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POTENTIAL USE OF FERMENTED DANGKE CHEESE TO IMPROVE GLYCEMIC CONTROL IN RATS FED WITH A HIGH-FAT GLUCOSE DIET AND PROPYLTHIOURACIL

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ABSTRACT

Dangke is a local cheese from Enrekang, South Sulawesi, traditionally made with buffalo milk and papaya sap. Dangke is a good source of lactic acid bacteria, which may offer health benefits. This study aimed to investigate the anti-hyperglycemic effects of dangke in rats. In addition, this study also examined the effect of fermentation time on the number of lactic acid bacteria obtained from dangke. Dangke was prepared by boiling buffalo milk and papaya sap until solidified and fermented in a jar for 1, 3, 7, and 14 days at room temperature. The number of lactic acid bacteria from dangke was determined using a Total Plate Count method. Hyperglycemia in rats was induced with propylthiouracil, a high-fat diet, and dextrose 10% in drinking water for 14 days, followed by dangke cheese (0.5 or 1.5 gram per 200 grams of rats' body weight) treatments for another 14 days along with high-fat diet and dextrose. It was found that the lactic acid bacteria counts obtained from dangke after 1, 3, 7 and 14 days of fermentation were 0.62 x 10^8 CFU/mL, 0.912 x 10^8 CFU/mL, 0.263 x 10^8 CFU/mL, 0.14 x 10^8 CFU/mL, respectively. The administration of dangke at the dose of 1.5 grams showed the highest percentage of blood glucose reduction among the treatment groups ($p<0.05$). In conclusion, three days of fermentation of dangke cheese produced the largest amount of lactic acid bacteria, and a 1.5 g dose of dangke cheese is required to effectively reduce blood glucose in hyperglycemic rats.

1. Introduction

Diabetes Mellitus (DM) is a chronic metabolic disease due to reduced production of insulin or increased resistance to insulin [1]. DM is associated with numerous complications, thereby, lowering the quality of life of people with diabetes. Moreover, the number of deaths associated with DM is reported to have increased over the past decades. World Health Organization reported that DM has caused a fatality in approximately 1.5 million people worldwide [1]. Among Southeast Asia countries, Malaysia and Indonesia have the highest incidence of insulin resistance with a prevalence of more than 50% and 42%, respectively [2].

Dangke cheese is a traditional food of Enrekang in South Sulawesi, Indonesia made from buffalo milk and papaya sap by fermentation technique. Many studies have identified that dangke contains probiotic lactic acid bacteria [3- 5]. A meta-analysis study reports that routine consumption of probiotics may improve blood glucose control [6]. Recent data have shown that probiotic supplementation provides benefits to improve glycemic control and reduce insulin resistance in healthy or pregnant women [7]. Supporting this, the supplementation of probiotics in experimental animals also led to reduce glucose and improve insulin sensitivity [8].

Since probiotics have been shown to provide great benefits for glycaemic controls, it is believed food products containing probiotics may possess an anti-hyperglycemia effect. In addition to probiotics, the nutritional value of dangke is very promising as functional food, including oleic acid, linoleic acid, stearic acid, and palmitic acid [9]. Therefore, the present study aimed to examine the anti-hyperglycemic effect of dangke cheese in hyperglycemia rats induced by propylthiouracil, a high-fat diet, and 10% dextrose. In addition, the effect of the fermentation time of dangke on the lactic acid bacteria counts was also determined.

2. MATERIALS AND METHODS

2.1 Materials

Buffalo milk was obtained from the Enrekang regency of South Sulawesi, Indonesia. Propylthiouracil (PTU) and Acarbose were obtained from a local pharmacy (OGB Dextrose (10%) and sodium carboxymethyl cellulose (NaCMC) was purchased from a chemical store in Makassar (CV Intraco). A reagent kit for glucose level measurement was obtained from Human Diagnostic Worldwide, Germany.

