Antihyperglycemic Activities of Fermented Milk Enriched with Gembili (*Dioscorea esculenta*)

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Abstract. Gembili is one variety of local tubers originating in Indonesia, where its utilization is not in its most use. This tuber has bioactive compounds such as dioscorin, diosgenin, inulin and water-soluble polysaccharides that function as immunomodulators, including those which prevents metabolic diseases such as hyperglycaemia. The addition of gembili tubers in fermented milk has more value and is an alternative nutraceutical food for people who need a healthy diet because it contains probiotics and bioactive components. This study aims to determine the antihyperglycemic activity of fermented milk enriched with gembili flour in animal models. Probiotics contained in fermented milk are *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Streptococcus thermophilus*. The experimental animals used in this study are healthy male white rats, not deformed, and had never been used in previous experiments. The animals in experiment were grouped into five treatment groups namely P1= negative control, P2= positive control, P3= 1 ml/rat/day of fermented milk with the addition of gembili tubers, P4= 2 ml/rat/day of fermented milk with the addition of gembili tubers, and P5= 3 ml/rat/day of fermented milk with the addition of gembili tubers (P5). The results of this study prove that the addition of fermented milk enriched with gembili showed significant results (p<0.05) on the decrease in blood sugar levels with the highest decrease of 43.59% in the P5 group.

1. Introduction

Hyperglycemia includes in cases of degenerative diseases with a prevalence number that keeps increasing in Indonesia [1]. The International Diabetes Federation predicts that the disease will double by 2030 [2]. Hyperglycemia occurs due to changes in lifestyle both physical activity and diet, where the condition can further develop into diabetes mellitus. Change in lifestyle is very important to control blood glucose levels [3]. Adjusting your diet or replacing carbohydrate-rich foods with fiber-rich foods can help control glucose levels [4]. The viscosity of the fiber has an effect to clear the contains inside the stomach longer, causing a longer feeling of fullness and reduce the speed of glucose absorption in the intestine [5].

One alternative that can be done to reduce glucose levels in the blood is by modifying the diet. Dietary management is the basis of every intervention in all cases and is the most essential thing that should not be forgotten [5]. Diet modification can be done by consuming fermented milk added with lactic acid bacteria as probiotics. Some research result shows that fermented milk can reduce blood glucose levels significantly [6,7,8,9].
Probiotic drinks can be combined with prebiotics from tubers to provide perfect health benefits. Gembili (Dioscorea esculenta) is a local food that has the potential as a prebiotic. Gembili is a local tuber plant from Indonesia that has not been widely used. Gembili flour contains 5.05% water soluble food fiber; 8.21% water-insoluble food fiber; 29.53% water-soluble polysaccharides; and 150.44 mg/100 g diosgenin [10]. The fiber content in gembili is approximately 2.29% [11]. Gembili tubers contain bioactive compounds which have positive effects towards the absorption of blood glucose such as water-soluble polysaccharides, food fiber, inulin and diosgenin. Water soluble polysaccharides in gembili can reduce blood glucose in experimental mice with hyperglycemia conditions [12].

Based on the description above, this study is conducted to determine the effect of fermented milk with lactic acid bacteria namely Lactobacillus acidophilus, Lactobacillus plantarum and Streptococcus thermophilus enriched with prebiotic from gembili tubers to decrease blood glucose levels in male wistar rats treated with hyperglycemia conditions.

2. Materials and Methods

The materials used in this study include skim milk, full cream milk, Lactobacillus acidophilus, Lactobacillus plantarum and Streptococcus thermophilus as lactic acid bacteria, gembili tuber flour, mineral water, and 70% of alcohol. Other materials used in the in vivo test are modified AIN-93M standard feed and husks.

The instruments used in this research were Incubators, glass bottles, stoves, pasteurization, measuring cups, stirrers, refrigerators, blood glucose instrument (Easy Touch), analytical scales (Camry), sonde needles (force feeding needles) for rat, syringes, micro tubes, micro tips, micro pipette, cage, food container, rat drinking bottle, cabinet dryer, meat grinder (Kenwood) to process feed.

2.1. Gembili tubers flour

Gembili is peeled and then washed clean to remove impurities. The Gembili is then thinly cut around 1-2 mm to ease the drying process. Next, the gembili is soaked using salt water, drained and dried using an oven at 60 ºC. After drying, the gembili is grounded using a grinding machine, sifted and stored in a sealed package [26].

2.2. Fermented milk with gembili tuber flour

This stage begins by preparing the cultivation of Lactobacillus acidophilus, Lactobacillus plantarum and Streptococcus thermophilus bacteria with a concentration of 2: 1: 1. The making of fermented milk is done by pasteurizing skim milk at a temperature of 80 ºC for 15 minutes. Gembili tuber flour is added as much as 1.5%. An addition of lactic acid bacteria starter as much as 3% then incubated for 24 hours at 37ºC [27].

