’s test using Minitab Ver.17.0. The accepted statistical value was significant at p<0.05.

3. Results

3.1. Characteristic Analysis Porang Flour (PF) and Porang Flour Formulation (PFF)

The characteristics of the porang flour and porang flour formulation are shown in Table 1. The result of paired t-test showed that porang flour and porang flour formulation had a difference of soluble fiber 18.82 (%) (p-value = 0.000), water absorption capacity 7.81 (g H₂O/g) (p-value = 0.019), the disintegration time 1.50 s (p-value = 0.001), and viscosity 3486 cps (p-value = 0.000).

3.2. Fasting Plasma Glucose (FPG) and Malondialdehyde (MDA) Level

The effects of porang flour, porang flour formulation, and metformin on FPG, MDA, and the number of pancreatic β-cells are presented in Table 2. The anti-diabetes activity of porang flour, porang flour formulation, and metformin was pointed out by the changes in the FPG levels before and after the treatments. On the 28th day, diabetic rats’ FPG was treated with porang flour (300 mg/kg bw), middle-dose porang flour formulation (300 mg/kg bw), and high-dose porang flour formulation (500 mg/kg bw) was significantly lower (p = 0.000) than the DM control group and not significantly different
Metformin administration showed lower FPG levels than the DM control group. Notably, the FPG levels of diabetic rats treated with low-dose porang flour formulation (100 mg/kg bw) were not significantly different (p = 0.000) from the normal group rats. The MDA levels of the diabetic rats treated with porang flour, porang flour formulation, and metformin were significantly lower than the DM control group. It means treatment with PF 300 mg/kg bw and PFF 300 mg/kg reduced FPG and MDA levels compared to other treatments.

### 3.3. Pancreas Immunohistochemical (IHC) Study

The result of IHC staining showed the Langerhans islet structure and the insulin secretion function improvements in the diabetic rats treated with porang flour and middle-dose porang flour formulation (300 mg/kg bw) compared to the diabetic control rats, as revealed in Figure 1. Moreover, the diabetic rats treated with porang flour and middle-dose porang flour formulation (300 mg/kg bw) improved the size, shape, distribution, and number of the β-cells. Furthermore, the diabetic rat's Langerhans islet size and shape were smaller than the normal group. They also had an extremely low immunoreactive response (brown color) against anti-insulin, indicating a low insulin production level.

The number of pancreatic β-cells that produce insulin in the diabetic rats was significantly lower (p<0.05) than those in the normal group rats supporting this fact, as presented in Table 2. The diabetic rats treated with porang flour and middle-dose porang flour formulation (300 mg/kg bw) had the number of pancreatic β-cells producing significantly higher insulin (visualized as brown color in Figure 1) (p = 0.000) than those in the diabetic control rats.

### 3.4. Lipid Profile Level

The effect of porang flour, porang flour formulation, and metformin on lipid profile including levels of HDL cholesterol, LDL cholesterol, triglyceride (TG), and total cholesterol (TC) of the normal rats and DM rats, were not significant (p>0.05) as presented in Table 3. The levels of lipid profiles produced by all groups were normal. However, the treatment of porang flour and porang flour formulation showed a trend of increasing HDL levels and decreasing LDL, TG, and TC levels.

### 3.5. Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) Level

The effects of porang flour, porang flour formulation, and metformin on the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in rats were examined further (Table 4).
Figure 1. (A) normal rats, (B) DM rats, (C) DM + PF 300 mg/kg bw, (D) DM + PFF 100 mg/kg bw, (E) DM + PFF 300 mg/kg bw, (F) DM + PFF 500 mg/kg bw, and (G) DM + metformin 51.38 mg/kg bw. Red arrow: pancreatic β–cells which have immunoreactive to anti-insulin. Green arrow: endocrine cells which do not show immunoreactive to anti-insulin

