



## Administration of Porang (*Amorphophallus Muelleri*) Tuber Flour Solution in the Prevention of Weight Increase and Adiposity in Male Wistar Rats (*Rattus Norvegicus*) Administrated with A High-Fat Diet

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### ABSTRACT

Obesity serves as a predisposing factor for various diseases, including diabetes, metabolic syndrome, cardiovascular conditions, and accelerated *Amorphophallus muelleri* (*A. muelleri*) is a plant rich in glucomannan, a water-soluble fiber that exhibits multiple physiological effects, including nutrient absorption in the intestine, enhancing satiety and suppressing appetite, as well as improving insulin sensitivity and reducing cholesterol absorption in the gastrointestinal tract. The objective of this study was to investigate the potential of porang tuber flour (derived from *A. muelleri*) as a preventive therapy for obesity by mitigating weight gain and adiposity.

This experimental study employed 30 male Wistar rats (*Rattus norvegicus*) aged 2-3 months. The rats were fed a high-fat diet and allocated into three groups: a control group receiving a placebo, a group administered with porang tuber flour at a dose of 200 mg/kgBW, and a group receiving porang tuber flour at a dose of 400 mg/kgBW for 28 days. The study outcomes encompassed the final body weight and the diameter of adipocytes.

The findings of this study indicated that porang tuber flour did not significantly impact body weight ( $p = 0.083$ ). Among the groups, the smallest mean difference in body weight was observed in the group receiving porang tuber flour at a dose of 200 mg/kgBW, followed by the control group and the group receiving porang tuber flour at a dose of 400 mg/kgBW. However, the administration of porang tuber flour resulted in a significant reduction in the diameter of adipocytes ( $p = 0.0001$ ). The group receiving porang tuber flour at a dose of 400 mg/kgBW and 200 mg/kgBW exhibited significantly smaller adipocyte diameter ( $44.58 \pm 6.40$  mm and  $56.98 \pm 12.31$  mm) compared to the control group with a diameter of  $127.84 \pm 18.27$  mm. However, considering the duration of this study was relatively short, further research is strongly recommended to address certain limitations.

**Keywords:** adiposity; *Amorphophallus muelleri*; glucomannan; konjac; obesity

### Introduction

Changes in human diet have a significant impact on bodily functions. With the proliferation of fast-food restaurants in various locations, offering a wide range of food and beverages, coupled with the convenience of online food delivery applications, physical activity has decreased. This sedentary lifestyle, combined with a lack of awareness about the health benefits of exercise, contributes to the prevalence of obesity and other chronic diseases. Consequently, obesity has become a prevalent issue leading to numerous health problems and accelerated aging processes.[1]

Adiposity refers to the excessive accumulation of fat deposits in the body. Adipocytes and adipose tissue serve as the primary reservoirs for storing body fat, including triglycerides and free cholesterol. When adipocytes undergo hypertrophy and excessive accumulation of adipose tissue occurs, it can result in pathogenic effects on adipocytes and adipose tissue (adiposopathy). These effects can lead to abnormal levels of circulating lipids, with dyslipidemia being a significant risk factor for atherosclerotic coronary heart disease.

Obesity is closely associated with various diseases, including aging and degenerative conditions. The aging process involves complex interactions between the environment and the body's systems, which gradually impairs the ability of tissues to repair, replace, and maintain normal structure and function. Consequently, degenerative diseases such as cancer, diabetes, hypertension, and Alzheimer's disease can arise. The aging process often coincides

with obesity and is characterized by low-grade chronic inflammation. Cytokines released from preadipose cells influence the function of adipose tissue and the recruitment of immune cells, contributing to the inflammatory response. The relationship between obesity and age has been demonstrated, as a high-calorie diet has been shown to increase age-related phenotypes and decrease lifespan in various organism models. Thus, caloric restriction has been found to extend the lifespan of organisms, ranging from fungi to mammals.[2] Anti-aging medicine has shifted the paradigm regarding the aging process, suggesting that it can be slowed down and prevented through proper diet, nutrition, regular physical activity, and an active lifestyle.

