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Research Article

Effect of Porang (*Amorphophallus muelleri* Blume) Flour Diet on Postprandial Blood Sugar Rates and Insulin Resistance in Male Wistar Rats (*Rattus norvegicus*) Diabetes Mellitus

Nathasia[1*], Ida Ayu Alit Widhiartini[2], I Made Pande Dwipayana[3], Nila Wahyuni[4], Ni Putu Tesi Maratni[5], Made Agus Hendrayana[6]

[1]*Master Program in Biomedical Science, Anti-Aging Medicine, Faculty of Medicine, Udayana University, Denpasar 80234, Bali, Indonesia*
[2]*Department of Pharmacology and Therapy, Faculty of Medicine, Udayana University, Denpasar 80234, Bali, Indonesia*
[3]*Department of Internal Medicine, Faculty of Medicine, Udayana University, Denpasar 80234, Bali, Indonesia*
[4]*Department of Physiology, Faculty of Medicine, Udayana University, Denpasar 80234, Bali, Indonesia*
[5]*Department of Biochemistry, Faculty of Medicine, Udayana University, Denpasar 80234, Bali, Indonesia*
[6]*Department of Microbiology, Faculty of Medicine, Udayana University, Denpasar 80234, Bali, Indonesia*

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ABSTRACT

Medical nutrition therapy is essential in diabetes management, especially as diabetes is often linked with aging. Porang tuber flour contains glucomannan, a compound with potential glucose-lowering effects. This study aimed to evaluate the effect of porang flour feeding frequency on 2-hour postprandial blood glucose (2hPPBG) and HOMA-IR in diabetic rats. Using a quasi-experimental pre-test and post-test control group design, 21 male Wistar rats were induced with diabetes via streptozotocin and nicotinamide, then randomly assigned to three groups: G0 (control, given aquadest), G1 (porang flour 300 mg/kg BW daily), and G2 (porang flour 300 mg/kg BW every two days). Five rats died during treatment. Wilcoxon test results showed a difference in 2hPPBG levels between each group before and after treatment. There was no decrease in G0 ($p = 0.893$), but a decrease in G1 ($p = 0.043$) and G2 ($p = 0.028$). The difference between HOMA-IR before and after treatment did not decrease in groups G0 ($p = 0.345$), G1 ($p = 0.138$), and G2 ($p = 0.249$). Post hoc test for 2hPPBG levels between groups showed a significant difference between G0 and G1 ($p<0.001$), a significant difference between G0 and G2 ($p<0.001$), and no significant difference between G1 and G2 ($p = 0.135$). One-way ANOVA test of HOMA-IR showed no significant difference between G0, G1, and G2 ($p = 0.148$). It was concluded that porang flour can reduce 2hPPBG, but not HOMA-IR. There was no difference in 2hPPBG levels between administering porang flour daily and every other day.

1. Introduction

Diabetes mellitus (DM) is a chronic disease that is a global health problem. An estimated 536.6 million sufferers aged 20 to 79 years in 2021, and the number is predicted to increase to 783.2 million in 2045 (Sun *et al.* 2022). Indonesia is expected to be the country with the seventh highest number of DM in the world by 2030 (Rusminingsih and Purnomo 2022). In 2019, 1.5 million deaths occurred, with 48% occurring in people under 70 years old (WHO 2023). High blood sugar levels can be caused by a high sugar diet (Syauqy *et al.* 2023). Other factors contributing to diabetes include lifestyle, diet, age, genetics, and other medical conditions (Banday *et al.* 2020). DM complications can impact morbidity and mortality (Perkeni 2021).