2.2 Preparation Dangke

Preparation of dangke was conducted in the Food Product Technology Laboratory (Faculty of Animal Husbandry). One liter of milk was heated at 85°C with 3 mL of papaya sap to coagulate the milk and was stirred until the milk was coagulated. The mixture was put into a mold until hardening.

2.3 Lactic Acid Bacteria Analysis from Fermented Dangke

Dangke was fermented for 14 days in a sealed container at room temperature. After 1, 3, 7, and 14 days, a sample was taken to determine the number of lactic acid bacteria in each day of fermentation using a total plate count method. The samples were dissolved in water and diluted at a concentration of 10^{-4}, 10^{-5}, and 10^{-6}. Each concentration was pipetted (0.1 mL) into a petri dish containing de Man Rogosa Sharpe Agar (MRSA) media, and $CaCO_3$ was added before being incubated at 37°C. After 48 hours, the growth of the

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colony in the MRSA media was observed. The presence of lactic acid bacteria was identified by white or cream-colored round bacterial colonies with smooth surface with flat edges. Colonies were counted using a colony counter tool and then calculated using the total plate count formula.

$$TPC = N \times V \times 1/Fp$$

TPC = Total plate count
N = number of colonies growing on MRS agar medium
V = dilution volume
1/Fp = dilution factor

2.4 Preparation of Animals

The experimental animals used were 30 male Wistar rats with a minimum weight of 150 grams. They were acclimatized to the laboratory environment for 7 days before treatment, during the adaptation period the rats were given standard feed and water for drinking. All protocols complied with the standard protocols for experimental animal use and care, and this study has been granted an ethical clearance number of 22/UN4.6.4.5.31./PP36/2023.

2.5 Experimental Protocols

Rats were randomly divided into 5 groups, each group consisting of 6 rats. A normal control was given normal feed and aquadest. The negative control was fed with a high-fat diet plus PTU and 10% dextrose in drinking water. The treatment groups were given a high-fat diet plus PTU and 10% dextrose then dangke 0.5 or 1.5 grams per 200 grams of rats' body weight. Whereas, the positive control was given a high-fat diet plus PTU and 10% dextrose then acarbose 1.1 gram per 200 grams of rats' body weight.

The high-fat diet contains 42% carbohydrates, 24% protein, 16.50% fat, 10% sugar, and 5% minerals and vitamins (Pa'commo, Makassar), and it was given 20 grams/rat daily. The induction of hyperglycemia was conducted for 14 days [10], [11]. After hyperglycemic condition was induced, dangke or acarbose treatment was initiated. Prior to administration, dangke was pulverized and dissolved in aquadest and administered by oral gavage to the experimental animals at a volume of 2mL/200-gram rat body weight. During the administration of dangke, the animals were still given a high-fat diet and 10% dextrose.

2.6 Measurement of blood glucose levels

Measurement of serum levels was carried out after the induction of hyperglycemia and the administration of treatments (28 days in total). Blood serum was obtained from rats that had been fasted for ± 12 hours. Blood sample withdrawal was through orbital veins (3 mL) and then centrifuged for 20 minutes at a rate of 2500 rpm. Blood serum was transferred into an Eppendorf tube, and measured on a Humalyzer 3500 (Human®) using a standard reagent for blood glucose [13].

2.7 Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA). The differences between groups were further analyzed using post hoc Tukey's HSD test. All statistical significance was achieved if the P value < 0.05.

3. RESULTS

3.1 Lactic Acid Bacteria Counts in Dangke

The growth of lactic acid bacteria colonies on MRSA medium after 48 hours of incubation is depicted in

Figure 1. It was found that the lactic acid bacteria counts obtained from dangke after 1, 3, 7 and 14 days of fermentation were 0.62 x 10^8 CFU/mL, 0.912 x 10^8 CFU/mL, 0.263 x 10^8 CFU/mL, 0.14 x 10^8 CFU/mL (Table 1). The organoleptic test showed that dangke fermented for up to 3 days still had a white color, milky taste, and mild flavor. After 7 days, the dangke cheese started to show discoloration with a strong odor and strong taste.