Pharmacological therapy for obesity aims to reduce caloric intake, increase cellular caloric consumption, or both. One crucial aspect of obesity treatment is reducing energy intake. Orlistat, an obesity drug, functions by inhibiting the intestinal lipase enzyme, thereby impeding fat absorption. Consequently, more triglycerides, cholesterol, and unabsorbed fat are excreted in the feces. However, the effectiveness of orlistat is often limited, requiring long-term use in combination with other drugs and presenting potential side effects. Besides medical treatment, lifestyle changes and herbal therapies remain primary options for weight loss interventions.[3]

*Amorphophallus muelleri*, a member of the Araceae family, is an edible plant commonly known as porang, native to Indonesian tropical forests. In Java, it is recognized as porang and is cultivated, with reported annual production of porang tubers, particularly in Madiun, reaching 8,100 tons in 2013 (Perhutani). Porang tuber (*A. muelleri*) contains a significant amount of glucomannan soluble fiber (15–64% on a dry basis). Glucomannan is a hydrophilic polysaccharide that can be derived from various natural plants. One well-known source is konjac glucomannan, often sold in the form of shirataki.[4]

Glucomannan, a soluble fiber, offers various health benefits, including enhanced digestive function and immune system support, reduced cholesterol and blood sugar levels, and assistance in weight loss. Individuals with hypertension and diabetes can incorporate porang root into their diet due to its high fiber content and low cholesterol levels. Glucomannan has received approval from the European Food Safety Authority (EFSA) as a weight loss agent for overweight or obese individuals. The primary mechanism of action for glucomannan involves inducing satiety by slowing gastric emptying. Furthermore, glucomannan exhibits therapeutic potential through its antioxidant, anticancer, anti-inflammatory, immunomodulatory, hypoglycemic, type 2 antidiabetic, and antibacterial properties.[5]

In addition to glucomannan, porang is recognized for its presence of various phytochemicals, including flavonoids, phenol tannins, alkaloids, saponins, steroids, and terpenoids.[6, 7] These compounds exhibit antioxidant properties that play a role in preventing and mitigating obesity through their impact on different stages of the adipocyte cycle.[8]

Several studies have investigated the effectiveness of administering porang tuber flour as a potential treatment for obesity. One such study conducted by Meiliana et al.[9] reported that the administration of porang tuber flour did not lead to a reduction in body weight in obese rats. Therefore, the objective of this research is to explore whether the administration of porang tuber flour (*A. muelleri*) can serve as a preventive therapy by preventing the increase in adiposity and the number of adipocyte cells in male rats (*Rattus norvegicus*).

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## Methods

This is an *in vivo* study conducted at the Integrated Biomedical Laboratory, Faculty of Medicine, Universitas Udayana, Bali, Indonesia, employing a post-test only control group design. Male Wistar rats (*Rattus norvegicus*) of 2-3 months old were utilized for the study. A total of 30 rats that satisfied the inclusion criteria were randomly assigned to three groups: a control group, a group administered with 200 mg/kgBW of porang tuber flour, and a group administered with 400 mg/kgBW of porang tuber flour. Each group consisted of 10 subjects. The inclusion criteria encompassed the following: (1) male Wistar rats (*Rattus norvegicus*), (2) aged 2-3 months, (3) weighing between 150-180 grams, and (4) demonstrating good health and activity levels. The study aimed to measure the impact of porang tuber flour on body weight and adipocyte diameter.

### *Subject preparation*

Forty Wistar rats that met the inclusion criteria underwent a period of acclimatization for seven days at the Animal Laboratory Unit, Pharmacology Department, Faculty of Medicine, University of Udayana, to facilitate environmental adaptation. Out of the initial 40 rats, 30 were randomly selected and divided into three groups, each consisting of 10 subjects. Body weight measurements were conducted one day prior to the commencement of the experiment. Each subject was housed in an individual cage and received equal treatment, except for their food. The control group was provided ad libitum access to a high-fat diet, receiving 20 grams of food once daily, along with a placebo (aquadest) administered via sonde for 28 days. The Porang 200 mg/kgBW group received the high-fat diet along with 200 mg/kgBW of porang tuber flour administered via sonde for 28 days. Similarly, the Porang 400 mg/kgBW group received the high-fat diet in addition to 400 mg/kgBW of porang tuber flour via sonde for 28 days. The high-fat feeds were prepared using a standard diet (Chicken Feed 594), supplemented with 6 grams of liquefied lard, comprising 18% protein, 13% fat, 8% starch, 7% ash, and 13% water, provided by the Pharmacology Laboratory of Universitas Udayana, Denpasar, Bali.

