[TiS] Manuscript Decision

External Inbox

Tidarat Kaewjaijong 20 Mar
to me, Faridah, Farzana, Harnavi

Dear Dr. Rinita Amelia, Faridah Mohd Said, Farzana Yasmin, Harnavi Harun:

We have reached a decision regarding your submission to Trends in Sciences, **"The Potential of West Sumatran Dadiah as The Novel to Alleviate Hyperglycemia, Hypercholesterolemia, and Reducing NF-kB Expression in Nephropathy Diabetes Rat Model"**. Please revise the manuscript carefully. The manuscript should be resubmitted along with point-by-point explanation according to reviewers'comments. If you disagree with any of the comments, please state your reasons. All corrections are mandatory and **must be differentiated with red colour** and submit it.

Our decision is to: Resubmit for Review

We request that you send a revised manuscript **within 20 days**, otherwise it may be considered withdrawn.

[TiS] New notification from Trends in Sciences

External Inbox

Trends in Sciences (TiS) 3 Aug

to me

You have a new notification from Trends in Sciences:

You have been added to a discussion titled "[Proofreading Request] Trends in Sciences (formerly know as WJST) Volume 20 (2023) ID 6812" regarding the submission "The Potential of West Sumatran Dadiah as The Novel to Alleviate Hyperglycemia, Hypercholesterolemia, and Reducing NF-kB Expression in Nephropathy Diabetes Rat Model".

Link: https://tis.wu.ac.th/index.php/tis/authorDashboard/submission/6812

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https://doi.org/

The Potential of West Sumatran *Dadiah* as The Novel to Alleviate Hyperglycemia, Hypercholesterolemia, and Reducing NF-kB Expression in Nephropathy Diabetes Rat Model

Highlights

Dadiah is a naturally fermented buffalo milk product in bamboo tubes. Dadiah (Probiotic) originating from West Sumatra, Indonesia acts as an antidiabetic. Dadiah and its metabolites significantly reduced hyperglycemia and serum cholesterol and inhibited oxidative stress by reducing NF-kB expression in kidney tissue after treatment. Dadiah probiotics should be considered as a nutritional companion in diabetic nephropathy and as a future therapeutic target for DM patients to prevent the development of microvascular complications and hypercholesterolemia.

Abstract

Diabetic nephropathy (ND) is the most common microvascular complication in diabetes mellitus (DM) patients. The main mechanism for the development of ND is an inflammatory reaction as indicated by increased expression of NF-kB in kidney tissue due to chronic hyperglycemia and hypercholesterolemia. Hyperglycemia is related to changes in the composition of the microbiota which can cause dysbiosis. Thus, the therapeutic approach in DM sufferers using probiotics needs to be considered. Dadiah is a naturally fermented buffalo milk product in bamboo tubes. Dadiah comes from West Sumatra, Indonesia, there has not been much research on the clinical and biomolecular effects of various metabolic diseases. Even though this probiotic has health benefits, its mechanism as an antidiabetic is not widely known. This study aims to reduce blood sugar levels, cholesterol, and the inflammatory marker NF-kB. This research is an experimental animal study that aims to determine the effect of Dadiah and its probiotic metabolites on diabetic rats induced by intraperitoneal Alloxan 100 mg/kg b. w. ND rats were treated with low and high doses of Dadiah, LAB (Lactic Acid Bacteria), and Bacteriocin for eight weeks. Next, we tested their effect on blood glucose levels, serum cholesterol, and NF-kB antibody expression in kidney tissue using immunohistochemistry assays. The results demonstrated the potential of Dadiah and its metabolites to significantly reduce hyperglycemia and serum cholesterol and inhibit oxidative stress by reducing NF-kB expression in kidney tissue after treatment. Dadiah probiotics should be considered as a nutritional companion in diabetic nephropathy and as a future therapeutic target for DM patients to prevent the development of microvascular complications and hypercholesterolemia.

Keywords: Dadiah, hyperglycemia, hypercholesterolemia, NF-kB, diabetes neuropathy, probiotic

Introduction

Diabetes Mellitus (DM) is a cause of premature death, blindness, heart disease, and kidney failure for sufferers. According to the International Diabetes Federation (IDF), the number of people with Diabetes Mellitus in Indonesia is expected to continue to increase from 9.1 million people in 2014 to 14.1 million people in 2035. DM is a group of metabolic diseases that cause chronic hyperglycemia [1]. DM consists of 2 types, namely, type 1 DM (T1DM) as a result of an autoimmune reaction to pancreatic cell proteins, and type 2 DM (T2DM) as a result of a combination of genetic factors and environmental factors, such as obesity, overeating, lack of food, exercise, stress, and aging [2]. Generally, patients with T2DM experience complications, and cardiovascular complications that cause morbidity and mortality. T2DM patients experience impaired insulin secretion and/or action, causing hyperglycemia and hyperinsulinemia [3]. Theincreasing prevalence of T2DM is becoming a major cause of microvascular such as retinopathy and macrovascular complications such as peripheral vascular disease, and diabetic nephropathy [4-5].

Diabetic nephropathy (ND) is a condition of decreased kidney function and the main cause of end-stage kidney disease [6]. DN is triggered by genetic, environmental, cellular, and molecular mechanisms that play a role in kidney damage in diabetes [7]. DN is a clinical syndrome characterized by persistent albuminuria and progressive decline in kidney function. 50% of patients with DN will experience end-stage kidney disease (ESKD) requiring treatment with dialysis or kidney transplantation which is associated with

Commented [-1]: DN

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significantly increased cardiovascular morbidity and mortality. The main risk factors for the development of DN are chronic hyperglycemia, hypercholesterolemia, and reduced expression of NF kappa B (NF-kB). Chronic hyperglycemia in DM sufferers is followed by damage, and impaired function of the eyes, kidneys, nerves, heart, and blood vessels. The diagnosis of diabetes mellitus is made based on the high level of glucose in the blood plasma [8-9]. Hypercholesterolemia can lead to atherosclerosis, coronary heart disease, pancreatitis, thyroid disorders, liver disease & disease [10]. NF-kB plays a role in the development and various complications of DM for sufferers, such as diabetic cardiomyopathy, retinopathy, nephropathy, and DM neuropathy. Many therapeutic approaches for DM sufferers have been developed, such as the use of several antioxidants, flavonoids, and probiotics. Probiotics are promising candidates for improving glycemic management, inflammatory systems, and lipid profiles in individuals with type 2 diabetes. Probiotic supplementation improves glycemic control and cardiometabolic risk markers. One possible mechanism for the hypocholesterolemia impact of probiotics has been suggested by direct cholesterol interaction or assimilation by probiotics [11]. The effectiveness and safety of probiotics for glycemic control in patients with impaired glucose control, including prediabetes and type 2 diabetes mellitus [12].

Many studies have used experimental models to evaluate the impact of supplementation with probiotics and prebiotics on various risk factors for metabolic syndrome [13]. The search for safer non-pharmacological therapies with cholesterol-lowering effects continues to be carried out by utilizing bacteria. Probiotic bacteria from the lactic acid group and Bifidobacterium can regulate serum cholesterol potential [14]. The results of the study showed that Gaio was able to lower cholesterol. Gaio is a yogurt product that utilizes the ability of *Enterococcus faecium* strains and *Streptococcus thermophilus* strains [15]. Decreased serum lipid concentrations with probiotic intake based on studies of various bacterial strains [16]. Probiotics are one of the most commonly used nutritional supplements around the world. One of the probiotics, Dadiah comes from West Sumatra, Indonesia, which is known as a traditional food.

Dadiah is a type of traditional fermented milk and has the potential to be developed as a functional food source of probiotics. Dadiah is made from buffalo milk, through a natural fermentation process involving lactic acid bacteria. Dadiah produced in West Sumatra, Indonesia is made from buffalo milk by relying on microbes that exist in nature as an inoculant or without a starter. The fermentation of the curd is carried out by microbes originating from bamboo, banana leaves, and milk [17]. Bamboo segments contain several microbes consisting of mold, yeast, lactic acid-forming microorganisms, protein breakers, and spore formers [18-19]. The use of antioxidants in DM cases needs to be considered to prevent the development of DM into diabetic nephropathy. Probiotics can enhance antioxidant absorption and antioxidant-related activity. Many studies have been conducted by local and national researchers regarding the nutritional components and their antimicrobial activity in Dadiah. However, only a few have studied clinically and scientifically confirmed its effects on various diseases, especially metabolic diseases. Although this probiotic has beneficial properties, its presumed anti-diabetic mechanism is unknown. This study aims to reduce blood sugar levels, cholesterol, and the inflammatory marker NF-kB.

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Materials and methods

Instruments of research

The instruments of research digital scale (ACS) with 0.01gram accuracy to weigh rats. Experimental animal cages, food and water containers for experimental animals, sonde to inject Dadiah and LAB isolated sampled from Dadiah 1 ml/day and 2 ml/day. The tools used in this research are a luminometer, pipettes, microscope, microtome, slide glass, razor blades/scissors, aluminum foil, metal basket, rotary tissue processor, refrigerator, water heater, processor cassette, autoclave (Hirayama), incubator (Fisher), hot plate, Eppendrof, bunsen, vortex, Erlenmeyer tubes, glucometer (Glucose blood level and cholesterol) and urine protein stick (UriScan).

Experimental animals

Wistar-strain male white rats (*Rattus norvegicus*) aged 2-3 months with a weight of ± 300-gram, standard feed as daily food, and Ad libitium drinking water. Dadiah. Lactic Acid Bacteria dan bacteriocin isolated sampled in Dadiah from Tanjung Bonai, Lintau Tanah Datar, West Sumatra. The examination results obtained information that Dadiah contained Lactic Acid Bacteria of 7,1 X 1010 CFU/g [20].

Preparation of Dadiah

Dadiah dosage for rats = conversion value x Dadiah dosage for humans. The dosage of administration, based on the recommended dosage of fermented milk in humans with a body weight of 70 kg, was 100-200 mL per day. The density (ρ) of Dadiah was 1.04 g/mL. The recommended Dadiah dosage is 104 g/70 kg of human. The Laurence table (2008), the conversion value of 70 kg of Human weight to 200 g of Rat weight is 0,018. The calculation of Dadiah dosage for the rat is Dadiah dosage for rat = conversion value x Dadiah dosage for humans. The recommended Dadiah dosage is 104/70 kg of human. Dadiah dosage for rat = conversion value x Dadiah dosage for human = 0,018 x 104 =1,87 g/200 g of rat weight. 1.87 g of Dadiah/200 g of Rat weight = 9.35 g/kg b. w. The weight of the male white rat (*Rattus norvegicus*) is ± 300 g = 0,3 kg Dadiah solution containing 1 g/mL was made by suspending Dadiah with aqua dest. The material in this experimental study is Dadiah 3 mL.

Preparation of LAB

Isolate *L. fermentum* is rejuvenated first, then propagated in the medium MRS broth at a temperature of 370ºC for 24 hours, and calculated the number of bacterial cells is by diluting up to 108 CFU/mL. Dilution results are calculated on the MRS medium so that it is included at a temperature of 37ºC for 2 x 24 hours in the incubator to find out the number of LAB to be induced. Following previous *in vitro* research obtained for 1 g Dadiah, there is a LAB colony of 7.1 x 10^{10} CFU/mL.