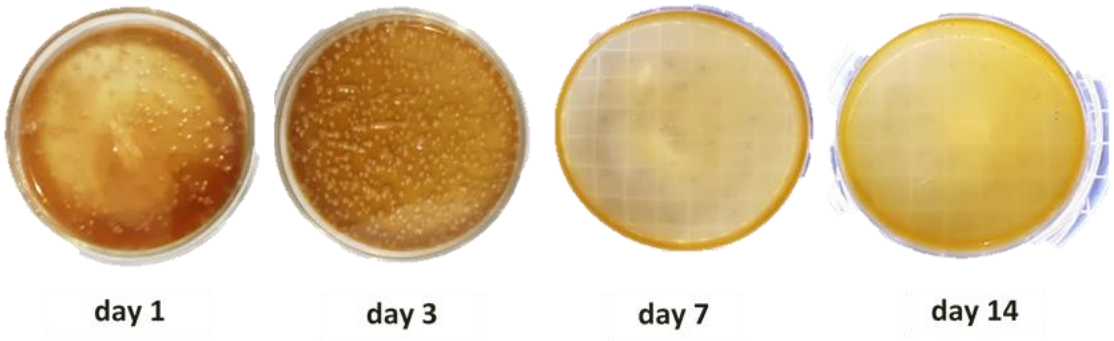

Figure 1. The growth of dangke's lactic acid bacteria on MRSA medium after 1,3,7, and 14 days of fermentation

Table 1. The pH and lactic acid bacteria colony counts in dangke after 1,3,7, and 14 days of fermentation

Fermentation day	pH	Colony counts
Day-1	5.0	0.62 x 10^8 CFU/mL
Day-3	5.0	0.91 x 10^8 CFU/mL
Day-7	6.0	0.26 x 10^8 CFU/mL
Day-14	6.0	0.14 x 10^8 CFU/mL

3.2 Anti-hyperglycemia of Dangke

Blood glucose levels after the induction of hyperglycemia and treatments are shown in Figure 2. The healthy controls had mean fasting blood glucose (FBG) levels of 97.37±17.3 mg/dl. In contrast, the negative controls that received PTU and a high-fat diet plus 10% glucose for 28 days experienced a high level of FBG, with a mean of 150.25±23.7 mg/dl ($p<0.05$). After 28 days of PTU and high-fat diet daily administration, all rats treated with dangke for 14 days showed lower FBG levels ($p<0.05$), especially those treated with 1.5 g/200 g body weight. Indeed, dangke at a 1.5 g dose could lower the FBG levels comparable to that of treated with acarbose.

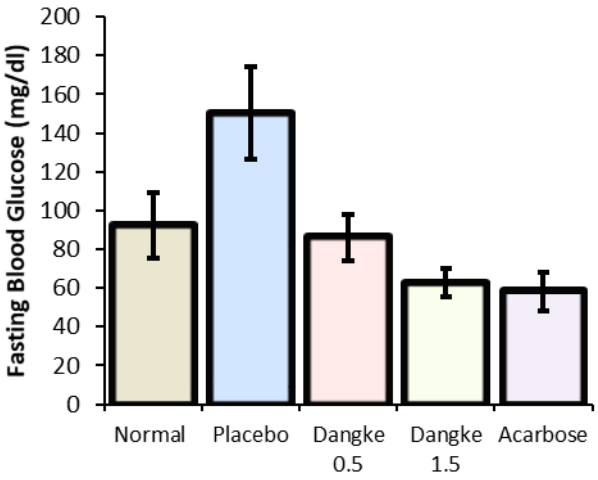

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Figure 2. The levels of fasting blood glucose after treatments for 14 days.
* P<0.05, ** P<0.01 compared to the placebo group

4. DISCUSSION

Dangke naturally contains indigenous lactic acid bacteria. Based on previous studies, lactic acid bacteria species that were isolated from dangke cheese included *L. fermentum, L. plantarum,* and *L. acidophilus* [3-5]. Lactic acid bacteria ferment sugar or carbohydrates to produce large amounts of lactic acid. Lactic acid bacteria are naturally found in various habitats such as fermented foods, animal digestive tracts, or dairy products [14], [15].