After the 28-day experimental period, all subjects underwent body weight measurements and were euthanized using ketamine (87.5 mg/kgBW) and xylazine (12.5 mg/kgBW). The abdominal cavity was then opened, and the visceral fat was extracted.

### *Adiposity measurement*

The extracted visceral fat obtained from the dissected abdominal cavity of the subjects was sliced to a thickness ranging from 3 to 5 mm and subsequently soaked in phosphate-buffered saline for a duration of 24 hours. The soaked samples were sliced further and carefully arranged into appropriately labeled tissue cassettes. Paraffin blocking was performed by pouring a small quantity of liquid paraffin into either a plastic mold or an L-shaped metal plate, after which the tissue slices were promptly inserted. Once inserted, additional paraffin was poured to complete the blocking process. Subsequently,

hematoxylin-eosin staining was carried out using an autostainer. The stained adipocytes were then examined under a microscope with a magnification of 400x to accurately measure the number per field of view and average diameter of the adipocytes.

#### Porang tuber flour preparation

Fresh and mature tubers of *Amorphophallus muelleri* (A. muelleri) were carefully selected, peeled, and thoroughly washed. Subsequently, the tubers were sliced into thin chips with a thickness of 2-3 mm. These porang tuber chips were then subjected to boiling in a 15% NaCl solution at a temperature of 80°C for 25 minutes, followed by a thorough rinse with water. Following the rinsing process, the chips were soaked in a 0.02% sodium bisulfide (Natrium bisulfide) solution for 10 minutes. After another round of washing, the chips were sun-dried for approximately 7.5 hours, or until the chips emitted a characteristic "crack" sound indicating complete drying. The drying process was continued overnight at room temperature. After 7 days, the dried chips were subjected to analysis to determine the level of calcium oxalate using permanganometry titration. Finally, the tuber chips were ground into flour using a flour-making machine.

#### Data analysis

Univariate analysis was employed to examine the characteristics of the data. To assess normality, the Shapiro-Wilk test was utilized since the sample size was less than 30. The results were considered normally distributed if the p-value was greater than 0.05, and non-normally distributed if  $p < 0.05$ . For variables that exhibited normal distribution such as body weight before and after the experiment, the average diameter of adipocytes, and feed residue, multivariate analysis using One-Way ANOVA was conducted. However, for variables that did not follow a normal distribution, such as the mean difference of body weight (obtained by subtracting the body weight before the experiment from the body weight after the experiment) and the number of adipocytes, the Kruskal-Wallis test was employed. Post-hoc analysis using Tamhane or Tukey test was carried out for significant multivariate analysis results. A significance level of  $p < 0.05$  was considered statistically significant for all analyses. The IBM® SPSS® (Statistical Package for the Social Sciences) software was utilized to perform all statistical analyses.

## Results

Thirty male Wistar rats (*Rattus norvegicus*) were randomly divided into three treatment groups: control, 200 mg/kgBW porang tuber flour, and 400 mg/kgBW porang tuber flour. The experiment lasted for 28 days. Two rats in the 200 mg/kgBW porang tuber flour group died during the treatment and were excluded from the analysis.

After the treatment, the average body weight increased to  $209.90 \pm 19.11$  grams in the control group,  $186.00 \pm 22.24$  grams in the 200 mg/kgBW porang tuber group, and  $189.80 \pm 28.63$  grams in the 400 mg/kgBW porang tuber group (Table I). The p-value obtained was 0.083, indicating no significant difference. Table I shows that the group receiving 400 mg/kgBW porang tuber experienced the greatest increase in body weight, followed by the control group, while the group receiving 200 mg/kgBW porang tuber exhibited the smallest increase in weight after the treatment. The mean differences were  $16.00 \pm 11.70$  grams,  $15.40 \pm 10.45$  grams, and  $11.75 \pm 10.19$  grams, respectively. However, the mean differences did not show significant variation among the groups ( $p = 0.524$ ).