Preparation of Bacteriocin (Production of crude Bacteriocin)

The LAB of Dadiah was cultivated in MRS broth (1000 ml) seeded with 10% inoculum of overnight culture and incubated at 37ºC for 24 hours. Following incubation, the entire broth was centrifuged for 16 minutes at 10.000 X g find the cell-free supernatant was used as crude Bacteriocin [21]. The amount of LAB and Bacteriocin used in this study was 1 mL and 2 mL per day.

Methods

This research is an experimental study base on animal trials with a post-test-only control-group design. Male *Rattus norvegicus* strain wistar rats were procured from Pharmacology Department, Universitas Andalas, Padang, West Sumatra, Indonesia. The research samples have the criteria, healthy with glowing eyes, active and having a good appetite, 2-3 months old, and weigh 200-300 grams. All rats were maintained at 23-25ºC, with both a standard pellet diet and water ad libitum. After acclimatization for two weeks, except for the negative control group, all other groups were injected with alloxan 100 mg/Kg b. w. All groups of mice were fed with standard pellets. Furthermore, the treatment group will be given Dadiah, LAB, and Bacteriocin. The experiment was conducted with five treatment groups and two control groups. In this study, rats were divided into five groups with the number of each group of six rats. So that six DN rats were treated with Dadiah 3 mL/day (P1 group) and isolated samples of LAB and Bacteriocin from Dadiah 1 mL and 2 mL/day (P2-P5 group). Control groups were three DN rats without being treated (Positive control/C+) and three normal rats (control negative/C-) who did not have DN (without alloxan injection). The number of samples obtained by 42 rats.

Induction of diabetes and *in vivo* experimental

Before the experiment began, all the rats were weighed, and measured blood glucose levels were cut off the rat's tail's 1 mm end. After that, the blood dropped on the glucose stick of the glucometer (OneTouch Merck; accuracy ISO 15197:2003) and the test of proteinuria by UriScan Test Strips (Biosys Laboratories, INC). After all the data have recorded, we had the first experiment that made rats into clinically marked ND for hyperglycemia (> 200 mg/dL) and proteinuria. In a preliminary study, rats kept on fasting for 12 hours received a single injection of freshly dissolved alloxan in 1.0 mL of sodium citrate buffer (0.1 M pH4.5) intraperitoneally (i. p), at a rate of 100 mg/kg b. w. The blood was withdrawn from the tail vein of rats, then the measurement of fasting blood glucose concentration and cholesterol serum every two weeks along the experimental protocol (56 days/8 weeks). After 7 days of alloxan induction, animals with fasting glucose > 200 mg/dL and proteinuria were considered diabetic nephropathy and grouped accordingly with an average of 6 rats per group and orally administered with Dadiah, LAB, and Bacteriocin isolated from Dadiah for eight weeks or 56 consecutive days.

The dissection of experimental animals

Dissection was performed after 56 days of treatment is given where male white rats (*Rattus norvegicus*) were killed using Anesthesia with ether. The method was by mixing the concentrated ether solution with 2% NaCl solvent or 10-25% in NaCl and a dose of 300 mg/kg or 1-1.25 g/kg placed on the bottom of the desiccator. Then put the rat in a closed container, wait until it became immobile, and its pupillary mydriasis and eyes were closed. If the rat lost consciousness, then brought ride inside the container, then laparotomy and neck pressure were done to kill it while pulling it anteriorly (dislocation Atlanta-occipitalis. Identification and nephrectomy were carried out, then directly put into a 10% BNF solution, after the kidney organ was removed.

Tissue processing

Rat renal tissue was processed into paraffin blocks and cut with a microtome with a thickness of 4 mm. The preparations were stained with hematoxylin-eosin and Sirius red. Measurements were taken by photo-shooting hematoxylin-eosin preparations with Olympus BX 51 light microscope at 400x (objective 40x) and 1000x (objective 100x) magnifications. Photomicrographs were taken in representative areas.

The techniques of immunohistochemical preparations

Kidneys were removed, trimmed, and weighed and the relative weight of the organ was calculated. The relative weight of the organ (%) was calculated as gram/100 gram of body weight. Specimens from the kidney were fixated immediately in 10% buffered formalin for immunohistochemical testing of NF-kB.

Data analyze

A comparison of the test was conducted using the average difference test, namely the one-way ANOVA test. Before the test, the underlying assumption was the normality of the data the Kolmogorov-Smirnov test. If the data used does not meet any or all of the assumptions, a replacement test will be conducted, that is, the Kruskal Wallis test. If the results of the one-way ANOVA are significantly different, the Duncan test will be carried out, as well as the further test for the Kruskal Wallis test, that is, Mann-Whitney. If the notation of the results of the further test between the two treatments is different, then the two treatments are significantly different. Meanwhile, if the notation between the two treatments is the same, then the two treatments are not significantly different test between treatments.

Results and discussion

Dadiah, traditional food from West Sumatra, Indonesia has health benefits due to probiotics and peptides inhibiting NF-kB expression in rat kidney tissue modeled diabetic. Dadiah's clinical efficacy in lowering blood sugar and serum cholesterol indicates that it may be used as a future therapy to prevent diabetic progression.

The NF-kB expression with immunohistochemistry in the kidney

The expressions of NF-kB appeared brown on the IHC staining and the staining pattern was mainly in the form of cytoplasmic staining (**Figure 1**). The microscopic assessment used the Olympus BX51 light microscope at 400x magnification (40x objective) by assessing the positive intracytoplasmic brown staining on the representative area. Each sample was observed in 5 different fields of view. In each field of view (40x objective).

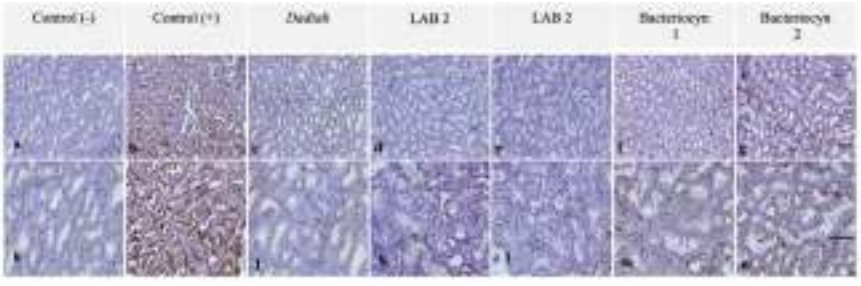

Figure 1 The NF-kB expression with immunohistochemistry in the kidney

The proportion of epithelial cells with positive intracytoplasmic brown staining was calculated, then compared to all epithelial cells per field of view. The staining intensity was reported in 4 intensity levels (negative, weak, moderate, and strong). The NF-kB immunohistochemical staining in the kidney of the animal model. The negative control group (a, h) and the positive control (b, i), the treatment with Dadiah (c, j), low-dosage lactic acid bacteria (d, k) and the high dosage (e, l), and low-dosage bacteriocin (f, m) and the high dosage (b, i). The NF-kB was intracytoplasmic expressed in a few tubular epithelial cells in the control animals with weak to moderate expressions, and some cells in the stroma and endothelium. The induction with alloxan showed an increase in the NF-kB expression with most of the tubular cells expressing moderate to strong. The treatment in the animal model showed a decrease in the NF-kB expression in the tissues compared to the positive control, both administered by Dadiah, lactic acid bacteria, and Bcteriocin. The NF-kB expression appeared to be lower in the treatment with Dadiah compared to other treatments. The immune peroxidase, using the low magnification with 10x objective lens (top) and the high magnification with 40x objective lens (bottom) at 200 µm scale.

NF-kB expression numbers in each group

NF-κB is a transcription factor that regulates the gene expression of several proinflammatory proteins. Based on Figure 2, the highest average of NF-kB expression in the C+ treatment (induced by alloxan + without any treatment) was 77.50 ± 8.80, and the lowest average of NF-kB expression was in the C– treatment (not induced by alloxan and not given a treatment), namely 20.83 ± 8.01. To prove whether there was a statistically significant difference in the average number of NF-kBexpressions, the Kruskal Wallis statistical analysis would be carried out. Based on the results of the Kruskal Wallis test, the p-value was smaller than (0.000 < 0.050), so it can be concluded that there was a significant difference in the average NF-kB expression number between treatments. It was observed that the positive control groups had significantly higher averages of NF-kB expression than the C-, P1, P2, P3, P4, and P5 groups.

Conversely, the hostile control groups (C-) had considerably lower average NF-kB expression than the positive control groups (C+), P2, and P3 but were not significantly different from the P1, P4, and P5 groups. NF-kB is a core nuclear transcription factor in the inflammatory response, increasing the expression of various cytokines and chemical substances involved in the formation and development of ND, In addition, a more recent study found that antioxidants inhibited the activity of NF-kB and decreased the production of particular pro-inflammatory mediators, especially the Tumor Necrosis Factor and Interleukin-6 (TNF and IL-6) [22, 23]. NF-kB is a ubiquitously distributed transcription factor that affects inflammation, apoptosis, adhesion, angiogenesis, and cycle cells. Inflammation is one of the key mechanisms responsible for the development and progression of ND. Many inflammation-related proteins are regulated by NF-kB [24]. Dadiah is known to contain probiotics and antioxidants, so it has been proven that Dadiah can reduce oxidative stress and inflammation.

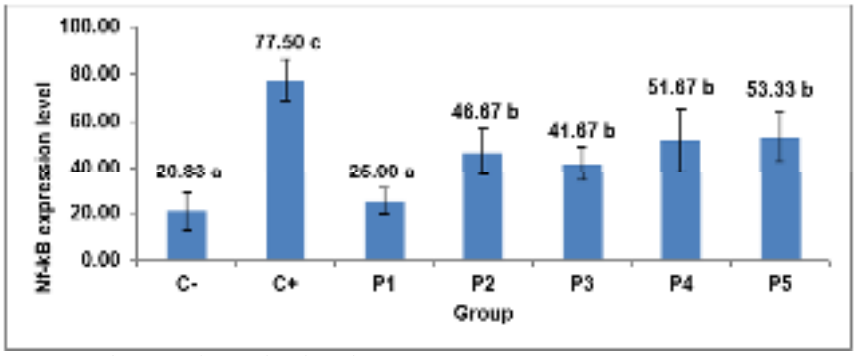

Figure 2 NF-kB expression numbers in each group

Even Dadiah itself contains a peptide that can stimulate endogenous antioxidants to inhibit the production of NF-kB. Administration of Dadiah, an isolateof lactic acid bacteria, and Bacteriocin has been shown to reduce macrophage activation in the production of proinflammatory cytokines. In addition, NF-kB expression shown in immunohistochemical examinationof kidney tissue decreases significantly close to the negative control [25].

Blood glucose levels

Blood sugar levels are an increase in glucose in the blood or an increase in serum glucose. Blood glucose levels in each treatment can be seen from the results of the research that has been carried out (**Figure 3**).