In this study, dangke cheese was fermented for 14 days. During the fermentation, the pH of dangke varied within the range of 5.0-6.0 depending on the time of fermentation. Lactic acid bacteria could grow at a pH of around 3.5 – 10.0, with most strains optimally growing at pH 4.4 although in general lactic acid bacteria has the optimum growth in the range of pH 5 .5 -6.5 [16].

MRSA media is a media of choice for growing lactic acid bacteria because it contains a component that optimally supports the growth of lactic acid bacteria, including ammonium citrate, dextrose, meat extract, yeast extract, and peptone [17]. The content of ammonium citrate at low pH supports the lactic acid bacteria growth [18]. Meanwhile, peptone, meat, and yeast are a source of nutrition for growth as the sources of nitrogen, vitamins, minerals, and amino acid, while dextrose provides energy from carbohydrates [19]. The addition of CaCO3 to the MRSA media is done to initially select and identify the presence of lactic acid bacteria. CaCO3 is alkaline, which can neutralize the acid production produced by lactic acid bacteria [3], this will help to form a clear zone around the colonies of lactic acid bacteria [20].

In this study, the growth of lactic acid bacteria was the most optimum on days 1 and 3 of fermentation. It is believed that the pH of dangke during fermentation gradually decreases and becomes more acidic when stored longer. As a result, the pH was too acidic for lactic acid bacteria to live. Therefore, it is suggested that the fermentation time of dangke should be limited to 3 days to obtain the optimal number of lactic acid bacteria.

The anti-hyperglycemia of the fermented dangke was tested against PTU- and high-fat diet-induced hyperglycemic rats. A long-term administration of a high-fat diet does not only change lipid profiles but also alters blood glucose levels, reduced pH, and promotes oxidative stress in rats [21]. The increase in blood glucose by PTU combined with a high-fat diet and dextrose has been demonstrated in some studies, which mechanism is related to hypothyroidism and insulin insensitivity [22]. This mechanism is different from alloxan, a diabetogenic agent that induces pancreatic cell damage via oxidative stress [23], [24]. With PTU and a high-fat diet, it requires a longer period to achieve a hyperglycemic state (14 days at least) compared to one to three days in alloxan-treated rats [25]. In our study, the placebo group had a high level of BFG after PTU treatment for 14 days and high-fat diets for 28 days. It was shown that dangke at doses 0.5 g and 1.5 g decreased the BFG levels by around 21% and 29%, respectively, when compared to the placebo ($p<0.05$), indicating its anti-hyperglycemic effect. Moreover, the FBG levels of dangke-treated rats (1.5 g) were comparable to that of the positive control receiving acarbose 1.1 mg. Acarbose is one of the standard antidiabetic oral that can lower blood glucose levels by inhibiting the alpha-glucosidase enzyme, thereby, delaying carbohydrates metabolism and glucose absorption, resulting in a reduction of postprandial glucose blood concentrations [26].

Dangke's antihyperglycemic effect may be associated with the ability of probiotics contained in dangke.

The mechanism involved may include the inhibition of α-glucosidase. To date, the antihyperglycemic effects of probiotics or lactic acid bacteria have been proposed in many studies. A report has shown that oral administration of *L. casei* for 12 weeks can inhibit pro-inflammatory mediators and thus prevent beta-pancreatic dysfunction in an experimental model of diabetes mellitus [27]. [28] found that *L. rhamnosus* was able to reduce insulin resistance in streptozotocin-treated rats. Furthermore, *L. acidophilus* and *L. casei* were demonstrated to protect insulin-producing cells and increased antioxidant levels in the liver and pancreatic tissues [29].

5. CONCLUSION

The number of lactic acid bacteria obtained from dangke cheese was the highest after three days of fermentation with a total colony of 0.912 x 10^8 CFU/mL. Fermented dangke has the effect of anti-hyperglycemia, shown by reduced fasting blood glucose in rats treated with propylthiouracil and a high-fat diet after the administration of dangke cheese for 14 days. It is found that the dose of 1.5 g/200 g of rat body weight is more effective than the lower dose. The anti-hyperglycemia of dangke may find clinical benefits in humans.