The control group exhibited the largest average diameter of adipocyte cells, measuring  $127.84 \pm 18.27$   $\mu\text{m}$ , based on adipocyte tissue dissection of sacrificed rats. The group receiving 200 mg/kgBW porang tuber ranked second with an average diameter of  $56.98 \pm 12.31$   $\mu\text{m}$ , while the group receiving 400 mg/kgBW porang tuber had an average diameter of  $44.58 \pm 6.40$   $\mu\text{m}$ . A significant difference was observed among the three groups, with a p-value of 0.0001. Post-hoc testing using the Tamhane method indicated that the adipocyte diameter in the group receiving 200 mg/kgBW porang tuber did not significantly differ from the group receiving 400 mg/kgBW porang tuber.

Table 1. Body weight analysis among the treatment groups

| Variable   | Treatment Group     |  |   | P-value |
|--|---------------------|--|---|---------|
|  | Control<br>(n = 10) | Porang tuber<br>200 mg/kgBW<br>(n = 8) | Porang tuber<br>400 mg/kgBW<br>(n = 10) |         |
| Post-experimental weight (gram)                            | $209.90 \pm 19.11$  | $186.00 \pm 22.24$                     | $189.80 \pm 28.63$                      | 0.083   |
| Mean difference of pre and post-experimental weight (gram) | $15.40 \pm 10.45$   | $11.75 \pm 10.19$                      | $16.00 \pm 11.70$                       | 0.524   |

The statistical analysis (Table II) confirmed significant differences of adipocytes number between the control group and the treatment groups (200 mg or 400 mg porang tubers). Further post-hoc analysis revealed significant differences among the groups, suggesting that adipocyte cell changes caused by porang tuber ingestion were clinically and statistically significant. The group receiving 400 mg/kgBW of porang tubers exhibited the most notable clinical decrease.

The control group exhibited the lowest average remaining feed, with a value of  $0.18 \pm 0.19$  grams. The group administered with 200 mg/kgBW porang tubers had the second lowest average remaining feed of  $0.84 \pm 0.18$  grams, whereas the group receiving 400 mg/kgBW porang tubers had the highest average remaining feed of  $1.12 \pm 0.59$  grams. A significant difference was observed among the three groups, with a p-value of 0.0001. Subsequent analysis using the Tukey method as a post-hoc test revealed that the remaining feed in the group administered with 200 mg/kgBW of porang tuber did not differ significantly from the group receiving 400 mg/kgBW of porang tuber. However, both treatment groups differed significantly from the control group.

Table 2. Adiposity analysis among the treatment groups

| Variable                               | Treatment Group     |  |   | P-value |
|--|---------------------|--|---|---------|
|  | Control<br>(n = 10) | Porang tuber<br>200 mg/kgBW<br>(n = 8) | Porang tuber 400<br>mg/kgBW<br>(n = 10) |         |
| Adipocyte diameter (mm)                | 127.84 ± 18.27      | 56.98 ± 12.31                          | 44.58 ± 6.40                            | 0.0001  |
| Number of adipocytes per field of view | 65 (62 – 81)        | 42.5 (40 – 46) <sup>a</sup>            | 35 (33 – 38) <sup>a,b</sup>             | <0.001  |

<sup>a</sup> statistically significant difference ( $p < 0.001$ ) was observed compared to the control group

<sup>b</sup> statistically significant difference ( $p < 0.001$ ) was observed compared to the 200 mg/kgBW porang tuber group

## Discussion

This study revealed a significant difference in the initial body weight of the rats before treatment ( $p = 0.013$ ), indicating the presence of selection bias. However, after administration of 200 mg/kgBW and 400 mg/kgBW of porang tubers, the final body weight did not exhibit significant differences between the two treatment groups, as well as when compared to the control group ( $p = 0.083$ ). Although the group receiving 400 mg/kgBW porang tubers showed the highest increase in body weight compared to the control group, and the group receiving 200 mg/kgBW porang tubers displayed the smallest increase, the differences in weight changes among the three groups were not statistically significant ( $p = 0.524$ ). These findings indicate that the utilization of porang tubers did not lead to a significant reduction or control of weight gain. This aligns with a study by Meiliana et al. [9] that reported no significant weight loss effect between groups receiving porang tuber flour at doses of 25 mg/kg, 50 mg/kg, and 100 mg/kg, as well as negative and positive control groups (orlistat 15.6 mg/kg), with a p-value of 0.599. It is worth noting that Meiliana's study had a shorter duration of 14 days compared to the present study.