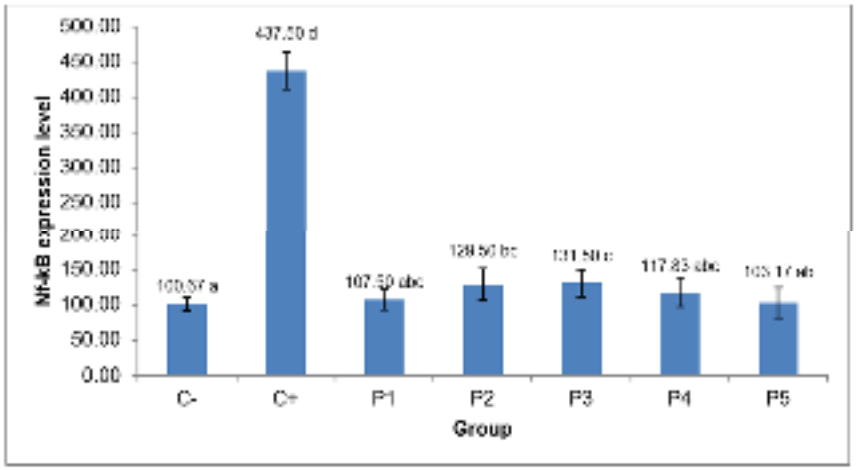

Figure 3 Blood glucose levels in each treatment

Based on **Figure 3**, it can be shown that the highest average blood glucose level in the C+ treatment (induced by alloxan + proteinuria) was 437.50 ± 26.70, and the lowest average blood glucose level was in the C– treatment (not induced by alloxan and not given any treatment), namely 100.67 ± 9.05. The highest average of blood glucose levels in the C+ treatment was significantly different from the C-, P1, P2, P3, P4, and P5 treatments. The lowest average of blood glucose levels in the C- treatment was significantly different from the C+, P2, and P3 treatments, but the C- treatment was not significantly different from the P1, P4, and P5 treatments. Hyperglycemia-induced oxidative stress has been linked to various diabetes complications, including ND. There is significant evidence that oxidative stress and the inflammatory response have a role in DN development. Sustained hyperglycemia induces oxidative stress and generates substantial reactive oxygen species (ROS) in renal tissues, activating the nuclear transcription factor NF-kB and resulting in kidney inflammation. Probiotic-based antidiabetic therapy has been proposed, and its influence on glycation is being explored. *L. fermentum* ME-3 may be used therapeutically to inhibit the formation/accumulation of certain glycation products in the kidneys and to ameliorate certain frequent disease-related complications [26].

Probiotic-fermented blueberry juice protects mice fed a high-fat diet from obesity and hyperglycemia by altering the gut flora. In addition, in HFD-fed mice, blueberry juices markedly improved hyperlipidemia and insulin resistance. Another study found the effect of Yogurt containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (LBST) on metabolic risk indicators is either beneficial or neutral. Increased blood pressure, increased blood glucose, abnormal blood lipids, subclinical inflammation (TNF and IL-6), overweight, and obesity are all metabolic indicators [27, 28]. Similarly, Probiotic Yogurt significantly lowered fasting blood glucose ($p = 0.01$) and HbA1c ($p = 0.05$) levels and boosted the activities of erythrocyte superoxide dismutase and glutathione peroxidase. Probiotic Yogurt made with *Lactobacillus acidophilus* and *Bifidobacterium lactis*. These data imply that probiotic Yogurt is a functional food with potential anti-diabetic and antioxidant effects [24]. Furthermore, other research investigated whether giving probiotics and selenium to GDM patients for six weeks improved their hyperglycemic status and lipid profiles [29].

Several studies demonstrate that treating diabetes patients with Voglibose (0.3 mg/kg) and probiotics (75 mg/kg) significantly decreased blood glucose and total cholesterol levels when compared to the diabetes group treated with only Voglibose (0.3 mg/kg). Similarly, research indicates that administering probiotic L sakei OK67 effectively prevents hyperglycemia development. the anti-diabetic effects of 14 probiotics in db/db mice resulted in improved intestinal barrier function and increased GLP-1 production, indicating that

these probiotics may be suitable for preventing and treating diabetes. Other studies have discovered that consuming probiotic Yogurt can help lower fasting blood glucose levels. These findings suggest that consuming probiotic Yogurt regularly may have a beneficial effect on treating metabolic syndrome [30]; [31, 32].

Serum cholesterol levels

The result of serum cholesterol levels showed that the C+ group has the highest average cholesterol of 166.42, while the P1 group as treated with Dadiah has the lowest average cholesterol of 116.24.66 (**Figure 4**). To prove a statistically significant difference in average cholesterol, a Kruskal Wallis statistical analysis will be performed. Based on the Kruskal Wallis test results, we obtained a p-value smaller than α (0.003 < 0.050), so it can be concluded that there is a significant difference in average cholesterol between treatments.

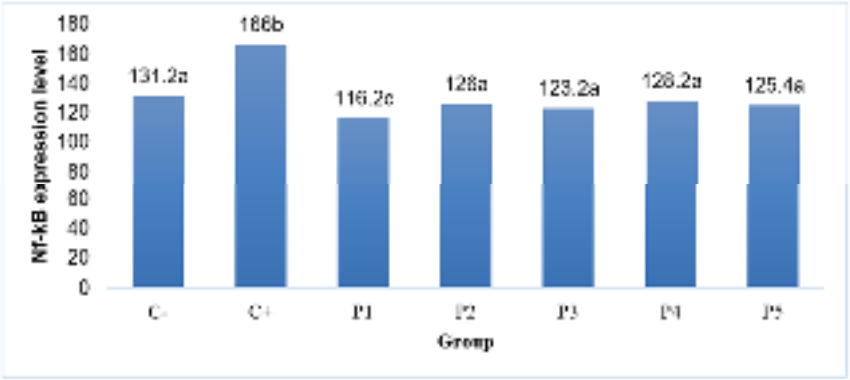

Figure 4. Serum cholesterol levels in each group

This study shows that the group of rats given the treatment of Dadiah can lower the cholesterol levels of mice-modeled diabetic nephropathy compared to other groups. Lactobacillus species are the most often utilized bacteria in probiotic treatments, and studies have shown that they can decrease cholesterol levels in humans. Consumption of probiotics may have a positive effect on managing cholesterol levels. The consumption of probiotic yogurt (300 g per day) containing *L. acidophilus* La5 (~4.14 × 106CFU/g) and *B. lactis* Bb12 (~3.61 × 106 CFU/g) for six weeks significantly improved the lipid profile of type 2 diabetes mellitus (T2D) patients. In addition, the results suggested that the regular consumption of probiotic yogurtcould improve the cholesterol level of T2D patients.

The study concluded that probiotic consumption amended the glycemic control, inflammatory system, and lipid profile in T2D subjects [33-35]. In vitro studies have also shown that L. acidophilus and B. lactis can lower cholesterol absorption. Similarly, in a study obtained, after four weeks ofintake of *L. fermentum* ME3 containing food supplement probiotics, all subjects' LDL cholesterol, total cholesterol, and ox-LDL levels reduced dramatically, while HDL cholesterol showed a potential to improve. The activity of the bile salt hydrolase (BSH) enzyme can be utilized to screen new probiotics for functional properties such as hypocholesterolemia activity and colonization potential [36-37]. According to a recent study, probiotics from fermented camel milk significantly improved blood glucose and lipid parameters and the morphological changes in the pancreas, liver, and kidney [38].

Conclusions

The use of Dadiah containing *L. fermentum* strains has been demonstrated to reduce inflammatory reactions associated with diabetic complications (DN). This study can be observed in the lower expression of NF-kB antibodies as proinflammatory biomarkers that rise with hyperglycemia. The outcomes of providing Dadiah alone against probiotics alone or LAB metabolites such as bacteriocin revealed the same improvement in inflammation, blood glucose, and cholesterol. However, the gift of Dadiah had the most significant impact on the control group. These results demonstrate that Dadiah with a comprehensive composition has a more substantial effect on biomolecular and clinical outcomes. For this reason, probiotics, and new strategies from Dadiah need to prevent and treat metabolic diseases and

prevent the progression of complications in DM.

Acknowledgments

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The Potential of West Sumatran *Dadiah* as The Novel to Alleviate Hyperglycemia, Hypercholesterolemia, and Reducing NF-kB Expression in Nephropathy Diabetes Rat Model

Highlights

Dadiah is a naturally fermented buffalo milk product in bamboo tubes. Dadiah (Probiotic) originating from West Sumatra, Indonesia acts as an antidiabetic. Dadiah and its metabolites significantly reduced hyperglycemia and serum cholesterol and inhibited oxidative stress by reducing NF-kB expressionin kidney tissue after treatment. Dadiah probiotics should be considered as a nutritional companion in diabetic nephropathy and as a future therapeutic target for DM patients to prevent the development of microvascular complications and hypercholesterolemia.

Abstract

Diabetic nephropathy (ND) is the most common microvascular complication in diabetes mellitus (DM) patients. The main mechanism for the development of ND is an inflammatory reaction as indicated by increased expression of NF-kB in kidney tissue due to chronic hyperglycemia and hypercholesterolemia. Hyperglycemia is related to changes in the composition of the microbiota which can cause dysbiosis. Thus, the therapeutic approach in DM sufferers using probiotics needs to be considered. Dadiah is a naturally fermented buffalo milk product in bamboo tubes. Dadiah comes from West Sumatra, Indonesia, there has not been much research on the clinical and biomolecular effects of various metabolic diseases. Even though this probiotic has health benefits, its mechanism as an antidiabetic is not widely known. This study aims to reduce blood sugar levels, cholesterol, and the inflammatory marker NF-kB. This research is an experimental animal study that aims to determine the effect of Dadiah and its probiotic metabolites on diabetic rats induced by intraperitoneal Alloxan 100 mg/kg b. w. ND rats were treated with low and high doses of Dadiah, LAB (Lactic Acid Bacteria), and Bacteriocin for eight weeks. Next, we tested their effect on blood glucose levels, serum cholesterol, and NF-kB antibody expression in kidney tissue using immunohistochemistry assays. The results demonstrated the potential of Dadiah and its metabolites to significantly reduce hyperglycemia and serum cholesterol and inhibit oxidative stress by reducing NF-kB expression in kidney tissue after treatment. Dadiah probiotics should be considered as a nutritional companion in diabetic nephropathy and as a future therapeutic target for DM patients to prevent the development of microvascular complications and hypercholesterolemia.

Keywords: Dadiah, hyperglycemia, hypercholesterolemia, NF-kB, diabetes neuropathy, probiotic

Introduction

Diabetes Mellitus (DM) is a cause of premature death, blindness, heart disease, and kidney failure for sufferers. According to the International Diabetes Federation (IDF), the number of people with Diabetes Mellitus in Indonesia is expected to continue to increase from 9.1 million people in 2014 to 14.1 million people in 2035. DM is a group of metabolic diseases that cause chronic hyperglycemia [1]. DM consists of 2 types, namely, type 1 DM (T1DM) as a result of an autoimmune reaction to pancreatic cell proteins, and type 2 DM (T2DM) as a result of a combination of genetic factors and environmental factors, such as obesity, overeating, lack of food, exercise, stress, and aging [2]. Generally, patients with T2DM experience complications, and cardiovascular complications that cause morbidity and mortality. T2DM patients experience impaired insulin secretion and/or action, causing hyperglycemia and hyperinsulinemia [3]. The increasing prevalence of T2DM is becoming a major cause of microvascular such as retinopathy and macrovascular complications such as peripheral vascular disease, and diabetic nephropathy [4-5].