2.3. Preparation of experimental animal

Experimental animals used in this study were Rattus norvegicus Wistar strain adapted for 1 week before being treated and observed for 4 weeks, then the effect of giving fermented tuber fermented milk is monitored. The reason to rat animals being chosen is the resemblance of mouse animals to humans. The relativity includes mammal groups, omnivores, easy to breed, and is easily treated. The experimental animals used for the study were male Rattus norvegicus wistar strain with the physical features of white fur, red eyes, wide head, healthy, and body weight ranging from 150-200 grams. Wistar rats used in the study were obtained from Malang Murine Farm. The ingredients used to create hyperglycemic conditions in wistar rats are sterile alloxan and distilled water. The alloxan dose given is 100 mg / kgBW intraperitoneally. After being injected with alloxan, rats were given 5% glucose drink for 24 hours [13].
2.4. Research design
In this study a completely randomized design (CRD) research method was used under one factor of the dosage form or type of sample given to the rat group. The research design used was the Pre and Post Test with Control Group Design, the control group that did not receive treatment and the experimental group that received treatment. This design makes it possible to find out how much change is due to the treatment given. The rats used for the study were divided into 5 groups. Each group consisted of 6 rats, the total number of rats used was 30. The treatment groups are as follows: negative control (P1) without alloxan injection; positive control (P2) by injection of alloxan; fermented milk treatment group with the addition of gembili tubers (P3) 1 ml / rat / day; fermented milk treatment group with the addition of gembili tubers (P4) 2 ml / rat / day; and the fermented milk treatment group with the addition of gembili tubers (P5) 3 ml / rat / day. Each group was given modified AIN-93M as standard feed and drinking water in ad libitum during treatment. Weighing the rats was carried out on each group of rats with the aim to see changes in the increase or decrease in body weight of the rats and to adjust the volume of the sample to be given. The weighing of rats was carried out on 0, 7, 14 and 21 days.

2.5. Measuring blood glucose level
Blood collection and measurement of blood glucose levels from fasting are performed every 7 days. Blood sampling of rats was carried out through rat tail (intravenously). The measurement of blood glucose levels was performed by using a glucotest tool. Glucotest (Easy Touch) is turned on and glucose sticks are added to glucotest. Blood is drawn through the tip of the tail and then dropped on a glucotest stick, within 10 seconds blood glucose levels will be measured automatically and the results can be read through the monitor in mg / dL units. The mechanism of action of this glucotest tool that works enzymatically involves the reaction of glucose oxidase, this reaction produces the color intensity which is detected by this tool [28].

2.6. Data analysis
The data obtained were tabulated and analyzed statistically through Analysis of Variants (ANOVA) using the Microsoft Excel 2007 program. If there is a difference, then a further BNT test with a 5% level is performed to see the difference from each treatment.

3. Results and Discussions
3.1 Yield of gembili tubers flour
The yield of gembili tuber flour is 27%. There are several factors that affect the yield of gembili tubers flour. The level of tuber yield is influenced by the interaction between the age of the harvest and the clones of the tuber. Yield can also be affected by water content because each ingredient has an optimal water content to achieve optimal flour of yield [29].

3.2 Antihyperglycemic activity of fermented milk enriched with gembili flour in animal models
Based on the results of ANOVA analysis, from the various treatments given to the group of rats, shows that there was no significant effect (p > 0.05) on feed consumption and body weight of rats during treatment. The amount of rat feed consumption was obtained by weighing the remaining rat feed every day during the treatment period. The average amount of feed intake consumed by rats in each treatment group can be seen in Figure 1. Based on Figure 1 it can be seen that the consumption of rat feed in all treatment groups shows an increase in feed intake every week. Increased feed consumption occurs because in conditions of high blood sugar levels, the body will lack energy because glucose does not diffuse easily through the pores of cell membranes without the help of insulin [14]. This condition will increase hunger, and this correlates with the increase in the amount of feed intake.

Changes in body weight patterns of rats in the treatment group are presented in Figure 2. Based on the ANOVA analysis of variance, the treatment group did not show any significant effect (p > 0.05)
towards body weight of rats. The results showed that the treatment showed an increase in body weight. Weight gain can occur due to good absorption of feed intake. Weight gain occurs because the food given and consumed by rats is in accordance with their abilities and proportion to the energy used, therefore the available energy is used for growth and increase in tissue mass [15]. In the positive control group or rats with hyperglycaemic conditions, shows weight loss on days 7 to 21 days. This happened due to the food being consumed was unable to be processed into energy, resulting in weight loss and increasing blood glucose levels. Weight loss is one of the characteristics of people with diabetes mellitus (DM). This is the impact of the reduction in insulin hormone. Insulin hormone deficiency can reduce the performance of the hexokinase enzyme, causing the use of blood glucose as an energy source to reduce and the body tends to obtain energy from other pathways such as fat breakdown. In addition, weight loss in people with DM is also caused by the breakdown of body protein [16].