Medication adherence is crucial for diabetes control, as only 34.14% of 331 diabetes patients were adherent to their medication regimen (Sahoo *et al.* 2022). Diet is also an essential factor. Choosing a diet with calorie restriction and low carbohydrates can lead to persistent hunger (Salvia and Quatromoni 2023). White rice, a

* Corresponding Author
E-mail Address: nathasia.w@gmail.com

staple food in Indonesia, has a high glycemic index that can cause blood sugar spikes (Nur *et al.* 2020; Solan 2021). As an alternative, porang tuber flour, which has a low glycemic index of 20.6, can be considered for diabetic diets (Lukitaningsih 2015). Glucomannan from the porang tuber can help reduce weight, improve blood sugar levels, and alleviate constipation. The gel form of glucomannan can slow intestinal absorption and insulin spikes (Wicaksono *et al.* 2023). Glucomannan, a complex polysaccharide consisting of long chains of glucose and mannose molecules bonded together, forms a gel that coats the intestinal wall. It slows down gastric emptying, which reduces glucose absorption (Saleh *et al.* 2015; Fang *et al.* 2023). Research shows that porang tuber flour can be used in adjunctive therapy for DM.

Previous studies did not explain the frequency of porang tuber flour administration. In other studies, porang-based rice was considered crunchier, chewier, and slightly harder than rice (Widjanarko *et al.* 2023; Lubis *et al.* 2024). Normal gut transit time in humans ranges from 14-58 hours, and foods high in fiber will take longer to digest (Asnicar *et al.* 2021; Ioniță-Mîndrican *et al.* 2022). This study used a reference dose from previous research by Widjanarko *et al.* which stated that porang tuber flour at a dose of 300 mg/kgBB/day was able to reduce fasting blood glucose (FBG) and Malondialdehyde (MDA), and increase the number of pancreatic β-cells in DM rats (Widjanarko *et al.* 2023). This reason is one of the considerations for conducting research on the administration of porang tuber flour, either daily or every other day. Further research is needed to confirm the frequency of porang tuber flour administration in managing diabetes using a diabetes-induced rat model.

2. Materials and Methods

2.1. Research Design

This research employs a quasi-experimental design with a pre-test-post-test control group. Twenty-one rats with inclusion criteria were white male Wistar rats (Rattus norvegicus), weighing 150-200 grams, aged 8-12 months, with FBG ≥135 mg/dL, and active behavior. The dropout criteria were if the rats were sick and died during the study. FBG for the animal model was checked by using the EasyTouch GCU® brand glucometer. The Research Ethics Committee of the Faculty of Medicine, Udayana University, has approved this study (reference number 2690/UN14.2.2.VII.14/LT/2024, dated 11th November 2024). This research was conducted at the Laboratorium Biomedik Terpadu, Faculty of Medicine, Udayana University, from November 13, 2024, to December 30, 2024.

2.2. Treatment Samples

The rats were induced DM once before treatment using streptozotocin (STZ) 65 mg/kg BW and nicotinamide (NAD) 230 mg/kg BW. The DM rats were divided into three groups: the negative control group (G0), which received a placebo aquadest via oral gavage feeding tube; treatment group 1 (G1), which received porang tuber flour at 300 mg/kg BW once daily via oral gavage feeding tube. Treatment group 2 (G2) was given porang tuber flour 300 mg/kg BW once every 2 days using an oral gavage feeding tube for four weeks. All groups received standard feed (PT Pokphan 594 chicken feed with 13% water content, 17.5-19.5% protein, 3% fat, 8% fiber, 7% ash, 0.9% calcium, and 0.9% phosphorus), 12-20 grams/day, and drink ad libitum.

2.3. Porang Tuber Flour

The porang tuber flour used in this study was collected from Karanganyar, Central Java. After examination at the Faculty of Agricultural Technology, Gadjah Mada University, this porang tuber flour was found to have a glucomannan content of 29.13% (2^{nd} quality). The calcium oxalate content contained in this flour is 47.24 ppm (3^{rd} quality). Moisture content was 10.39% (1^{st} quality). Ash content was 4.05% (2^{nd} quality). The quality of this porang tuber flour is categorized according to the Indonesian National Standard (SNI 2020).

2.4. Postprandial Blood Sugar Rate Test

The examination of 2hPPBG levels was performed using a colorimetric method. 1 mL of venous blood was collected through the orbital sinus using a microcapillary tube and then examined in the laboratory using the glucose oxidase method.