Diabetic nephropathy (ND) is a condition of decreased kidney function and the main cause of end-stage kidney disease [6]. DN is triggered by genetic, environmental, cellular, and molecular mechanisms that play a role in kidney damage in diabetes [7]. DN is a clinical syndrome characterized by persistent albuminuria and progressive decline in kidney function. 50% of patients with DN will experience end-stage kidney disease (ESKD) requiring treatment with dialysis or kidney transplantation which is associated with

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significantly increased cardiovascular morbidity and mortality. The main risk factors for the development of DN are chronic hyperglycemia, hypercholesterolemia, and reduced expression of NF kappa B (NF-kB). Chronic hyperglycemia in DM sufferers is followed by damage, and impaired function of the eyes, kidneys, nerves, heart, and blood vessels. The diagnosis of diabetes mellitus is made based on the high level of glucose in the blood plasma [8-9]. Hypercholesterolemia can lead to atherosclerosis, coronary heart disease, pancreatitis, thyroid disorders, liver disease & disease [10]. NF-kB plays a role in the development and various complications of DM for sufferers, such as diabetic cardiomyopathy, retinopathy, nephropathy, and DM neuropathy. Many therapeutic approaches for DM sufferers have been developed, such as the use of several antioxidants, flavonoids, and probiotics. Probiotics are promising candidates for improving glycemic management, inflammatory systems, and lipid profiles in individuals with type 2 diabetes. Probiotic supplementation improves glycemic control and cardiometabolic risk markers. One possible mechanism for the hypocholesterolemia impact of probiotics has been suggested by direct cholesterol interaction or assimilation by probiotics [11]. The effectiveness and safety of probiotics for glycemic control in patients with impaired glucose control, including prediabetes and type 2 diabetes mellitus [12].

Many studies have used experimental models to evaluate the impact of supplementation with probiotics and prebiotics on various risk factors for metabolic syndrome [13]. The search for safer non-pharmacological therapies with cholesterol-lowering effects continues to be carried out by utilizing bacteria. Probiotic bacteria from the lactic acid group and Bifidobacterium can regulate serum cholesterol potential [14]. The results of the study showed that Gaio was able to lower cholesterol. Gaio is a yogurt product that utilizes the ability of *Enterococcus faecium* strains and *Streptococcus thermophilus* strains [15]. Decreased serum lipid concentrations with probiotic intake based on studies of various bacterial strains [16]. Probiotics are one of the most commonly used nutritional supplements around the world. Oneof the probiotics, Dadiah comes from West Sumatra, Indonesia, which is known as a traditional food.

Dadiah is a type of traditional fermented milk and has the potential to be developed as a functional food source of probiotics. Dadiah is made from buffalo milk, through a natural fermentation process involving lactic acid bacteria. Dadiah produced in West Sumatra, Indonesia is made from buffalo milk by relying on microbes that exist in nature as an inoculant or without a starter. The fermentation of the curd is carried out by microbes originating from bamboo, banana leaves, and milk [17]. Bamboo segments contain several microbes consisting of mold, yeast, lactic acid-forming microorganisms, protein breakers, and spore formers [18-19]. The use of antioxidants in DM cases needs to be considered to prevent the development of DM into diabetic nephropathy. Probiotics can enhance antioxidant absorption and antioxidant-related activity. Many studies have been conducted by local and national researchers regarding the nutritional components and their antimicrobial activity in Dadiah. However, only a few have studied clinically and scientifically confirmed its effects on various diseases, especially metabolic diseases. Although this probiotic has beneficial properties, its presumed anti-diabetic mechanism is unknown. This study aims to reduce blood sugar levels, cholesterol, and the inflammatory marker NF-kB.

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Materials and methods

Instruments of research

The instruments of research digital scale (ACS) with 0.01gram accuracy to weigh rats. Experimental animal cages, food and water containers for experimental animals, sonde to inject Dadiah and LAB isolated sampled from Dadiah 1 ml/day and 2 ml/day. The tools used in this research are a luminometer, pipettes, microscope, microtome, slide glass, razor blades/scissors, aluminum foil, metal basket, rotary tissue processor, refrigerator, water heater, processor cassette, autoclave (Hirayama), incubator (Fisher), hot plate, Eppendrof, bunsen, vortex, Erlenmeyer tubes, glucometer (Glucose blood level and cholesterol) and urine protein stick (UriScan).

Experimental animals

Wistar-strain male white rats (*Rattus norvegicus*) aged 2-3 months with a weight of ± 300-gram, standard feed as daily food, and Ad libitium drinking water. Dadiah. Lactic Acid Bacteria dan bacteriocin isolated sampled in Dadiah from Tanjung Bonai, Lintau Tanah Datar, West Sumatra. The examination results obtained information that Dadiah contained Lactic Acid Bacteria of 7,1 X 1010 CFU/g [20].

Preparation of Dadiah

Dadiah dosage for rats = conversion value x Dadiah dosage for humans. The dosage of administration, based on the recommended dosage of fermented milk in humans with a body weight of 70 kg, was 100-200 mL per day. The density (ρ) of Dadiah was 1.04 g/mL. The recommended Dadiah dosage is 104 g/70 kg of human. The Laurence table (2008), the conversion value of 70 kg of Human weight to 200 g of Rat weight is 0,018. The calculation of Dadiah dosage for the rat is Dadiah dosage for rat = conversion value x Dadiah dosage for humans. The recommended Dadiah dosage is 104/70 kg of human. Dadiah dosage for rat = conversion value x Dadiah dosage for human = 0,018 x 104 =1,87 g/200 g of rat weight. 1.87 g of Dadiah/200 g of Rat weight = 9.35 g/kg b. w. The weight of the male white rat (*Rattus norvegicus*) is ± 300 g = 0,3 kg Dadiah solution containing 1 g/mL was made by suspending Dadiah with aqua dest. The material in this experimental study is Dadiah 3 mL.

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Preparation of LAB

Isolate *L. fermentum* is rejuvenated first, then propagated in the medium MRS broth at a temperature of 370ºC for 24 hours, and calculated the number of bacterial cells is by diluting up to 108 CFU/mL. Dilution results are calculated on the MRS medium so that it is included at a temperature of 37ºC for 2 x 24 hours in the incubator to find out the number of LAB to be induced. Following previous *in vitro* research obtained for 1 g Dadiah, there is a LAB colony of 7.1 x 10^{10} CFU/mL.

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Preparation of Bacteriocin (Production of crude Bacteriocin)

The LAB of Dadiah was cultivated in MRS broth (1000 ml) seeded with 10% inoculum of overnight culture and incubated at 37ºC for 24 hours. Following incubation, the entire broth was centrifuged for 16 minutes at 10.000 X g find the cell-free supernatant was used as crude Bacteriocin [21]. The amount of LAB and Bacteriocin used in this study was 1 mL and 2 mL per day.

Methods

This research is an experimental study base on animal trials with a post-test-only control-group design. Male *Rattus norvegicus* strain wistar rats were procured from Pharmacology Department, Universitas Andalas, Padang, West Sumatra, Indonesia. The research samples have the criteria, healthy with glowing eyes, active and having a good appetite, 2-3 months old, and weigh 200-300 grams. All rats were maintained at 23-25ºC, with both a standard pellet diet and water ad libitum. After acclimatization for two weeks, except for the negative control group, all other groups were injected with alloxan 100 mg/Kg b. w. All groups of mice were fed with standard pellets. Furthermore, the treatment group will be given Dadiah,LAB, and Bacteriocin. The experiment was conducted with five treatment groups and two control groups. In this study, rats were divided into five groups with the number of each group of six rats. So that six DN rats were treated with Dadiah 3 mL/day (P1 group) and isolated samples of LAB and Bacteriocin from Dadiah 1 mL and 2 mL/day (P2-P5 group). Control groups were three DN rats without being treated (Positive control/C+) and three normal rats (control negative/C-) who did not have DN (without alloxan injection). The number of samples obtained by 42 rats.

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Induction of diabetes and *in vivo* experimental

Before the experiment began, all the rats were weighed, and measured blood glucose levels were cut off the rat's tail's 1 mm end. After that, the blood dropped on the glucose stick of the glucometer (OneTouch Merck; accuracy ISO 15197:2003) and the test of proteinuria by UriScan Test Strips (Biosys Laboratories, INC). After all the data have recorded, we had the first experiment that made rats into clinically marked ND for hyperglycemia (> 200 mg/dL) and proteinuria. In a preliminary study, rats kept on fasting for 12 hours received a single injection of freshly dissolved alloxan in 1.0 mL of sodium citrate buffer (0.1 M pH 4.5) intraperitoneally (i. p), at a rate of 100 mg/kg b. w. The blood was withdrawn from the tail vein of rats, then the measurement of fasting blood glucose concentration and cholesterol serum every two weeks along the experimental protocol (56 days/8 weeks). After 7 days of alloxan induction, animals with fasting glucose > 200 mg/dL and proteinuria were considered diabetic nephropathy and grouped accordingly with an average of 6 rats per group and orally administered with Dadiah, LAB, and Bacteriocin isolated from Dadiah for eight weeks or 56 consecutive days.

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The dissection of experimental animals

Dissection was performed after 56 days of treatment is given where male white rats (*Rattus norvegicus*) were killed using Anesthesia with ether. The method was by mixing the concentrated ether solution with 2% NaCl solvent or 10-25% in NaCl and a dose of 300 mg/kg or 1-1.25 g/kg placed on the bottom of the desiccator. Then put the rat in a closed container, wait until it became immobile, and its pupillary mydriasis and eyes were closed. If the rat lost consciousness, then brought ride inside the container, then laparotomy and neck pressure were done to kill it while pulling it anteriorly (dislocation Atlanta-occipitalis. Identification and nephrectomy were carried out, then directly put into a 10% BNF solution, after the kidney organ was removed.

Tissue processing

Rat renal tissue was processed into paraffin blocks and cut with a microtome with a thickness of 4 mm. The preparations were stained with hematoxylin-eosin and Sirius red. Measurements were taken by photo-shooting hematoxylin-eosin preparations with Olympus BX 51 light microscope at 400x (objective 40x) and 1000x (objective 100x) magnifications. Photomicrographs were taken in representative areas.

The techniques of immunohistochemical preparations

Kidneys were removed, trimmed, and weighed and the relative weight of the organ was calculated. The relative weight of the organ (%) was calculated as gram/100 gram of body weight. Specimens from the kidney were fixated immediately in 10% buffered formalin for immunohistochemical testing of NF-kB.

Data analyze

A comparison of the test was conducted using the average difference test, namely the one-way ANOVA test. Before the test, the underlying assumption was the normality of the data the Kolmogorov-Smirnov test. If the data used does not meet any or all of the assumptions, a replacement test will be conducted, that is, the Kruskal Wallis test. If the results of the one-way ANOVA are significantly different, the Duncan test will be carried out, as well as the further test for the Kruskal Wallis test, that is, Mann-Whitney. If the notation of the results of the further test between the two treatments is different, then the two treatments are significantly different. Meanwhile, if the notation between the two treatments is the same, then the two treatments are not significantly different test between treatments.

Results and discussion

Dadiah, traditional food from West Sumatra, Indonesia has health benefits due to probiotics and peptides inhibiting NF-kB expression in rat kidney tissue modeled diabetic. Dadiah's clinical efficacy in lowering blood sugar and serum cholesterol indicates that it may be used as a future therapy to prevent diabetic progression.

The NF-kB expression with immunohistochemistry in the kidney

The expressions of NF-kB appeared brown on the IHC staining and the staining pattern was mainly in the form of cytoplasmic staining (**Figure 1**). The microscopic assessment used the Olympus BX51 light microscope at 400x magnification (40x objective) by assessing the positive intracytoplasmic brown staining on the representative area. Each sample was observed in 5 different fields of view. In each field of view (40x objective).