However, these results differ from the research conducted by Nissa [10] which reported a significant difference in average body weight among groups administered with porang flour at doses of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW ( $p = 0.0001$ ) over an 8-week period. The lowest body weight was observed in the group of obese rats that received porang at a dose of 400 mg/kgBW ( $237.3 \pm 4.46$  grams), while the obese control group without porang supplementation had the highest final body weight ( $379 \pm 4.77$  grams). Similar trends were observed in the comparison between the non-porang-administered normal rat group and the normal rat group receiving porang, with the former exhibiting higher body weight. Another finding in Nissa's [10] study was the decrease in body weight among the group of obese rats receiving porang tuber flour compared to their initial body weight.

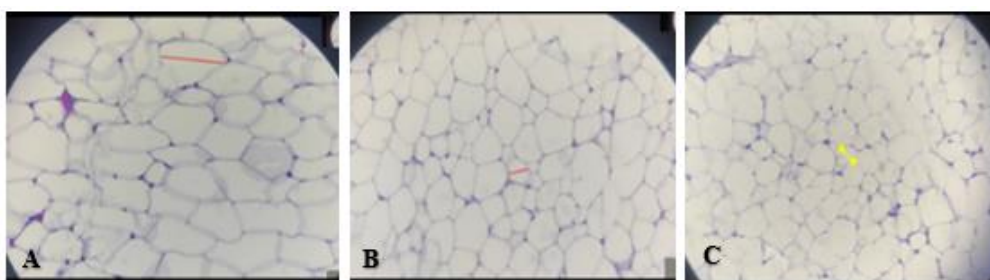


Fig. 1. In hematoxylin & eosin staining diameter of adipocytes in treatment groups (B & C) appears smaller than control group (A) at 400x

The leftover feed consumption by the rats was analyzed as an indicator of satiety level. The group receiving porang tubers at a dose of 400 mg/kgBW showed the highest average residue ( $1.12 \pm 0.59$  grams), followed by the group receiving porang tubers at a dose of 200 mg/kgBW ( $0.84 \pm 0.18$  grams), and the control group with the lowest remaining feed ( $0.18 \pm 0.19$  grams). These findings are consistent with Nissa [10], which indicated a higher amount of food residue in the group of rats consuming porang tubers compared to the normal group, particularly in the group receiving porang at a dose of 400 mg/kgBW. Similarly, Au's [11] study demonstrated lower daily food intake in rats receiving a Konjac solution compared to the control group, as Konjac solution promotes satiety due to its digestibility.[12]

*A. muelleri* has been documented to possess a substantial concentration of glucomannan, as reported by Wahidah et al.[13] Glucomannan is a polysaccharide that is both water-soluble and fermentable, commonly found within the genus *Amorphophallus*, including porang tubers. The genus comprises several well-known variants worldwide, such as *Amorphophallus konjac*, *A. rivieri*, and *A. muelleri* which are widely distributed in Indonesia.[14, 15]

Glucomannan is recognized for its high water-absorption capacity, with the ability to absorb 100 grams of water per gram of glucomannan. This property leads to the formation of a gel-like substance, increasing the viscosity of the digestive tract and subsequently reducing the absorption of food in the intestines. Similar to other water-soluble fibers, glucomannan acts by replacing energy derived from other nutrients, promoting satiety through the formation of a gel-like mass that expands in the digestive tract. Consequently, it reduces appetite and can improve glycemic parameters in the body. Glucomannan has also been linked to the reduction of total cholesterol and low-density lipoprotein (LDL) levels by stimulating the excretion of cholesterol and bile acids in feces, as well as decreasing cholesterol absorption in the gastrointestinal tract.[12, 14, 16]

Furthermore, glucomannan exhibits laxative effects, similar to other dietary fibers. By absorbing water, it increases stool volume, enhances the growth of beneficial intestinal flora, and stimulates peristalsis, facilitating regular bowel movements. Glucomannan also contributes to intestinal cleansing by absorbing toxic substances and waste products, potentially leading to weight reduction.[15, 17] The European Food Safety Authority (EFSA) has recognized glucomannan as a nutraceutical or health-promoting food, capable of reducing body weight by 3 grams per day in overweight individuals.[9, 18] Nevertheless, studies involving human subjects have not consistently shown significant differences in weight loss between groups receiving glucomannan and control groups.[12]