2.5. HOMA-IR Test

The HOMA-IR test requires fasting blood sugar (FBG) and plasma insulin tests. FBG examination is performed using a colorimetric method. 1 mL of venous blood was collected through the orbital sinus using a

microcapillary tube and then examined in the laboratory using the glucose oxidase method. Plasma insulin examination was performed using 1 mL of venous blood taken through the orbital sinus with a microcapillary tube, then examined using the Rat Insulin ELISA kit E0707 Ra from Bioassay Technology Laboratory.

2.6. Data Analysis

Data from this study were analyzed using SPSS version 27 for descriptive analysis tests, normality tests, homogeneity tests, and treatment effect tests. Descriptive data were used to determine the characteristics of the research sample. The normality test used was the Shapiro-Wilk test, and the distribution was considered normal if p>0.05. Due to abnormal data, data transformation was carried out, but the results remained abnormal. The homogeneity test used Levene's test and found all data homogeneous. The pre-test to post-test change for each group was tested using the Wilcoxon nonparametric test. As for the change in pre-test to post-test scores between groups, since the data were normal and homogeneous, it was analyzed using one-way ANOVA. If a difference was found, the LSD post-hoc test was used for further analysis. Assessment was carried out with a 95% confidence interval (CI) and a p-value of 0.05. HOMA-IR calculation was obtained using the formula.

$$\text{HOMA - IR} = \frac{\text{fasting insulin (mIU/L)} \times \text{fast blood glucose (mg/dL)}}{405}$$

3. Results

Twenty-one male Wistar rats, 2-3 months old and weighing 150-200 grams, were randomly divided into three groups. Two rats in the negative control group (G0), two rats in treatment group I (G1), and one rat in treatment group 2 (G2) died due to illness. Therefore, the total number of rats was 16.

3.1. Postprandial Blood Sugar Levels (2hPPBG)

The mean of 2hPPBG pre-test and post-test for each group are shown in Table 1. Changes in 2hPPBG of each group were conducted using the Wilcoxon nonparametric test for all groups (Figure 1).

Based on the results of the 2hPPBG change test for each group, it was found that, between before and after treatment, group G0 showed no significant difference

Table 1. Baseline characteristics results on research sample 2hPPBG and HOMA-IR

Variable	Negative control group	Group I	Group II
2hPPBG pre-test (mg/dL)	166.087±1.776	164.980±0.770	164.097±0.582
2hPPBG post-test (mg/dL)	167.527±7.426	134.194±12.709	123.297±9.776
HOMA-IR pre-test	2.285±1.208	3.514±1.547	3.020±1.081
HOMA-IR post-test	4.238±3.135	2.320±0.944	2.356±1.495

(p = 0.893), group G1 showed a significant difference (p = 0.043), and group G2 showed a significant difference (p = 0.028). The One-way ANOVA test results for changes in 2hPPBG between groups yielded a significance value of p<0.001, indicating a statistically significant difference between groups. The analysis continued with the LSD Post Hoc test because the data of each group was homogeneous (Figure 2).

Based on the results of the LSD post hoc test of changes in 2hPPBG between groups, it was found that after the 2hPPBG intervention, the group G0 showed a significant difference compared to the group G1 and G2 (p<0.001). The group G1 showed a significant difference from group G0 (p<0.001). The group G1 was higher than the group G2, but the difference was not significant (p = 0.135). The group G2 showed a significant difference from the group G0 (p<0.001). The group G2 was lower than the group G1, but the difference was not significant (p = 0.135).

3.2. HOMA-IR

The mean of HOMA-IR pre-test and post-test for each group are shown in Table 1. The HOMA-IR change test for each group was conducted using the Wilcoxon nonparametric test for all groups (Figure 3).

Based on the test results of changes in HOMA-IR for each group, it was found that there was no significant difference between before and after treatment in group G0 (p = 0.345), group G1 (p = 0.138), and group G2 (p = 0.249). The One-way ANOVA test results for changes in HOMA-IR between groups yielded a significance value of p = 0.148, indicating no difference between groups (Figure 4).