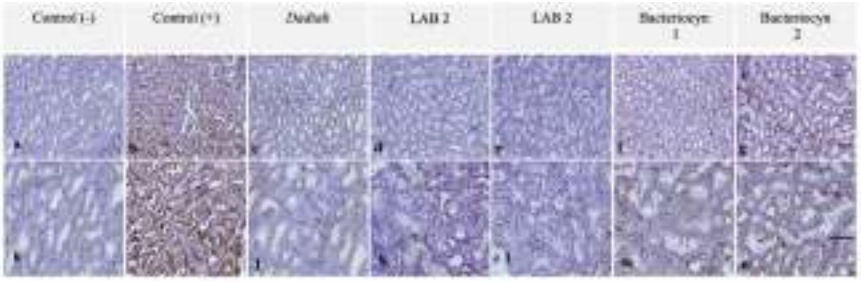

Figure 1 The NF-kB expression with immunohistochemistry in the kidney

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The proportion of epithelial cells with positive intracytoplasmic brown staining was calculated, then compared to all epithelial cells per field of view. The staining intensity was reported in 4 intensity levels (negative, weak, moderate, and strong). The NF-kB immunohistochemical staining in the kidney of the animal model. The negative control group (a, h) and the positive control (b, i), the treatment with Dadiah (c, j), low-dosage lactic acid bacteria (d, k) and the high dosage (e, l), and low-dosage bacteriocin (f, m) and the high dosage (b, i). The NF-kB was intracytoplasmic expressed in a few tubular epithelial cells in the control animals with weak to moderate expressions, and some cells in the stroma and endothelium. The induction with alloxan showed an increase in the NF-kB expression with most of the tubular cells expressing moderate to strong. The treatment in the animal model showed a decrease in the NF-kB expression in the tissues compared to the positive control, both administered by Dadiah, lactic acid bacteria, and Bcteriocin. The NF-kB expression appeared to be lower in the treatment with Dadiah compared to other treatments. The immune peroxidase, using the low magnification with 10x objective lens (top) and the high magnification with 40x objective lens (bottom) at 200 µm scale.

NF-kB expression numbers in each group

NF-κB is a transcription factor that regulates the gene expression of several proinflammatory proteins. Based on Figure 2, the highest average of NF-kB expression in the C+ treatment (induced by alloxan + without any treatment) was 77.50 ± 8.80, and the lowest average of NF-kB expression was in the C– treatment (not induced by alloxan and not given a treatment), namely 20.83 ± 8.01. To prove whether there was a statistically significant difference in the average number of NF-kBexpressions, the Kruskal Wallis statistical analysis would be carried out. Based on the results of the Kruskal Wallis test, the p-value was smaller than (0.000 < 0.050), so it can be concluded that there was a significant difference in the average NF-kB expression number between treatments. It was observed that the positive control groups had significantly higher averages of NF-kB expression than the C-, P1, P2, P3, P4, and P5 groups.

Conversely, the hostile control groups (C-) had considerably lower average NF-kB expression than the positive control groups (C+), P2, and P3 but were not significantly different from the P1, P4, and P5 groups. NF-kB is a core nuclear transcription factor in the inflammatory response, increasing the expression of various cytokines and chemical substances involved in the formation and development of ND, In addition, a more recent study found that antioxidants inhibited the activity of NF-kB and decreased the production of particular pro-inflammatory mediators, especially the Tumor Necrosis Factor and Interleukin-6 (TNF and IL-6) [22, 23]. NF-kB is a ubiquitously distributed transcription factor that affectsinflammation, apoptosis, adhesion, angiogenesis, and cycle cells. Inflammation is one of the key mechanisms responsible for the development and progression of ND. Many inflammation-related proteinsare regulated by NF-kB [24]. Dadiah is known to contain probiotics and antioxidants, so it has been proven that Dadiah can reduce oxidative stress and inflammation.

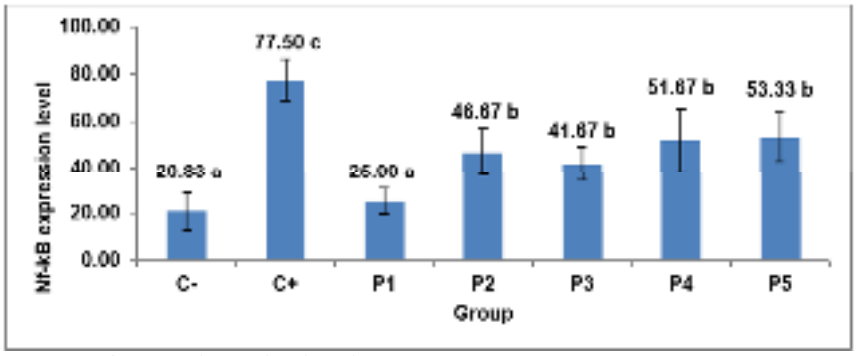

Figure 2 NF-kB expression numbers in each group

Even Dadiah itself contains a peptide that can stimulate endogenous antioxidants to inhibit the production of NF-kB. Administration of Dadiah, an isolateof lactic acid bacteria, and Bacteriocin has been shown to reduce macrophage activation in the production of proinflammatory cytokines. In addition, NF-kB expression shown in immunohistochemical examinationof kidney tissue decreases significantly close to the negative control [25].

Blood glucose levels

Blood sugar levels are an increase in glucose in the blood or an increase in serum glucose. Blood glucose levels in each treatment can be seen from the results of the research that has been carried out (**Figure 3**).

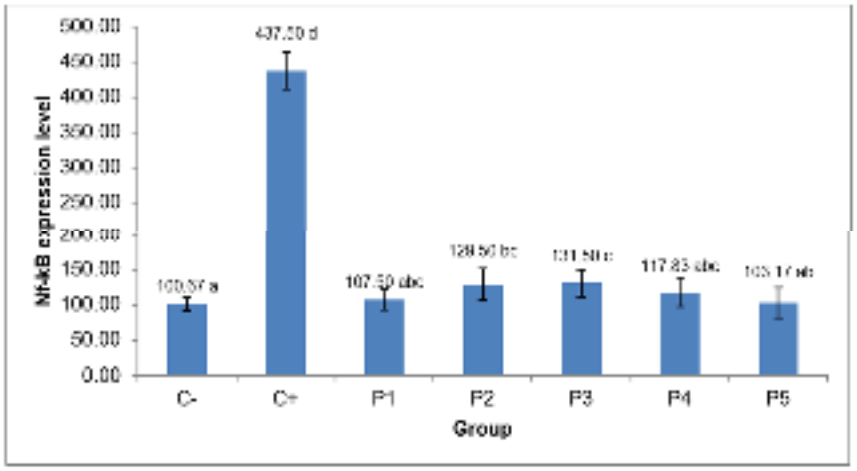

Figure 3 Blood glucose levels in each treatment

Based on **Figure 3**, it can be shown that the highest average blood glucose level in the C+ treatment (induced by alloxan + proteinuria) was 437.50 ± 26.70, and the lowest average blood glucose level was in the C– treatment (not induced by alloxan and not given any treatment), namely 100.67 ± 9.05. The highest average of blood glucose levels in the C+ treatment was significantly different from the C-, P1, P2, P3, P4, and P5 treatments. The lowest average of blood glucose levels in the C- treatment was significantly different from the C+, P2, and P3 treatments, but the C- treatment was not significantly different from the P1, P4, and P5 treatments. Hyperglycemia-induced oxidative stress has been linked to various diabetes complications, including ND. There is significant evidence that oxidative stress and the inflammatory response have a role in DN development. Sustained hyperglycemia induces oxidative stress and generates substantial reactive oxygen species (ROS) in renal tissues, activating the nuclear transcription factor NF-kB and resulting in kidney inflammation. Probiotic-based antidiabetic therapy has been proposed, and its influence on glycation is being explored. *L. fermentum* ME-3 may be used therapeutically to inhibit the formation/accumulation of certain glycation products in the kidneys and to ameliorate certain frequent disease-related complications [26].

Probiotic-fermented blueberry juice protects mice fed a high-fat diet from obesity and hyperglycemia by altering the gut flora. In addition, in HFD-fed mice, blueberry juices markedly improved hyperlipidemia and insulin resistance. Another study found the effect of Yogurt containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (LBST) on metabolic risk indicators is either beneficial or neutral. Increased blood pressure, increased blood glucose, abnormal blood lipids, subclinical inflammation (TNF and IL-6), overweight, and obesity are all metabolic indicators [27, 28]. Similarly, Probiotic Yogurt significantly lowered fasting blood glucose ($p = 0.01$) and HbA1c ($p = 0.05$) levels and boosted the activities of erythrocyte superoxide dismutase and glutathione peroxidase. Probiotic Yogurt made with *Lactobacillus acidophilus* and *Bifidobacterium lactis*. These data imply that probiotic Yogurt is a functional food with potential anti-diabetic and antioxidant effects [24]. Furthermore, other research investigated whether giving probiotics and selenium to GDM patients for six weeks improved their hyperglycemic status and lipid profiles [29].

Several studies demonstrate that treating diabetes patients with Voglibose (0.3 mg/kg) and probiotics (75 mg/kg) significantly decreased blood glucose and total cholesterol levels when compared to the diabetes group treated with only Voglibose (0.3 mg/kg). Similarly, research indicates that administering probiotic L sakei OK67 effectively prevents hyperglycemia development. the anti-diabetic effects of 14 probiotics in db/db mice resulted in improved intestinal barrier function and increased GLP-1 production, indicating that these probiotics may be suitable for preventing and treating diabetes. Other studies have discovered that

consuming probiotic Yogurt can help lower fasting blood glucose levels. These findings suggest that consuming probiotic Yogurt regularly may have a beneficial effect on treating metabolic syndrome [30]; [31, 32].

Serum cholesterol levels

The result of serum cholesterol levels showed that the C+ group has the highest average cholesterol of 166.42, while the P1 group as treated with Dadiah has the lowest average cholesterol of 116.24.66 (**Figure 4**). To prove a statistically significant difference in average cholesterol, a Kruskal Wallis statistical analysis will be performed. Based on the Kruskal Wallis test results, we obtained a p-value smaller than α (0.003 < 0.050), so it can be concluded that there is a significant difference in average cholesterol between treatments.

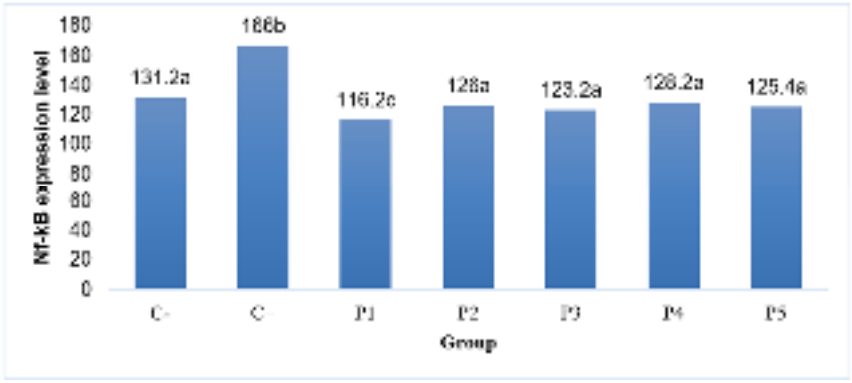

Figure 4. Serum cholesterol levels in each group

This study shows that the group of rats given the treatment of Dadiah can lower the cholesterol levels of mice-modeled diabetic nephropathy compared to other groups. Lactobacillus species are the most often utilized bacteria in probiotic treatments, and studies have shown that they can decrease cholesterol levels in humans. Consumption of probiotics may have a positive effect on managing cholesterol levels. The consumption of probiotic yogurt (300 g per day) containing *L. acidophilus* La5 (~4.14 × 106CFU/g) and *B. lactis* Bb12 (~3.61 × 106 CFU/g) for six weeks significantly improved the lipid profile of type 2 diabetes mellitus (T2D) patients. In addition, the results suggested that the regular consumption of probiotic yogurt could improve the cholesterol level of T2D patients.