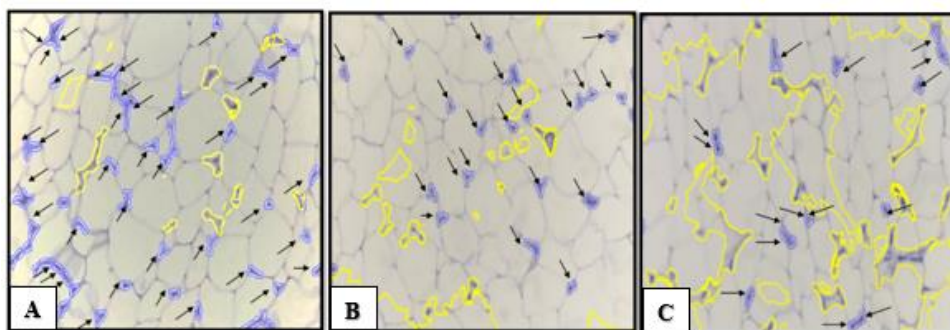


Fig. 2. Control group (A) showed significantly higher number of adipocytes (black arrows) compared to 200 mg/kgBW porang tuber group (B) and 400 mg/kgBW porang tuber group (C).

All figures should be numbered with Arabic numerals (1,2,...n). All photographs, schemas, graphs and diagrams are to be referred to as figures. Line drawings should be good quality scans or true electronic output. Low-quality scans are not acceptable. Figures must be embedded into the text and not supplied separately. Lettering and symbols should be clearly defined either in the caption or in a legend provided as part of the figure. Figures should be placed at the top or bottom of a page wherever possible, as close as possible to the first reference to them in the paper.

In addition to glucomannan, porang tubers contain various phytochemicals, such as antioxidants, phenols, and saponins, that offer numerous health benefits.[7] Porang leaves and stems are known to harbor flavonoids, tannins, alkaloids, saponins, steroids, and terpenoids. Antioxidants inhibit oxidation reactions by donating electrons, thereby neutralizing free radicals and promoting stability. [6] Free radicals are implicated in various diseases, including obesity, diabetes, and high cholesterol. Plant-derived antioxidants, such as flavonoids and curcumin, have demonstrated significant reductions in body weight in *in vivo* studies. [19] Saponins are also believed to be effective in preventing weight gain. Research conducted by Wen et al. [20] using saponins from sea cucumbers on rats showed significant reductions in body weight and prevention of weight gain.

Despite the evidence showcasing the potential of porang tuber flour in reducing or preventing weight gain, this particular study did not observe significant changes in body weight between rats fed with porang tuber flour and those on a normal diet. This discrepancy could be attributed to the specific variant of porang tubers used, which differed from previous studies, as well as the relatively short duration of treatment (28 days), whereas Nissa [10] demonstrated significant changes in body weight after 8 weeks of porang tuber administration. Therefore, further research with an extended treatment period is recommended. Additionally, the authors believe that sample standardization plays a crucial role in minimizing selection bias, as the available space and ratios in the current conditioning setup may differ from those in the study conducted by Nissa. [10]

Although the three groups consumed varying amounts of feed, with the 400 mg/kgBW porang tuber group consuming the less and the control group consuming the most, this difference did not account for the insignificant weight gain observed among the groups following treatment. The reduced daily food intake by the porang tuber group suggests that porang tuber flour might enhance satiety and decrease appetite, which would be expected to contribute to lower body weight in this group. However, the lack of significant differences in body weight could also be influenced by variations in the metabolic rates of the individual experimental subjects, a factor that was not investigated in the current study.

Considering the relatively short duration of the experiment (28 days), it is plausible that observable changes in body weight were not as pronounced compared to the study conducted by Nissa et al., which spanned 8 weeks. To enhance the accuracy of the study results, it is recommended to include a negative control group that does not receive high-fat feed, as well as a positive control group that is administered standard anti-obesity drugs such as

orlistat. By comparing the treatment group with these control groups, it would be possible to determine whether the insignificant results are indicative of the ineffectiveness of porang tuber flour or the suboptimal conditions of induction.