6. ACKNOWLEDGMENT

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7. REFERENCES

[1] World Health Organization, Diabetes, https://www.who.int/health-topics/diabetes#tab=tab_1 Accessed on January 2023.

[2] Goh LP, Sani SA, Sabullah MK, Gansau JA. The Prevalence of Insulin Resistance in Malaysia and Indonesia: An Updated Systematic Review and Meta-Analysis. Medicina. 2022;58(6):826.

[3] Nur F, Hatta M, Natzir R, Djide MN. Isolation of lactic acid bacteria as a potential probiotic in dangke, a traditional food from Enrekang, Indonesia. International Journal of Sciences: Basic and Applied Research. 2017;35(1):19-27.

[4] Malaka R, Laga A, Ako A, Zakariah M, Mauliah FU. Quality and storage time of traditional dangke cheese inoculated with indigenous lactic acid bacteria isolated from Enrekang District, South Sulawesi, Indonesia. Biodiversitas Journal of Biological Diversity. 2022;23(6).

[5] Jatmiko YD, Howarth GS, Barton MD. Assessment of probiotic properties of lactic acid bacteria isolated from Indonesian naturally fermented milk. AIP conference proceedings 2017; 1908(1): 050008.

[6] Ruan Y, Sun J, He J, Chen F, Chen R, Chen H. Effect of probiotics on glycemic control: a systematic review and meta-analysis of randomized, controlled trials. PloS one. 2015;10(7):e0132121.

[7] Pan YQ, Zheng QX, Jiang XM, Chen XQ, Zhang XY, Wu JL. Probiotic supplements improve blood glucose and insulin resistance/sensitivity among healthy and GDM pregnant women: a systematic review and Meta-analysis of randomized controlled trials. Evidence-Based Complementary and Alternative Medicine. 2021;2021.

[8] Tabuchi M, Ozaki M, Tamura A, Yamada N, Ishida T, Hosoda M, Hosono A. Antidiabetic effect of Lactobacillus GG in streptozotocin-induced diabetic rats. Bioscience, biotechnology, and biochemistry.

ISSN: 0005-2523
Volume 63, Issue 01, January, 2023

2003;67(6):1421-4.

[9] Yusuf M, Fitriani UN, Saleh R. Dangke: Local Indigenous Cheese from Enrekang, South Sulawesi Indonesia. IOP Conference Series: Earth and Environmental Science 2022; 1097(1): 012064.

[10] Moreno-Fernández, S., Garcés-Rimón, M., Vera, G., Astier, J., Landrier, J. F., & Miguel, M. (2018). High fat/high glucose diet induces metabolic syndrome in an experimental rat model. Nutrients, 10(10):1502.

[11] Syahputri, H., Silalahi, J., Harahap, U., & Satria, D. (2020). Antidiabetic Activity of Lactobacillus fermentum Bacteria from Dengke Naniura Goldfish (Cyprinuscarpio) in Nicotinamide-Streptozotosin Induced Rats. Asian Journal of Pharmaceutical Research and Development, 8(4):12-15.

[12] Al-Noory AS, Amreen AN, Hymoor S. Antihyperlipidemic effects of ginger extracts in alloxan-induced diabetes and propylthiouracil-induced hypothyroidism in (rats). Pharmacognosy research. 2013;5(3):157.

[13] Hardi H, Djabir YY, Setiawati H, Lallo S. The effect of breadfruit (Artocarpus altilis (Parkinson) Fosberg) leaf extract on blood glucose, lipid profiles, and weight loss in alloxan-induced diabetic rats. ACTA Pharmaceutica Sciencia.;60(4):234-40

[14] Bureenok, S., W. Suksombat, & Y. Kawamoto. 2011. Effects of the fermented juice of epiphytic lactic acid bacteria (FJLB) and molasses on digestibility and rumen fermentation characteristics of ruzigrass (Brachiaria ruziziensis) silages. Livest. Sci. 138: 266-271.