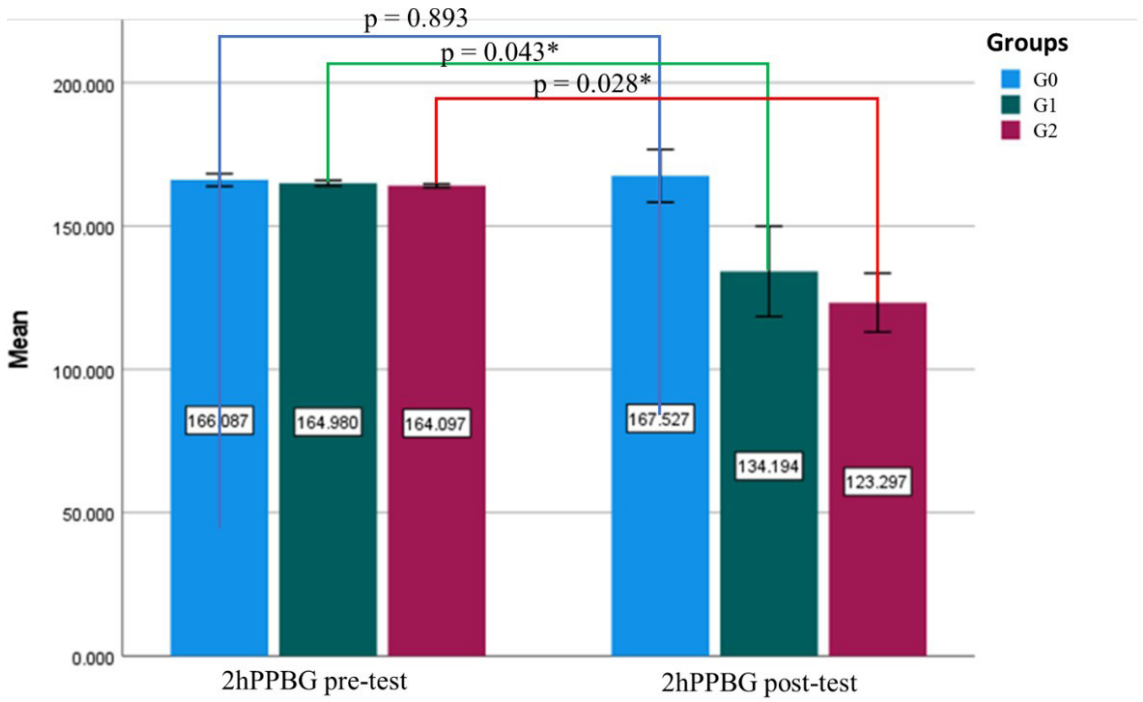

Figure 1. Bar chart of changes in 2hPPBG of each group. The value of p<0.05 indicates a significant difference. G0: negative control group given was placebo aquadest; G1: group 1 was given porang tuber flour 300 mg/kg BW once a day; G2: group 2 was given porang tuber flour 300 mg/kg BW once every 2 days. *: significant