Table 3. Data of HDL level, LDL level, TG level and TC level in the rats*

<table>
<thead>
<tr>
<th>Group</th>
<th>HDL level (mg/dl)</th>
<th>LDL level (mg/dl)</th>
<th>TG level (mg/dl)</th>
<th>TC level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>76.80±35.20a</td>
<td>12.45±4.18a</td>
<td>93.00±47.70a</td>
<td>86.00±16.39a</td>
</tr>
<tr>
<td>DM</td>
<td>89.50±11.70a</td>
<td>18.00±3.51a</td>
<td>98.80±48.80a</td>
<td>91.25±11.44a</td>
</tr>
<tr>
<td>DM+PF 300 mg/kg bw</td>
<td>77.50±22.20a</td>
<td>11.75±8.85a</td>
<td>73.50±11.47a</td>
<td>80.75±15.37a</td>
</tr>
<tr>
<td>DM+PFF 100 mg/kg bw</td>
<td>87.25±9.43a</td>
<td>16.60±2.81a</td>
<td>79.25±13.05a</td>
<td>86.50±7.77a</td>
</tr>
<tr>
<td>DM+PFF 300 mg/kg bw</td>
<td>73.50±7.85a</td>
<td>14.40±9.51a</td>
<td>74.80±23.60a</td>
<td>79.25±15.02a</td>
</tr>
<tr>
<td>DM+PFF 500 mg/kg bw</td>
<td>80.00±10.23a</td>
<td>14.45±10.08a</td>
<td>68.50±24.20a</td>
<td>79.25±18.48a</td>
</tr>
<tr>
<td>DM+metformin 51.38 mg/kg bw</td>
<td>76.50±17.31a</td>
<td>11.90±5.18a</td>
<td>76.50±29.00a</td>
<td>82.50±21.50a</td>
</tr>
</tbody>
</table>

*Values are mean ± standard deviations (n = 4 for each group). Values of HDL, LDL, TG, and TC levels were obtained from the 28th-day experiment. The animal experiments were designed as follows: normal rats, DM rats, DM+PF 300 mg/kg bw, DM + PFF 100 mg/kg bw, DM + PFF 300 mg/kg bw, DM + PFF 500 mg/kg bw, and DM + metformin 51.38 mg/kg bw for 28 days. Values in a column with the same letters are not significantly (p>0.05) different by honestly significance difference (HSD). PF: porang flour; PFF: porang flour formulation; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TG: triglyceride; TC: total cholesterol
levels of AST and ALT produced by all groups were not significant (p>0.05, AST p = 0.828 and ALT p = 0.122) from the DM rats. However, the data shows that AST and ALT levels were normal, except for the low-dose porang flour, which increased AST levels (191.50 U/L).

4. Discussion

The soluble fiber content in porang flour was higher than in the porang flour formulation. The soluble fiber that plays a role is glucomannan, a water-soluble food fiber with a strong hydrocolloid. Porang flour had a higher water absorption capacity than porang flour formulation. The water absorption of each material differs depending on the material’s ability to form hydrogen bonds. Moreover, the components of the materials influence viscosity. The higher the glucomannan content in porang flour, the higher the viscosity (Faridah and Widjanarko 2013; Witoyo et al. 2022). Porang flour had a higher viscosity than porang flour formulation because the most significant component of glucomannan in porang flour had the highest viscosity compared to other components.

The destruction of β–cells of islets of Langerhans in the pancreas and the lack of insulin secretion also increases plasma glucose caused by induction by using STZ (Srihari et al. 2013). Porang flour decreased the FPG level because of its high glucomannan content. The present study did not investigate the exact mechanism of how glucomannan could reduce postprandial blood glucose. However, it is believed to be the same as the other soluble dietary fibers, i.e., it increases the viscosity of the gastrointestinal contents, slows gastric emptying, and acts as a barrier to mucosal diffusion (Jenkins et al. 1978; Doi et al. 1979; Magnati et al. 1984). As reported by other studies, glucomannan might improve glycemic parameters by inhibiting appetite and slowing intestinal absorption due to the increased viscosity (Jenkins et al. 1994; Vuksan et al. 1999, 2000; Chearskul et al. 2009). It can delay stomach emptying by modulating the rate of nutrient absorption from the small bowel and increasing insulin sensitivity (Vuksan et al. 2001). As an edible A. konjac-based food, it will generate a more gradual dietary sugar absorption and reduce the blood sugar levels elevation. Therefore, glucomannan-enriched diets may improve overall diabetic control in patients with diabetes (Fang and Wu 2004).