The study concluded that probiotic consumption amended the glycemic control, inflammatory system, and lipid profile in T2D subjects [33-35]. In vitro studies have also shown that L. acidophilus and B. lactis can lower cholesterol absorption. Similarly, in a study obtained, after four weeks ofintake of *L. fermentum* ME3 containing food supplement probiotics, all subjects' LDL cholesterol, total cholesterol, and ox-LDL levels reduced dramatically, while HDL cholesterol showed a potential to improve. The activity of the bile salt hydrolase (BSH) enzyme can be utilized to screen new probiotics forfunctional properties such as hypocholesterolemia activity and colonization potential [36-37]. Accordingto a recent study, probiotics from fermented camel milk significantly improved blood glucose and lipid parameters and the morphological changes in the pancreas, liver, and kidney [38].

Conclusions

The use of Dadiah containing *L. fermentum* strains has been demonstrated to reduce inflammatory reactions associated with diabetic complications (DN). This study can be observed in the lower expression of NF-kB antibodies as proinflammatory biomarkers that rise with hyperglycemia. The outcomes of providing Dadiah alone against probiotics alone or LAB metabolites such as bacteriocin revealed the same improvement in inflammation, blood glucose, and cholesterol. However, the gift of Dadiah had the most significant impact on the control group. These results demonstrate that Dadiah with a comprehensive composition has a more substantial effect on biomolecular and clinical outcomes. For this reason, probiotics, and new strategies from Dadiah need to prevent and treat metabolic diseases and prevent the progression of complications in DM.

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The Potential of West Sumatran *Dadiah* as The Novel to Alleviate Hyperglycemia, Hypercholesterolemia, and Reducing NF-kB Expression in Nephropathy Diabetes Rat Model

Highlights

Dadiah is a naturally fermented buffalo milk product in bamboo tubes. Dadiah (Probiotic) originating from West Sumatra, Indonesia acts as an antidiabetic. Dadiah and its metabolites significantly reduced hyperglycemia and serum cholesterol and inhibited oxidative stress by reducing NF-kB expressionin kidney tissue after treatment. Dadiah probiotics should be considered as a nutritional companion in diabetic nephropathy and as a future therapeutic target for DM patients to prevent the development of microvascular complications and hypercholesterolemia.

Abstract

Diabetic nephropathy (ND) is the most common microvascular complication in diabetes mellitus (DM) patients. The main mechanism for the development of ND is an inflammatory reaction as indicated by increased expression of NF-kB in kidney tissue due to chronic hyperglycemia and hypercholesterolemia. Hyperglycemia is related to changes in the composition of the microbiota which can cause dysbiosis. Thus, the therapeutic approach in DM sufferers using probiotics needs to be considered. Dadiah is a naturally fermented buffalo milk product in bamboo tubes. Dadiah comes from West Sumatra, Indonesia, there has not been much research on the clinical and biomolecular effects of various metabolic diseases. Even though this probiotic has health benefits, its mechanism as an antidiabetic is not widely known. This study aims to reduce blood sugar levels, cholesterol, and the inflammatory marker NF-kB. This research is an experimental animal study that aims to determine the effect of Dadiah and its probiotic metabolites on diabetic rats induced by intraperitoneal Alloxan 100 mg/kg b. w. ND rats were treated with low and high doses of Dadiah, LAB (Lactic Acid Bacteria), and Bacteriocin for eight weeks. Next, we tested their effect on blood glucose levels, serum cholesterol, and NF-kB antibody expression in kidney tissue using immunohistochemistry assays. The results demonstrated the potential of Dadiah and its metabolites to significantly reduce hyperglycemia and serum cholesterol and inhibit oxidative stress by reducing NF-kB expression in kidney tissue after treatment. Dadiah probiotics should be considered as a nutritional companion in diabetic nephropathy and as a future therapeutic target for DM patients to prevent the development of microvascular complications and hypercholesterolemia.

Keywords: Dadiah, hyperglycemia, hypercholesterolemia, NF-kB, diabetes neuropathy, probiotic

Introduction

Diabetes Mellitus (DM) is a cause of premature death, blindness, heart disease, and kidney failure for sufferers. According to the International Diabetes Federation (IDF), the number of people with Diabetes Mellitus in Indonesia is expected to continue to increase from 9.1 million people in 2014 to 14.1 million people in 2035. DM is a group of metabolic diseases that cause chronic hyperglycemia [1]. DM consists of 2 types, namely, type 1 DM (T1DM) as a result of an autoimmune reaction to pancreatic cell proteins, and type 2 DM (T2DM) as a result of a combination of genetic factors and environmental factors, such as obesity, overeating, lack of food, exercise, stress, and aging [2]. Generally, patients with T2DM experience complications, and cardiovascular complications that cause morbidity and mortality. T2DM patients experience impaired insulin secretion and/or action, causing hyperglycemia and hyperinsulinemia [3]. The increasing prevalence of T2DM is becoming a major cause of microvascular such as retinopathy and macrovascular complications such as peripheral vascular disease, and diabetic nephropathy [4-5].

Diabetic nephropathy (ND) is a condition of decreased kidney function and the main cause of end-stage kidney disease [6]. DN is triggered by genetic, environmental, cellular, and molecular mechanisms that play a role in kidney damage in diabetes [7]. DN is a clinical syndrome characterized by persistent albuminuria and progressive decline in kidney function. 50% of patients with DN will experience end-stage kidney disease (ESKD) requiring treatment with dialysis or kidney transplantation which is associated with

significantly increased cardiovascular morbidity and mortality. The main risk factors for the development of DN are chronic hyperglycemia, hypercholesterolemia, and reduced expression of NF kappa B (NF-kB). Chronic hyperglycemia in DM sufferers is followed by damage, and impaired function of the eyes, kidneys, nerves, heart, and blood vessels. The diagnosis of diabetes mellitus is made based on the high level of glucose in the blood plasma [8-9]. Hypercholesterolemia can lead to atherosclerosis, coronary heart disease, pancreatitis, thyroid disorders, liver disease & disease [10]. NF-kB plays a role in the development and various complications of DM for sufferers, such as diabetic cardiomyopathy, retinopathy, nephropathy, and DM neuropathy. Many therapeutic approaches for DM sufferers have been developed, such as the use of several antioxidants, flavonoids, and probiotics. Probiotics are promising candidates for improving glycemic management, inflammatory systems, and lipid profiles in individuals with type 2 diabetes. Probiotic supplementation improves glycemic control and cardiometabolic risk markers. One possible mechanism for the hypocholesterolemia impact of probiotics has been suggested by direct cholesterol interaction or assimilation by probiotics [11]. The effectiveness and safety of probiotics for glycemic control in patients with impaired glucose control, including prediabetes and type 2 diabetes mellitus [12].

Many studies have used experimental models to evaluate the impact of supplementation with probiotics and prebiotics on various risk factors for metabolic syndrome [13]. The search for safer non-pharmacological therapies with cholesterol-lowering effects continues to be carried out by utilizing bacteria. Probiotic bacteria from the lactic acid group and Bifidobacterium can regulate serum cholesterol potential [14]. The results of the study showed that Gaio was able to lower cholesterol. Gaio is a yogurt product that utilizes the ability of *Enterococcus faecium* strains and *Streptococcus thermophilus* strains [15]. Decreased serum lipid concentrations with probiotic intake based on studies of various bacterial strains [16]. Probiotics are one of the most commonly used nutritional supplements around the world. Oneof the probiotics, Dadiah comes from West Sumatra, Indonesia, which is known as a traditional food.

Dadiah is a type of traditional fermented milk and has the potential to be developed as a functional food source of probiotics. Dadiah is made from buffalo milk, through a natural fermentation process involving lactic acid bacteria. Dadiah produced in West Sumatra, Indonesia is made from buffalo milk by relying on microbes that exist in nature as an inoculant or without a starter. The fermentation of the curd is carried out by microbes originating from bamboo, banana leaves, and milk [17]. Bamboo segments contain several microbes consisting of mold, yeast, lactic acid-forming microorganisms, protein breakers, and spore formers [18-19]. The use of antioxidants in DM cases needs to be considered to prevent the development of DM into diabetic nephropathy. Probiotics can enhance antioxidant absorption and antioxidant-related activity. Many studies have been conducted by local and national researchers regarding the nutritional components and their antimicrobial activity in Dadiah. However, only a few have studied clinically and scientifically confirmed its effects on various diseases, especially metabolic diseases. Although this probiotic has beneficial properties, its presumed anti-diabetic mechanism is unknown. This study aims to reduce blood sugar levels, cholesterol, and the inflammatory marker NF-kB.

Materials and methods

Instruments of research

The instruments of research digital scale (ACS) with 0.01gram accuracy to weigh rats. Experimental animal cages, food and water containers for experimental animals, sonde to inject Dadiah and LAB isolated sampled from Dadiah 1 ml/day and 2 ml/day. The tools used in this research are a luminometer, pipettes, microscope, microtome, slide glass, razor blades/scissors, aluminum foil, metal basket, rotary tissue processor, refrigerator, water heater, processor cassette, autoclave (Hirayama), incubator (Fisher), hot plate, Eppendrof, bunsen, vortex, Erlenmeyer tubes, glucometer (Glucose blood level and cholesterol) and urine protein stick (UriScan).

Experimental animals

Wistar-strain male white rats (*Rattus norvegicus*) aged 2-3 months with a weight of ± 300-gram, standard feed as daily food, and Ad libitium drinking water. Dadiah. Lactic Acid Bacteria dan bacteriocin isolated sampled in Dadiah from Tanjung Bonai, Lintau Tanah Datar, West Sumatra. The examination results obtained information that Dadiah contained Lactic Acid Bacteria of 7,1 X 1010 CFU/g [20].

Preparation of Dadiah

Dadiah dosage for rats = conversion value x Dadiah dosage for humans. The dosage of administration, based on the recommended dosage of fermented milk in humans with a body weight of 70 kg, was 100-200 mL per day. The density (ρ) of Dadiah was 1.04 g/mL. The recommended Dadiah dosage is 104 g/70 kg of human. The Laurence table (2008), the conversion value of 70 kg of Human weight to 200 g of Rat weight is 0,018. The calculation of Dadiah dosage for the rat is Dadiah dosage for rat = conversion value x Dadiah dosage for humans. The recommended Dadiah dosage is 104/70 kg of human. Dadiah dosage for rat = conversion value x Dadiah dosage for human = 0,018 x 104 =1,87 g/200 g of rat weight. 1.87 g of Dadiah/200 g of Rat weight = 9.35 g/kg b. w. The weight of the male white rat (*Rattus norvegicus*) is ± 300 g = 0,3 kg Dadiah solution containing 1 g/mL was made by suspending Dadiah with aqua dest. The material in this experimental study is Dadiah 3 mL.