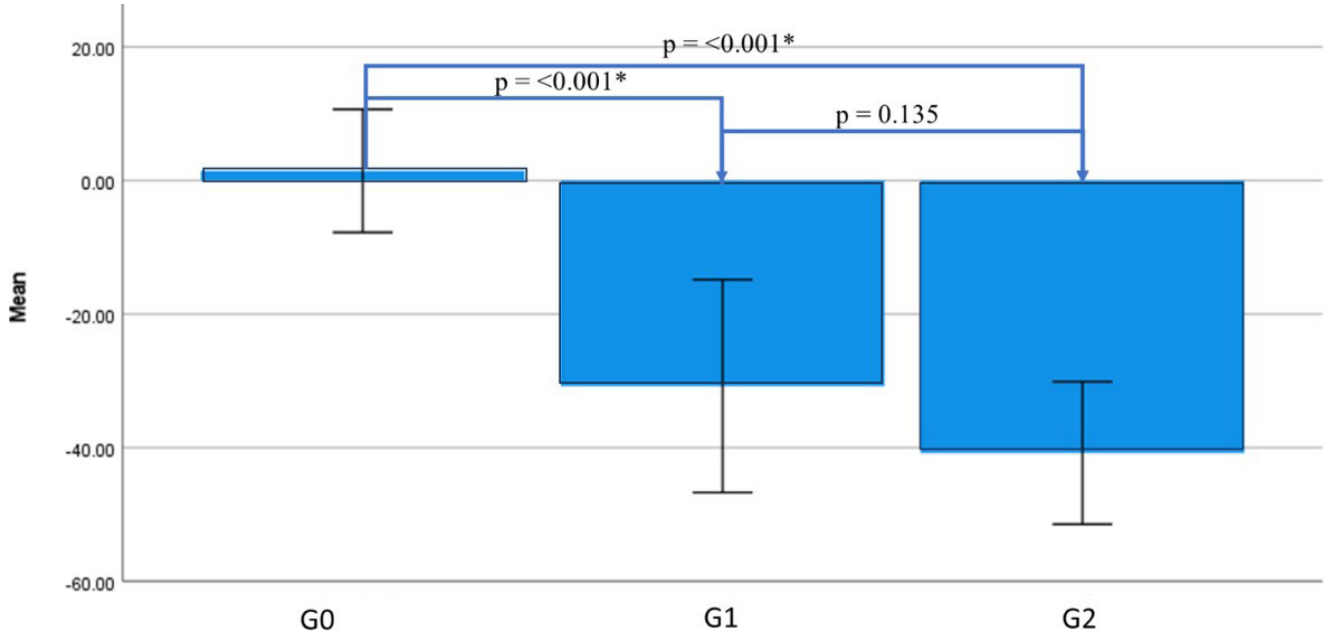

Figure 2. Bar chart of change in 2hPPBG between groups. The value of p<0.05 indicates a significant difference. G0: negative control group was given placebo aquadest; G1: group 1 was given porang tuber flour 300 mg/kg BW once a day; G2: group 2 was given porang tuber flour 300 mg/kg BW once every 2 days. *: significant