Middle-dose porang flour formulation decreased the level of FPG because, besides glucomannan, soluble fiber inulin and carrageenan in porang flour formulation also have advantages. In the colon, the gut microbiota degraded FOS or inulin into short-chain fatty acids such as acetate, propionate, and butyrate (Wolever et al. 1989). Generally, propionate and butyrate are metabolized in the colon and liver, which essentially affects the local gut and the functions of the liver. They also induce intestinal gluconeogenesis and sympathetic activity by improving glucose and energy homeostasis (Kimura et al. 2011; Mithieux and Gautier-Stein 2014). In the distal gut, short-chain fatty acid bind to coupled G-protein, which brings to the gut hormones PYY production and glucagon-like peptide 1 (GLP-1) by affecting satiety and glucose homeostasis. Various studies have reported the increase of plasma PYY and GLP-1 also the upregulation of proglucagon mRNA with inulin-type fructans in rodents (Delzenne et al. 2005; Urias-Silvas et al. 2008). GLP-1 has numerous anti-obesity and anti-diabetic actions, including food intake inhibition, delayed gastric emptying, insulin secretion stimulation, and β-cell proliferation induction (Drucker 2006).

Metformin is an anti-diabetic drug, and it works in hepatic. Metformin utilizes its glucose-lowering effect primarily by decreasing the production of hepatic glucose through gluconeogenesis suppression and enhancing insulin suppression of endogenous glucose production and, to a lesser extent, by reducing the absorption of intestinal
glucose and possibly improving glucose uptake and utilization by peripheral tissues such as skeletal muscle and adipose tissue (Natali and Ferrannini 2006).

Glucomannan and inulin reduced the lipid peroxidative product, MDA, in either the distal or proximal colon effectively. The enhanced glutathione peroxidase gene expression in the proximal and distal colonic mucosa and the catalase gene expression in the distal colonic mucosa likely mediated this reduced oxidative stress. Furthermore, KGM and inulin diets reduced the MDA levels in the liver, the metabolism site of many compounds absorbed from the gastrointestinal tract. These advantageous effects of fibers in the liver were associated with the enhanced hepatic gene expression of superoxide dismutase and catalase (Wu and Chen 2011).

The mechanism of how porang flour and porang flour formulation could increase pancreatic β-cells was not clear. However, it is believed to be the same with the other soluble dietary fibers, i.e., fiber intake induces increased satiety, delayed gastric emptying, reduced macronutrient absorption, and improved insulin sensitivity (Satija and Hu 2012). Secondly, dietary fiber has been identified to utilize protective roles against oxidative stress by ROS generation suppression (Ghanim et al. 2017). The involvement of oxidative stress in the pathogenesis of DM has been acclaimed (Bonomini et al. 2015). Furthermore, the phylogenetic structure and functional capacity of the gut microbiome can be affected by fiber supplementation (Holscher et al. 2015). Previous studies have implied that changes in the microbial community and functional alterations of the gut microbiome drive systemic inflammation and insulin resistance (Festi et al. 2014). Therefore, the practical benefits of fiber intake might be, at least in part, interceded through its gut microbiome regulation.

Porang flour and porang flour formulation treatment did not indicate any effects on the lipid profile. However, the treatment showed a trend of increasing HDL levels and decreasing LDL, TG, and CH levels. The mechanisms by which dietary fiber affects blood lipids have not been fully defined yet. Glucomannan lowered cholesterol via viscosity-mediated interference of cholesterol absorption and by incrementing faecal bile acid excretion (Jones 2008). The polysaccharide constrains cholesterol absorption, specifically in the jejunum and bile acid absorption in the ileum (Kiriyama et al. 1974; Venter et al. 1987). The glucomannan-induced viscosity might also contribute to less postprandial stimulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase (Jenkins et al. 1993). Fermentation of glucomannan in the large intestine predominantly increases propionate formation, which may decrease cholesterol synthesis (Connolly et al. 2010; Ishimwe et al. 2015). Acetic acid, the result of fermentation, may also contribute to decreasing LDL cholesterol, TG, and TC levels in the diabetic-rats serum (Yamashita et al. 2007). Additionally, acetic acid can constrain cholesterologenesis (acetate CoA to cholesterol) and lipogenesis (acetate CoA into fatty acids and subsequently stored as triglycerides) metabolic pathway in the liver, fatty acids oxidation and stimulates fecaalex creation of bile acids (Fushimi et al. 2006). In conclusion, treating porang flour, middle-dose porang formulation, and metformin reduced FPG by 75.31%, 67.81%, and 53.88%, respectively. Porang flour significantly increased the number of pancreatic β-cells compared to the other treatments. Porang flour (300 mg/kg bw) and middle-dose porang flour formulation have the potential as therapeutic agents for type-2 DM.

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