[15] Cobos, M. A., A. L. deCoss, N. D. Ramirez, S. S. Gonzalez, & R. F. Cerrato. 2011. Pediococcus acidilactici isolated from the rumen of lambs with rumen acidosis, 16S rRNA identification and sensibility to monensin and lasalocid. Res. Vet. Sci. 90: 26–30.

[16] Abdel-Rahman, M. A., Tashiro, Y., & Sonomoto, K. Recent advances in lactic acid production by microbial fermentation processes. Biotechnology Advances. 2013; 31(6), 877-902.

[17] Meruvu H, Harsa ST. Lactic acid bacteria: isolation–characterization approaches and industrial applications. Critical Reviews in Food Science and Nutrition. 2022;17:1-20.

[18] Bover-Cid S, Holzapfel WH. Improved screening procedure for biogenic amine production by lactic acid bacteria. International journal of food microbiology. 1999; 53(1):33-41.

[19] Atlas RM. Handbook of microbiological media. CRC press; 2004.

[20] Tjahjaningsih, W., Masithah, E. D., Pramono, H., & Suciati, P. Enzymatic Activity of Lactic Acid Bacteria Isolate from the Digestive Tract of Mud Crab (Scylla spp.) as a Candidate Probiotics. Marine and Fisheries Scientific Journal.2016; 8(2): 94-108

[21] Arsyad A, Idris I, Rasyid AA, Usman RA, Faradillah KR, Latif WO, Lubis ZI, Aminuddin A, Yustisia I, Djabir YY. Long-term ketogenic diet induces metabolic acidosis, anemia, and oxidative stress in healthy wistar rats. Journal of nutrition and metabolism. 2020:2020.

[22] Losacco MC, de Almeida CF, Hijo AH, Bargi-Souza P, Gama P, Nunes MT, Goulart-Silva F. High-fat diet affects gut nutrients transporters in hypo and hyperthyroid mice by PPAR-a independent mechanism. Life Sciences. 2018;202:35-43.

[23] Sari DR, Ahmad FF, Djabir YY, Yulianty R. Breadfruit leaves extract (Artocarpus altilis) effect on pancreatic damage in diabetic type II animal model induced by alloxan–nicotinamide. Medicina Clínica Práctica. 2020;3:100099.

[24] Djabir YY, Hardi H, Setiawati H, Lallo S, Yulianty R, Cangara MH, Hadju V. Artocarpus altilis leaf extract protects pancreatic islets and improves glycemic control in alloxan-induced diabetic rats. Journal of Reports in Pharmaceutical Sciences. 2021;10(1):87.

[25] Setiawati H, Djabir YY, Hardi H, Lallo S, Cangara MH. Potential Use of Breadfruit (Artocarpus altilis) Leaf Extract to Recover Hepatic and Renal Damage in Alloxan-Induced Diabetic Rats. Fabad Journal of Pharmaceutical Sciences. 2022;2(47):151-60.

[26] McIver LA, Preuss CV, Tripp J. Acarbose. [Updated 2022 Sep 21]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. 2022

[27] Matsuzaki T, Yamazaki R, Hashimoto S, Yokokura T. Antidiabetic effects of an oral administration of Lactobacillus casei in a non-insulin-dependent diabetes mellitus (NIDDM) model using KK-Ay mice. Endocrine journal. 1997;44(3):357-65.

[28] Tabuchi M, Ozaki M, Tamura A, Yamada N, Ishida T, Hosoda M, Hosono A. Antidiabetic effect of Lactobacillus GG in streptozotocin-induced diabetic rats. Bioscience, biotechnology, and biochemistry. 2003;67(6):1421-4.

[29] Yadav H, Jain S, Sinha PR. Antidiabetic effect of probiotic dahi containing Lactobacillus acidophilus and Lactobacillus casei in high fructose fed rats. Nutrition. 2007;23(1):62-8.