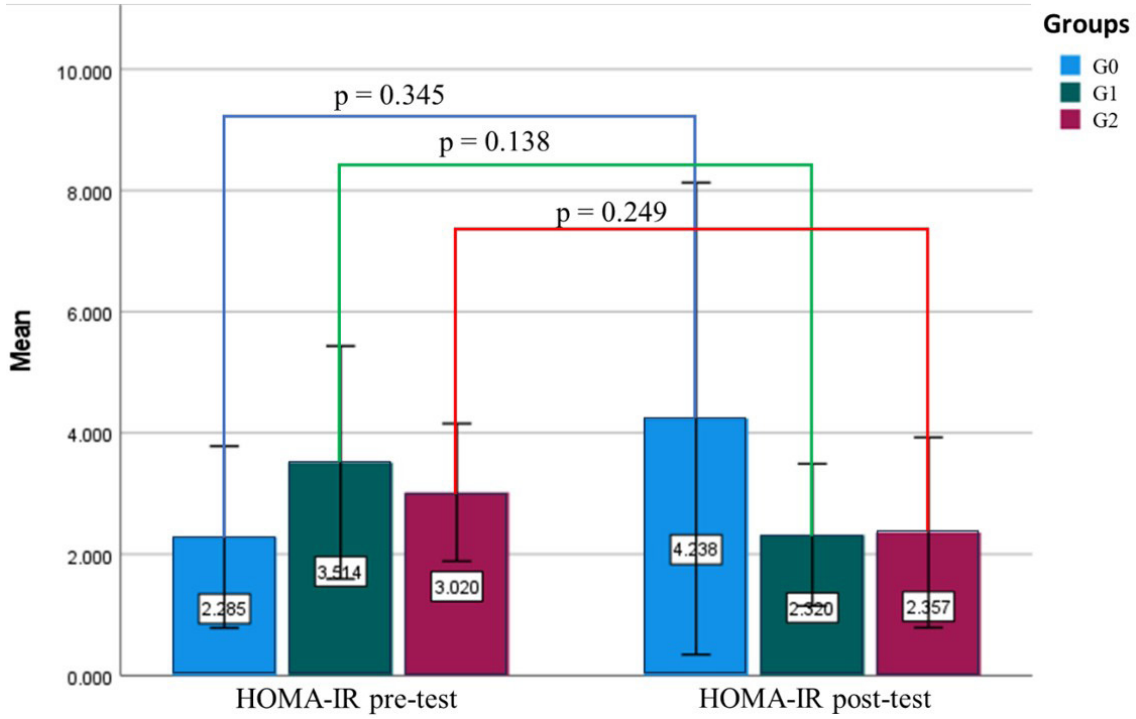

Figure 3. Bar chart of changes in HOMA-IR of each group. A p-value greater than 0.05 indicates no significant difference. G0: negative control group was given placebo aquadest; G1: group 1 was given porang tuber flour 300mg/kgBW once a day; G2: group 2 was given porang tuber flour 300 mg/kgBW once every 2 days

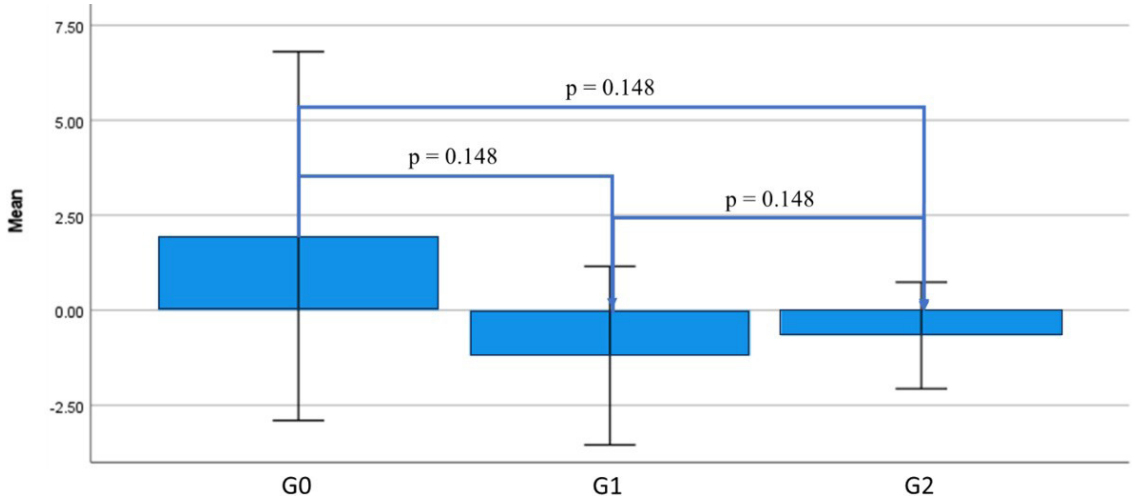

Figure 4. Bar chart of change in HOMA-IR between groups. A p-value greater than 0.05 indicates no significant difference. G0: negative control group was given placebo aquadest; G1: group 1 was given porang tuber flour 300mg/kgBW once a day; G2: group 2 was given porang tuber flour 300 mg/kgBW once every 2 days

4. Discussion

4.1. Effect of Porang Tuber Flour on 2hPPBG Levels

High blood sugar levels characterize DM. Blood sugar levels after a meal (postprandial) typically return to normal within 2 hours (Nakrani *et al.* 2025). Glucomannan in porang tuber flour is a water-soluble dietary fiber that can lower blood sugar levels and increase food viscosity. This fiber forms a gel that inhibits digestion, slows nutrient absorption and gastric emptying, and provides a longer feeling of fullness, which in turn lowers blood glucose levels (Fitriani and Setiarini 2024).