In the context of obesity, the expansion of adipose tissue mass is influenced by two mechanisms: adipocyte hypertrophy and adipocyte hyperplasia, collectively referred to as adipogenesis. Adipocyte hypertrophy involves the enlargement of existing adipocytes due to lipid accumulation within fat cells. On the other hand, adipocyte hyperplasia involves an increase in the number of adipocytes, typically observed during childhood and adolescence. Once adulthood is reached, the number of adipocytes remains relatively constant, even after weight loss occurs. Weight loss is generally associated with a reduction in adipocyte volume, making adipocyte volume a relevant parameter in studies focusing on adipocytes and obesity. [21]

This study revealed a significant difference in adipocyte diameter between the group receiving porang tuber flour and the control group ( $p = 0.0001$ ). Specifically, the treatment group administered with porang tuber flour at a dosage of 400 mg/kgBW exhibited the smallest adipocyte diameter. In comparison, the diameter was larger in the group receiving porang tuber flour at a dosage of 200 mg/kgBW and in the control group. However, the analysis indicated that there were no significant differences between the groups receiving porang tuber flour at 200 mg/kgBW and 400 mg/kgBW.

Interestingly, these findings contrast with the results reported by Au [11] where smaller adipose cell sizes were observed in the control group ( $97.1 \pm 16.6 \mu\text{m}$ ) compared to the Konjac group ( $106.9 \pm 9.1$  and  $103.7 \pm 10.2 \mu\text{m}$  in the 2.5% and 5% solution groups, respectively), albeit without statistical significance. Nevertheless, the measurements of adipose tissue mass in retroperitoneal, epididymal, and mesenteric fat tissues indicated lower mass in the Konjac solution group compared to the control group, particularly in retroperitoneal and mesenteric adipose tissues, which exhibited significant differences ( $p < 0.01$ ).

Additionally, this study identified a significant difference in adipocyte cell numbers between the control group and the treatment groups receiving porang tuber flour at 200 mg/kgBW or 400 mg/kgBW ( $p < 0.05$ ). Microscopic analysis revealed the smallest number of cells in the group administered porang tubers at a dosage of 400 mg/kgBW ( $66.9 \pm 0.65$  adipocyte cell numbers), followed by the group receiving porang tubers at a dosage of 200 mg/kgBW ( $42.6 \pm 1.85$  adipocyte cells), and finally the control group ( $35.1 \pm 1.66$  adipocytes). Post-hoc analysis confirmed the significant changes in adipocyte cell numbers among the three groups and those administered porang tuber flour or placebo.

The process of adipogenesis commences with the commitment of mesenchymal stem cells (MSCs), followed by recruitment, proliferation, and differentiation processes. Preadipocyte differentiation into adipocytes is regulated by several transcription factors that control the expression of proteins responsible for lipid accumulation, adipocyte phenotype development, and adipocyte maturation. Notably, peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) and CCAAT/enhancer-binding protein alpha (C/EBP $\alpha$ ) are two crucial transcription factors involved in this process. [22] PPAR $\gamma$  serves as an indicator of adipocyte hypertrophy, and previous studies have shown that glucomannan derived from porang reduces PPAR $\gamma$  expression in adipose tissue. [23] Furthermore, Chen et al. [17] demonstrated that glucomannan from porang exhibited antioxidant activity and mitigated pancreatic damage and adipocyte cell hypertrophy.

Phytochemical substances exert their preventive effects against obesity by targeting various stages of the adipocyte cycle, including the inhibition of adipogenesis, induction of lipolysis, stimulation of adipocyte apoptosis, and promotion of transdifferentiation from white adipocytes to brown-like adipocytes. [8] Several phytochemicals, such as flavonoids, phenols, and alkaloids, have been identified for their ability to reduce lipid accumulation, downregulate the expression of PPAR $\gamma$  and C/EBP $\alpha$ , and induce adipocyte apoptosis. Flavonoids are abundantly present in various plants, including green tea, soybeans, apples, onions, among others. In a study by Andersen et al., it was observed that green tea extract containing epigallocatechin-3-gallate (EGCG) at a concentration of 100  $\mu\text{M}$  hindered adipocyte cell proliferation. At levels  $\geq 50 \mu\text{M}$ , EGCG reduced lipid accumulation and the expression of PPAR $\gamma$  and C/EBP $\alpha$ , while concentrations exceeding 100  $\mu\text{M}$  induced lipolysis. Similarly, quercetin, a flavonoid found in apples, led to reduced body weight, triglyceride levels, and plasma cholesterol in rats, indicative of metabolic gene upregulation.