![Figure 1. Graph of changes in feed consumption during treatment](image1)

P1 = negative control, P2 = positive control, P3 = 1 ml/rat/day of fermented milk with the addition of gembili tubers, P4 = 2 ml/rat/day of fermented milk with the addition of gembili tubers, and P5 = 3 ml/rat/day of fermented milk with the addition of gembili tubers.

![Figure 2. Graph of changes in body weight during treatment](image2)

P1 = negative control, P2 = positive control, P3 = 1 ml/rat/day of fermented milk with the addition of gembili tubers, P4 = 2 ml/rat/day of fermented milk with the addition of gembili tubers, and P5 = 3 ml/rat/day of fermented milk with the addition of gembili tubers.
Increase in the blood sugar levels by induction of alloxan occurred due to the release of calcium ions from the mitochondria which in result disturbs the process of cell oxidation and cause homeostatic disorders in the cell. The results of the research testing the decrease in blood glucose levels of rats can be seen in Table 1, which presents that the treatment of P3, P4, P5 has a significant effect (p<0.05) towards the decrease in rat blood glucose levels. Table 1 shows that the addition of fermented milk with gembili tubers shows a decrease in rat blood glucose levels. The highest reduction in blood glucose levels was found in the P5 treatment, for approximately 147 ± 20.46 mg/dl or 43.59 ± 3.83%. Consumption of probiotic drinks for 16 days can reduce blood sugar levels by 47.4% [17].

Table 1. Average reduction in blood glucose levels

| Group | Treatment | Average Blood Glucose Level | Average Decrease in Blood Glucose Levels | (mg/dL)* | (%)* |
|-------|-----------|-----------------------------|-----------------------------------------|----------|------|
|       | Start (mg/dL) | Final (mg/dL) | Start (mg/dL) | Final (mg/dL) | Start (mg/dL) | Final (mg/dL) | (mg/dL)* | (%)* |
| P1    | 74.60     | 76.40          | 1.80±15.72  | 4.85±20.10b | 96.98±58.42a |
| P2    | 283.80    | 511.40         | 227.60±126.36 | -32.53±4.27c |
| P3    | 346.20    | 233.80         | -112.40±15.24 | -37.07±3.27bc |
| P4    | 322.00    | 202.00         | -120.00±22.93 | -43.59±3.83bc |
| P5    | 337.40    | 190.40         | -147.00±20.46 | -43.59±3.83bc |

*: different superscript indicates significant different (p<0.05)

Decreased blood glucose levels in hyperglycaemic rats is due to fermented milk drinks containing probiotics which are living microbes that have therapeutic abilities to improve microbial balance found in the human digestive tract. Probiotic bacteria can increase insulin sensitivity; therefore, it can significantly reduce blood sugar levels [18, 19, 20]. Probiotic bacteria can maintain intestinal biota which can provide good effectiveness in maintaining insulin sensitivity, by inhibiting the activity of the inflammatory pathway from the presence of pathogenic bacteria, lipopolysaccharides or free fatty acids [21]. In the fermentation process of making yogurt, vitamin K2 is produced by lactic acid bacteria such as Lactococcus, Lactobacillus, Enterococcus, Leuconostoc, and Streptococcus [22]. Vitamin K2 can have an effect on pancreatic β cell function and insulin sensitivity on health by regulating glucose metabolism through osteocalcin modulation [23].

Probiotic bacteria during their growth must also be supported by suitable substrates. Gembili tubers are local food ingredients that can support the growth of probiotic bacteria. Gembili is a tuber that contains bioactive compounds that are beneficial for health. The active compounds contained in gembili are water soluble polysaccharides, diosgenin, and dioscorin. Water soluble polysaccharides are water soluble food fibers that can improve the glycemic response. The thick, gel-forming nature of PLA can inhibit macronutrient absorption and decrease the postprandial glucose response. While diosgenin and dioscorin are active compounds that can be used to reduce metabolic diseases such as hypercholesterolemia, dyslipidaemia, diabetes, obesity, inflammation and cancer [24]. Diosgenin is part of the saponin steroid group which can undergo transformation into sapogenin which plays a role in reducing blood glucose levels [25].

4. Conclusion
In vivo test result shows that the addition of fermented milk with gembili tubers can reduce blood sugar levels with the highest decrease of 43.59% in the P5 group.

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