Porang tuber flour has a low glycaemic index (20.6), which can slow glucose absorption, reduce

postprandial hyperglycemia, and maintain stable blood glucose levels (Lukitaningsih 2015; Vlachos *et al.* 2020; Fang *et al.* 2023). The gel formed is not easily destroyed by heating, even at 85°C, which reduces glucose absorption in the intestine. Several previous studies have demonstrated the effect of porang tuber flour on reducing blood sugar levels in rats compared to untreated diabetic rats (Fatchiyah *et al.* 2019; Laksmitawati *et al.* 2024).

In diabetic rats, it was found that the enzyme hexokinase decreased, resulting in a reduction in the conversion of glucose into glucose-6-phosphate, and blood sugar levels remained high. Glucomannan in porang tuber flour enhances hexokinase activity, allowing for increased conversion of glucose into glucose-6-phosphate (Fang *et al.* 2023). Glucose-6-phosphate will be converted to pyruvate, and pyruvate will be converted to acetyl-CoA, thus increasing ATP production, which in turn will reduce blood sugar levels (Daghlas and Mohiuddin 2023). Glucose-6-phosphate concentration increases in people with DM (Bhagavan 2011). KGM administration was found to decrease the enzyme glucose-6-phosphatase, thereby reducing the conversion of glucose-6-phosphate into glucose (Fang *et al.* 2023). This process reduces blood sugar levels by inhibiting the gluconeogenesis process.

Porang tuber flour enhances aromatic amino acid (AAA) metabolism through the tryptophan metabolic pathway, which gut bacteria can degrade into various metabolites, such as indole, skatol, and indole-3-acetic acid, and is associated with gut bacteria (Lactobacillus, Ruminococcus-1, and Bifidobacterium), which are probiotic (Gao *et al.* 2018; Fang *et al.* 2023). Probiotic microorganisms primarily help maintain the balance of the gut microbiota. These bacteria can also aid digestion and boost the immune system (Tremblay *et al.* 2023). In patients with DM, there is an imbalance of gut microbiota (Asnicar *et al.* 2021). The gut microbiota can increase GLUT-4 expression and induce GLP-1, which stimulates insulin secretion and enhances insulin sensitivity. Fermentation of dietary fiber by gut bacteria produces short-chain fatty acids (SCFAs), which increase insulin secretion and insulin sensitivity and promote satiety through peptide YY (PYY). PYY will bind to neuropeptide Y receptors in the brain, increasing the sensation of satiety and indirectly reducing food consumption, thereby preventing weight gain. SCFAs have been shown to increase glycogen synthesis and reduce glycolysis and gluconeogenesis in the liver. SCFAs also increase GLUT-4 expression via adenosine monophosphate-activated protein kinase (AMPK) (Salamone *et al.* 2021).

4.2. Effect of Porang Tuber Flour on HOMA-IR

Type 2 DM is characterized by insulin resistance, which reduces the cells' sensitivity to insulin so that, despite increased insulin production, glucose has difficulty entering the cells and causing hyperglycemia. The pancreas continues to produce insulin, but over time, pancreatic beta cells can experience fatigue and dysfunction. Insulin resistance is also linked to dyslipidemia, hypertension, and chronic inflammation, which increases the risk of cardiovascular disease, chronic kidney disease, and other complications of diabetes (Lankatillake *et al.* 2019). The pathogenesis of insulin resistance depends on genetics, obesity, age, disease, and the effects of drugs (Zhao *et al.* 2023).