[8]

Resveratrol, a polyphenolic derivative present in grapes, demonstrated notable effects in reducing lipid accumulation and the expression of PPAR $\gamma$  and C/EBP $\alpha$  at concentrations  $\geq 25 \mu\text{M}$ . At a dose of 100  $\mu\text{M}$ , resveratrol promoted apoptosis, and concentrations exceeding 125  $\mu\text{M}$  suppressed lipogenesis and induced lipolysis. [24] Plants containing alkaloids, such as capsaicin and ephedrine, have also shown promise in reducing fat mass and body weight, inhibiting adipogenesis, lipid accumulation, and adipocyte differentiation while enhancing lipolysis. [22]

Although the outcomes of various studies may differ, it is known that porang tubers contain several phytochemicals, including flavonoids, phenol, tannins, alkaloids, saponins, steroids, and terpenoids. [6, 7] It is plausible that porang tubers operate through similar mechanisms as plants containing analogous phytochemicals, leading to a reduction in adipocyte volume by inhibiting adipogenesis or fat accumulation, promoting adipocyte lipolysis and apoptosis, and preventing adipocyte differentiation. Nonetheless, further research is necessary to validate these mechanisms. Furthermore, glucomannan, the predominant component of porang, exhibits the ability to decrease the expression of PPAR $\gamma$  and C/EBP $\alpha$ , both of which are proteins involved in adipocyte cell hypertrophy. By reducing the expression of these proteins, lipid accumulation and adipocyte maturation are mitigated, leading to diminished volume gain or adipocyte hypertrophy. The combined impact of glucomannan and the aforementioned phytochemicals culminates in a reduction in cell diameter. [22, 23]

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## Conclusion

Porang tuber flour did not demonstrate a significant effect in preventing weight gain in the experimental subject however, it did result in a significantly smaller adipocyte diameter compared to the control group, at both 200 mg/kgBW and 400 mg/kgBW doses. Moreover, the number of adipocytes was significantly lower in the group receiving porang tubers compared to the control group. These findings suggest that porang tuber flour possesses the potential to inhibit fat accumulation, which could eventually contribute to preventing weight gain and obesity. However, it is important to note that this study was conducted over a relatively short duration of 28 days, limiting the visibility of potential effects on weight gain prevention. Future research should extend the investigation over a longer timeframe to yield more accurate results. Additionally, a notable limitation of this study was the absence of a negative control group that did not receive a high-fat diet, as well as a positive control group that was administered a standard anti-obesity drug such as orlistat. This omission hindered the ability to discern whether the lack of significant changes in body weight observed in the study were attributed to the ineffectiveness of porang tuber flour or the relatively short duration of the research period.

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Table 3. Subject characteristics

|  | Group | Mean   | Median | SD    | Min    | Max    |
|--|-------|--------|--------|-------|--------|--------|
| Post-experimental weight (gram)                            | C     | 209.00 | 211.50 | 19.11 | 174.00 | 241.00 |
|  | A     | 186.00 | 185.00 | 22.24 | 160.00 | 220.00 |
|  | B     | 189.80 | 188.50 | 28.63 | 141.00 | 240.00 |
| Mean difference of pre and post-experimental weight (gram) | C     | 15.40  | 16.50  | 10.45 | -1.00  | 30.00  |
|  | A     | 11.75  | 13.00  | 10.19 | -9.00  | 23.00  |
|  | B     | 16.00  | 20.50  | 11.70 | -6.00  | 26.00  |
| Adipocytes diameter (mm)                                   | C     | 127.84 | 122.80 | 18.27 | 109.20 | 169.60 |
|  | A     | 56.98  | 54.60  | 12.31 | 43.60  | 77.80  |
|  | B     | 44.58  | 46.30  | 6.40  | 33.80  | 53.40  |
| Number of adipocytes (per field of view)                   | C     | 66.90  | 65.00  | 6.05  | 62.00  | 81.00  |
|  | A     | 42.63  | 42.50  | 1.85  | 40.00  | 46.00  |
|  | B     | 35.10  | 35.00  | 1.66  | 33.00  | 38.00  |
| Feed residue (gram)  | C     | 0.19   | -      | 0.19  | 0.01   | 0.52   |
|  | A     | 0.84   | -      | 0.18  | 0.57   | 1.18   |
|  | B     | 1.12   | -      | 0.59  | 0.28   | 2.12   |