Preparation of LAB

Isolate *L. fermentum* is rejuvenated first, then propagated in the medium MRS broth at a temperature of 370ºC for 24 hours, and calculated the number of bacterial cells is by diluting up to 108 CFU/mL. Dilution results are calculated on the MRS medium so that it is included at a temperature of 37ºC for 2 x 24 hours in the incubator to find out the number of LAB to be induced. Following previous *in vitro* research obtained for 1 g Dadiah, there is a LAB colony of 7.1 x 10^{10} CFU/mL.

Preparation of Bacteriocin (Production of crude Bacteriocin)

The LAB of Dadiah was cultivated in MRS broth (1000 ml) seeded with 10% inoculum of overnight culture and incubated at 37ºC for 24 hours. Following incubation, the entire broth was centrifuged for 16 minutes at 10.000 X g find the cell-free supernatant was used as crude Bacteriocin [21]. The amount of LAB and Bacteriocin used in this study was 1 mL and 2 mL per day.

Methods

This research is an experimental study base on animal trials with a post-test-only control-group design. Male *Rattus norvegicus* strain wistar rats were procured from Pharmacology Department, Universitas Andalas, Padang, West Sumatra, Indonesia. The research samples have the criteria, healthy with glowing eyes, active and having a good appetite, 2-3 months old, and weigh 200-300 grams. All rats were maintained at 23-25ºC, with both a standard pellet diet and water ad libitum. After acclimatization for two weeks, except for the negative control group, all other groups were injected with alloxan 100 mg/Kg b. w. All groups of mice were fed with standard pellets. Furthermore, the treatment group will be given Dadiah,LAB, and Bacteriocin. The experiment was conducted with five treatment groups and two control groups. In this study, rats were divided into five groups with the number of each group of six rats. So that six
DN rats were treated with Dadiah 3 mL/day (P1 group) and isolated samples of LAB and Bacteriocin from Dadiah 1 mL and 2 mL/day (P2-P5 group). Control groups were three DN rats without being treated (Positive control/C+) and three normal rats (control negative/C-) who did not have DN (without alloxan injection). The number of samples obtained by 42 rats.

Induction of diabetes and *in vivo* experimental

Before the experiment began, all the rats were weighed, and measured blood glucose levels were cut off the rat's tail's 1 mm end. After that, the blood dropped on the glucose stick of the glucometer (OneTouch Merck; accuracy ISO 15197:2003) and the test of proteinuria by UriScan Test Strips (Biosys Laboratories, INC). After all the data have recorded, we had the first experiment that made rats into clinically marked ND for hyperglycemia (> 200 mg/dL) and proteinuria. In a preliminary study, rats kept on fasting for 12 hours received a single injection of freshly dissolved alloxan in 1.0 mL of sodium citrate buffer (0.1 M pH 4.5) intraperitoneally (i. p), at a rate of 100 mg/kg b. w. The blood was withdrawn from the tail vein of rats, then the measurement of fasting blood glucose concentration and cholesterol serum every two weeks along the experimental protocol (56 days/8 weeks). After 7 days of alloxan induction, animals with fasting glucose > 200 mg/dL and proteinuria were considered diabetic nephropathy and grouped accordingly with an average of 6 rats per group and orally administered with Dadiah, LAB, and Bacteriocin isolated from Dadiah for eight weeks or 56 consecutive days.

The dissection of experimental animals

Dissection was performed after 56 days of treatment is given where male white rats (*Rattus norvegicus*) were killed using Anesthesia with ether. The method was by mixing the concentrated ether solution with 2% NaCl solvent or 10-25% in NaCl and a dose of 300 mg/kg or 1-1.25 g/kg placed on the bottom of the desiccator. Then put the rat in a closed container, wait until it became immobile, and its pupillary mydriasis and eyes were closed. If the rat lost consciousness, then brought ride inside the container, then laparotomy and neck pressure were done to kill it while pulling it anteriorly (dislocation Atlanta-occipitalis. Identification and nephrectomy were carried out, then directly put into a 10% BNF solution, after the kidney organ was removed.

Tissue processing

Rat renal tissue was processed into paraffin blocks and cut with a microtome with a thickness of 4 mm. The preparations were stained with hematoxylin-eosin and Sirius red. Measurements were taken by photo-shooting hematoxylin-eosin preparations with Olympus BX 51 light microscope at 400x (objective 40x) and 1000x (objective 100x) magnifications. Photomicrographs were taken in representative areas.

The techniques of immunohistochemical preparations

Kidneys were removed, trimmed, and weighed and the relative weight of the organ was calculated. The relative weight of the organ (%) was calculated as gram/100 gram of body weight. Specimens from the kidney were fixated immediately in 10% buffered formalin for immunohistochemical testing of NF-kB.

Data analyze

A comparison of the test was conducted using the average difference test, namely the one-way ANOVA test. Before the test, the underlying assumption was the normality of the data the Kolmogorov-Smirnov test. If the data used does not meet any or all of the assumptions, a replacement test will be conducted, that is, the Kruskal Wallis test. If the results of the one-way ANOVA are significantly different, the Duncan test will be carried out, as well as the further test for the Kruskal Wallis test, that is, Mann-Whitney. If the notation of the results of the further test between the two treatments is different, then the two treatments are significantly different. Meanwhile, if the notation between the two treatments is the same, then the two treatments are not significantly different test between treatments.

Results and discussion

Dadiah, traditional food from West Sumatra, Indonesia has health benefits due to probiotics and peptides inhibiting NF-kB expression in rat kidney tissue modeled diabetic. Dadiah's clinical efficacy in lowering blood sugar and serum cholesterol indicates that it may be used as a future therapy to prevent diabetic progression.

The NF-kB expression with immunohistochemistry in the kidney

The expressions of NF-kB appeared brown on the IHC staining and the staining pattern was mainly in the form of cytoplasmic staining (**Figure 1**). The microscopic assessment used the Olympus BX51 light microscope at 400x magnification (40x objective) by assessing the positive intracytoplasmic brown staining on the representative area. Each sample was observed in 5 different fields of view. In each field of view (40x objective).

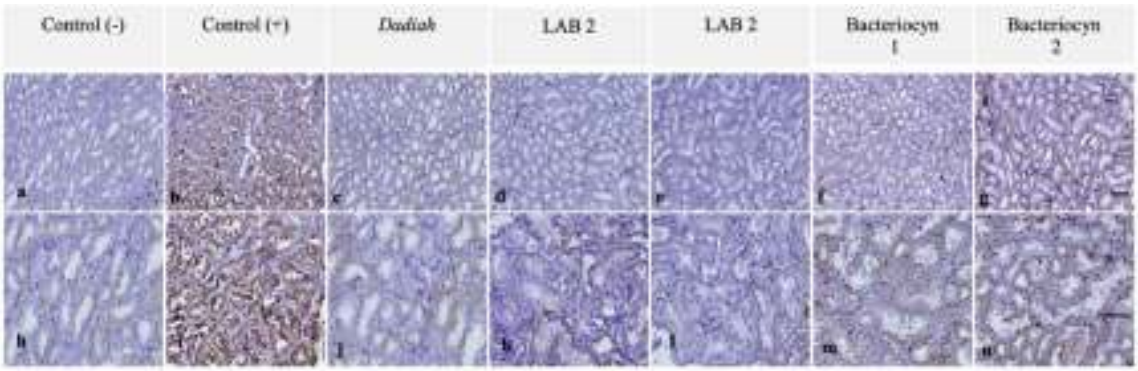

Figure 1 The NF-kB expression with immunohistochemistry in the kidney

The proportion of epithelial cells with positive intracytoplasmic brown staining was calculated, then compared to all epithelial cells per field of view. The staining intensity was reported in 4 intensity levels (negative, weak, moderate, and strong). The NF-kB immunohistochemical staining in the kidney of the animal model. The negative control group (a, h) and the positive control (b, i), the treatment with Dadiah (c, j), low-dosage lactic acid bacteria (d, k) and the high dosage (e, l), and low-dosage bacteriocin (f, m) and the high dosage (b, i). The NF-kB was intracytoplasmic expressed in a few tubular epithelial cells in the control animals with weak to moderate expressions, and some cells in the stroma and endothelium. The induction with alloxan showed an increase in the NF-kB expression with most of the tubular cells expressing moderate to strong. The treatment in the animal model showed a decrease in the NF-kB expression in the tissues compared to the positive control, both administered by Dadiah, lactic acid bacteria, and Bcteriocin. The NF-kB expression appeared to be lower in the treatment with Dadiah compared to other treatments. The immune peroxidase, using the low magnification with 10x objective lens (top) and the high magnification with 40x objective lens (bottom) at 200 µm scale.

NF-kB expression numbers in each group

NF-κB is a transcription factor that regulates the gene expression of several proinflammatory proteins. Based on Figure 2, the highest average of NF-kB expression in the C+ treatment (induced by alloxan + without any treatment) was 77.50 ± 8.80, and the lowest average of NF-kB expression was in the C– treatment (not induced by alloxan and not given a treatment), namely 20.83 ± 8.01. To prove whether there was a statistically significant difference in the average number of NF-kBexpressions, the Kruskal Wallis statistical analysis would be carried out. Based on the results of the Kruskal Wallis test, the p-value was smaller than (0.000 < 0.050), so it can be concluded that there was a significant difference in the average NF-kB expression number between treatments. It was observed that the positive control groups had significantly higher averages of NF-kB expression than the C-, P1, P2, P3, P4, and P5 groups.

Conversely, the hostile control groups (C-) had considerably lower average NF-kB expression than the positive control groups (C+), P2, and P3 but were not significantly different from the P1, P4, and P5 groups. NF-kB is a core nuclear transcription factor in the inflammatory response, increasing the expression of various cytokines and chemical substances involved in the formation and development of ND, In addition, a more recent study found that antioxidants inhibited the activity of NF-kB and decreased the production of particular pro-inflammatory mediators, especially the Tumor Necrosis Factor and Interleukin-6 (TNF and IL-6) [22, 23]. NF-kB is a ubiquitously distributed transcription factor that affectsinflammation, apoptosis, adhesion, angiogenesis, and cycle cells. Inflammation is one of the key mechanisms responsible for the development and progression of ND. Many inflammation-related proteinsare regulated by NF-kB [24]. Dadiah is known to contain probiotics and antioxidants, so it has been proven that Dadiah can reduce oxidative stress and inflammation.