Glucomannan slows down the absorption of branched-chain amino acids (BCAAs) from the gut into the bloodstream, and the activity of mTOR and S6 kinase is inhibited, resulting in decreased muscle protein synthesis. The inhibited S6 kinase is expected to have a positive effect on IRS-1 and reduce insulin resistance (Fang *et al.* 2023). Glucomannan enhances the insulin signalling pathway by increasing the expression of Insulin Receptor Substrate 1 (IRS1) and Phosphatidylinositol 3-kinase (PI3K). When blood glucose levels increase, the pancreas releases insulin, which binds to receptor tyrosine kinase (RTK) and activates the insulin signalling pathway. IRS and PI3K will bind. Activated PI3K will convert phosphatidylinositol (4,5)-bisphosphate (PIP2) into phosphatidylinositol (3,4,5)-trisphosphate (PIP3). PIP3 activates protein kinase B (AKT/PKB). AKT/PKB will transport to the vesicles, allowing vesicles containing glucose transporters (GLUT) to diffuse to the cell membrane, and more glucose will enter the muscle. In type 2 DM, insulin resistance interferes with this process, so the signal activation is not effective even though insulin is bound to the receptor (Świderska *et al.* 2020). Glucomannan inhibits inflammatory factor-related signalling pathways and reduces DM-associated inflammatory immune responses. DM is affected by oxidative stress and inflammation. The primary causes of insulin resistance include inflammatory markers such as tumor necrosis factor (TNF), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1) (Fang *et al.* 2023).

In previous studies, it has been proven that the administration of porang tuber flour can reduce

HOMA-IR in DM and obese rats when compared to the untreated group (Susanti *et al.* 2015; Zhu *et al.* 2019). Decreased insulin response occurs in the first 2 weeks after induction of DM rats with STZ (Nordquist and Sjöquist 2009). This study provides STZ and NAD as protectors against pancreatic β-cell damage. The research duration of only 28 days may be a factor in why HOMA-IR did not show significant changes. Although not statistically significant, the results of the pre-test and post-test changes showed that group G0 tended to increase, while groups G1 and G2 decreased. Research over a more extended period may be able to demonstrate the effect of porang tuber flour in reducing HOMA-IR. In this study, rats were not fed a high-fat or high-fructose diet, ensuring that the rats' condition during the study was not one of obesity. Insulin resistance is more pronounced in obese conditions than in normal conditions (Kim *et al.* 2023). This condition may have prevented HOMA-IR from increasing evenly in the pre-test of all groups in this study. The standardized diet contained <35% fat (low fat) and 45% carbohydrate (low carbohydrate) (Krisanits *et al.* 2020; Palma-Morales *et al.* 2022). Feed was given at a rate of 12-20 grams per day per mouse. In one rat cage, there are 2-3 rats, and it is unknown how much feed each rat consumes, which is one of the possible factors contributing to varying HOMA-IR values.

4.3. The Effect of Feeding Porang Tuber Flour on the Difference of Feeding Porang Tuber Flour Once a Day and Once Every Two Days

Normal gut transit time ranges from 14-58 hours (Asnicar *et al.* 2021). DM patients with glucose levels >200 mg/dL often experience DM gastroparesis, which can also cause abnormal small bowel motility. Acute hyperglycaemia in DM gastroparesis is associated with increased gastrointestinal sensitivity, such as nausea and bloating. Gastroparesis treatments include lifestyle modifications, glycemic control, eating smaller and more frequent meals, increasing fluid intake, and reducing the fat and fiber content (Aswath *et al.* 2023). Additionally, porang tuber flour in the form of processed foods is also considered less palatable (Lubis *et al.* 2024).

Frequent feeding of porang tuber flour may also cause bloating due to the presence of glucomannan gel. Additionally, porang flour is a food rich in fiber, so it is essential to determine the appropriate frequency of porang flour consumption in DM patients. In this study, giving porang once a day or once every two days had no difference in reducing 2hPPBG and HOMA-IR levels for people with DM. The decrease in 2hPPBG levels was more pronounced with the administration of porang tuber flour once every two days. Giving porang once every two days can be a nutritional therapy of choice for DM patients.

In conclusion, 300 mg/kg BW porang flour, administered once a day or every 2 days, similarly reduces 2hPPBG levels but does not significantly impact HOMA-IR. Both doses showed no significant difference in reducing 2hPPBG and HOMA-IR levels. A longer research time and higher glucomannan levels in porang flour are needed to prove the effect of porang on insulin resistance.

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