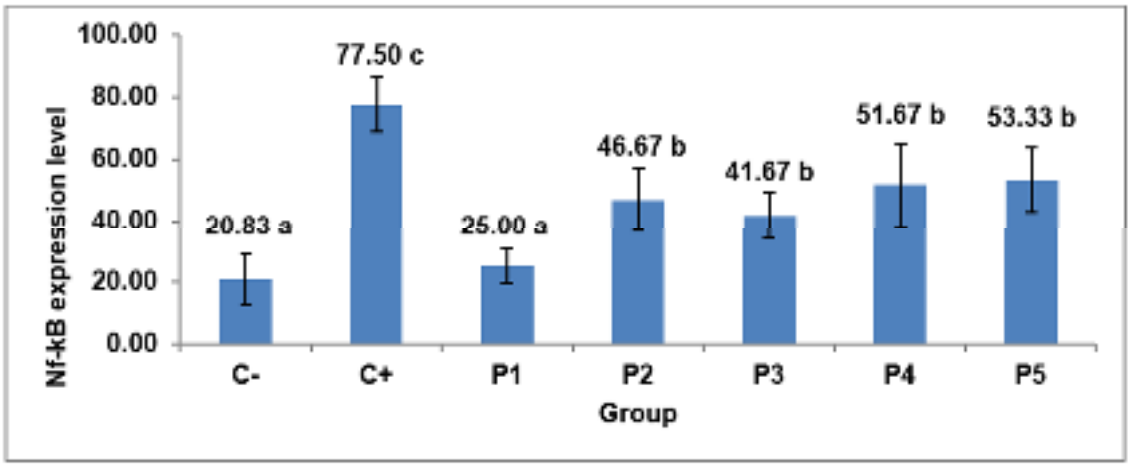

Figure 2 NF-kB expression numbers in each group

Even Dadiah itself contains a peptide that can stimulate endogenous antioxidants to inhibit the production of NF-kB. Administration of Dadiah, an isolateof lactic acid bacteria, and Bacteriocin has been shown to reduce macrophage activation in the production of proinflammatory cytokines. In addition, NF-kB expression shown in immunohistochemical examinationof kidney tissue decreases significantly close to the negative control [25].

Blood glucose levels

Blood sugar levels are an increase in glucose in the blood or an increase in serum glucose. Blood glucose levels in each treatment can be seen from the results of the research that has been carried out (**Figure 3**).

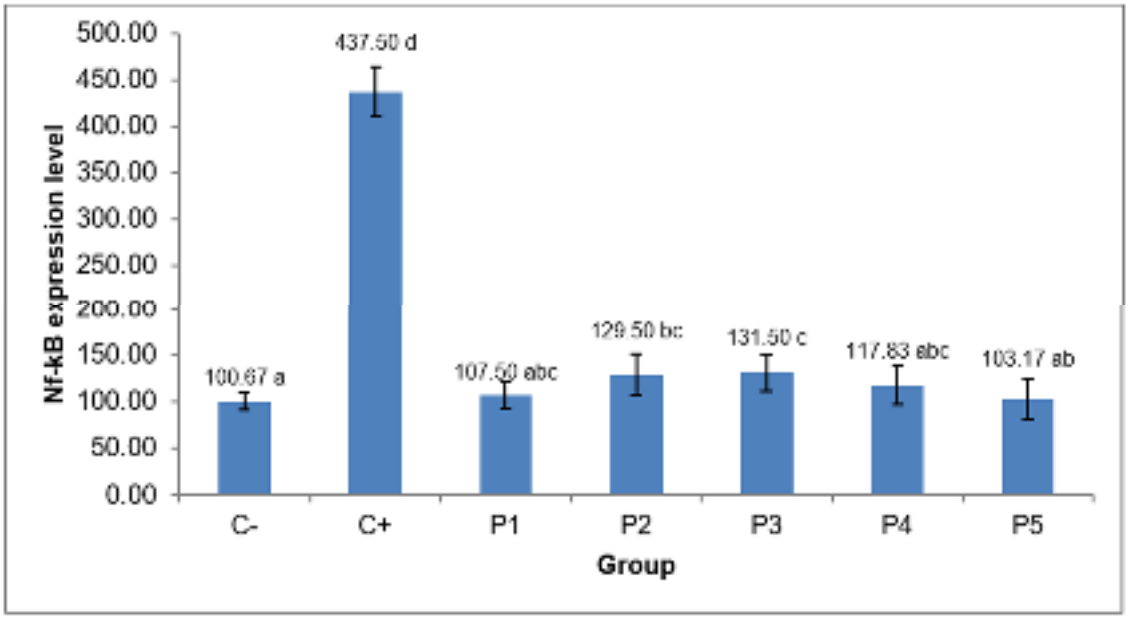

Figure 3 Blood glucose levels in each treatment

Based on **Figure 3**, it can be shown that the highest average blood glucose level in the C+ treatment (induced by alloxan + proteinuria) was 437.50 ± 26.70, and the lowest average blood glucose level was in the C– treatment (not induced by alloxan and not given any treatment), namely 100.67 ± 9.05. The highest average of blood glucose levels in the C+ treatment was significantly different from the C-, P1, P2, P3, P4, and P5 treatments. The lowest average of blood glucose levels in the C- treatment was significantly different from the C+, P2, and P3 treatments, but the C- treatment was not significantly different from the P1, P4, and P5 treatments. Hyperglycemia-induced oxidative stress has been linked to various diabetes complications, including ND. There is significant evidence that oxidative stress and the inflammatory response have a role in DN development. Sustained hyperglycemia induces oxidative stress and generates substantial reactive oxygen species (ROS) in renal tissues, activating the nuclear transcription factor NF-kB and resulting in kidney inflammation. Probiotic-based antidiabetic therapy has been proposed, and its influence on glycation is being explored. *L. fermentum* ME-3 may be used therapeutically to inhibit the formation/accumulation of certain glycation products in the kidneys and to ameliorate certain frequent disease-related complications [26].

Probiotic-fermented blueberry juice protects mice fed a high-fat diet from obesity and hyperglycemia by altering the gut flora. In addition, in HFD-fed mice, blueberry juices markedly improved hyperlipidemia and insulin resistance. Another study found the effect of Yogurt containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (LBST) on metabolic risk indicators is either beneficial or neutral. Increased blood pressure, increased blood glucose, abnormal blood lipids, subclinical inflammation (TNF and IL-6), overweight, and obesity are all metabolic indicators [27, 28]. Similarly, Probiotic Yogurt significantly lowered fasting blood glucose ($p = 0.01$) and HbA1c ($p = 0.05$) levels and boosted the activities of erythrocyte superoxide dismutase and glutathione peroxidase. Probiotic Yogurt made with *Lactobacillus acidophilus* and *Bifidobacterium lactis*. These data imply that probiotic Yogurt is a functional food with potential anti-diabetic and antioxidant effects [24]. Furthermore, other research investigated whether giving probiotics and selenium to GDM patients for six weeks improved their hyperglycemic status and lipid profiles [29].

Several studies demonstrate that treating diabetes patients with Voglibose (0.3 mg/kg) and probiotics (75 mg/kg) significantly decreased blood glucose and total cholesterol levels when compared to the diabetes group treated with only Voglibose (0.3 mg/kg). Similarly, research indicates that administering probiotic L sakei OK67 effectively prevents hyperglycemia development. the anti-diabetic effects of 14 probiotics in db/db mice resulted in improved intestinal barrier function and increased GLP-1 production, indicating that these probiotics may be suitable for preventing and treating diabetes. Other studies have discovered that

consuming probiotic Yogurt can help lower fasting blood glucose levels. These findings suggest that consuming probiotic Yogurt regularly may have a beneficial effect on treating metabolic syndrome [30]; [31, 32].

Serum cholesterol levels

The result of serum cholesterol levels showed that the C+ group has the highest average cholesterol of 166.42, while the P1 group as treated with Dadiah has the lowest average cholesterol of 116.24.66 (**Figure 4**). To prove a statistically significant difference in average cholesterol, a Kruskal Wallis statistical analysis will be performed. Based on the Kruskal Wallis test results, we obtained a p-value smaller than α (0.003 < 0.050), so it can be concluded that there is a significant difference in average cholesterol between treatments.

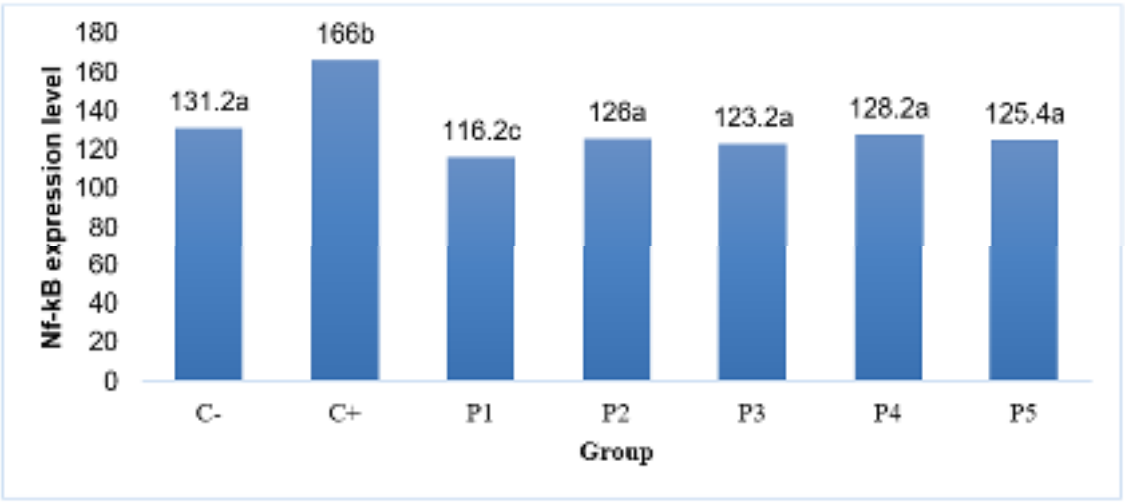

Figure 4. Serum cholesterol levels in each group

This study shows that the group of rats given the treatment of Dadiah can lower the cholesterol levels of mice-modeled diabetic nephropathy compared to other groups. Lactobacillus species are the most often utilized bacteria in probiotic treatments, and studies have shown that they can decrease cholesterol levels in humans. Consumption of probiotics may have a positive effect on managing cholesterol levels. The consumption of probiotic yogurt (300 g per day) containing *L. acidophilus* La5 (~4.14 × 106CFU/g) and *B. lactis* Bb12 (~3.61 × 106 CFU/g) for six weeks significantly improved the lipid profile of type 2 diabetes mellitus (T2D) patients. In addition, the results suggested that the regular consumption of probiotic yogurt could improve the cholesterol level of T2D patients.

The study concluded that probiotic consumption amended the glycemic control, inflammatory system, and lipid profile in T2D subjects [33-35]. In vitro studies have also shown that L. acidophilus and B. lactis can lower cholesterol absorption. Similarly, in a study obtained, after four weeks ofintake of *L. fermentum* ME3 containing food supplement probiotics, all subjects' LDL cholesterol, total cholesterol, and ox-LDL levels reduced dramatically, while HDL cholesterol showed a potential to improve. The activity of the bile salt hydrolase (BSH) enzyme can be utilized to screen new probiotics forfunctional properties such as hypocholesterolemia activity and colonization potential [36-37]. Accordingto a recent study, probiotics from fermented camel milk significantly improved blood glucose and lipid parameters and the morphological changes in the pancreas, liver, and kidney [38].

Conclusions

The use of Dadiah containing *L. fermentum* strains has been demonstrated to reduce inflammatory reactions associated with diabetic complications (DN). This study can be observed in the lower expression of NF-kB antibodies as proinflammatory biomarkers that rise with hyperglycemia. The outcomes of providing Dadiah alone against probiotics alone or LAB metabolites such as bacteriocin revealed the same improvement in inflammation, blood glucose, and cholesterol. However, the gift of Dadiah had the most significant impact on the control group. These results demonstrate that Dadiah with a comprehensive composition has a more substantial effect on biomolecular and clinical outcomes. For this reason, probiotics, and new strategies from Dadiah need to prevent and treat metabolic diseases and prevent the progression of complications in DM.

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