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We request that you send a revised manuscript **within 20 days**, otherwise it may be considered withdrawn.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

Editor of Trends in Sciences

Trends in Sciences

The Anti-inflammatory Activity of Probiotic *Dadiah* to Activate Sirtuin-1 in Inhibiting Diabetic Nephropathy Progression

Rinita Amelia[1,*], Faridah Mohd Said[2], Farzana Yasmin[2], Harnavi Harun[3] and Tofrizal[4]

[1]Medical Faculty, Baiturrahmah University Padang, West Sumatra, Indonesia.

[2]Lincoln University College, Petaling Jaya, Selangor, Malaysia.

[3]Internist Medicine Department of Andalas University, Padang, West Sumatra, Indonesia.

[4] Pathology Anatomy Department of Medical Faculty Andalas University, Padang, West Sumatra, Indonesia.

***Correspondence:** rinitaamelia@fk.unbrah.ac.id/rinitaamelia@gmail.com; Tel. +62 751 463 069

Abstract

Purpose: The activation of SIRT-1 in the kidney has become a new therapeutic target to increase resistance to many causal factors in DN development. Furthermore, antioxidative stress and anti-inflammation are essential to preventing renal fibrosis in DN. Therefore, finding "probiotic products" to treat and prevent DN is necessary. This study aimed to analyze the anti-inflammatory of probiotic *dadiah* to activate SIRT-1 in inhibiting DN progression.
Methods: This study is an experimental group designed with a post-test-only control group to observe the effect of *dadiah*, LAB, and bacteriocin on alloxan-induced nephropathy diabetic rats through two control groups and five intervention groups for eight weeks. The expression of antibodies SIRT-1 and TNF-α was examined using Immunohistochemistry and histopathology of kidney tissue. All data were analyzed using ANOVA test.
Results: The treatment of *dadiah*, lactic acid bacteria, and bacteriocin showed a higher expression of Sirtuin-1 than the positive control. They also, reduce TNF-α expression varies significantly between treatments. The highest average of interstitial fibrosis in the C+ groups was substantially different from all groups, but all treatments showed decreased kidney fibrosis. Although all treatments showed a decrease in interstitial kidney fibrosis found in the control group, the treatment using *dadiah* showed the highest result.
Conclusions: *Dadiah* has the potential to the prevention of fibrosis on kidney tissue of alloxan-induced nephropathy diabetic rats. The findings could be to develop novel treatments for DN that aim to reduce the cascade of oxidative stress and inflammatory signals in kidney tissue.

Keywords: *Dadiah*; Sirtuin-1; TNF-α; Diabetic Nephropathy

1. Introduction

Diabetes mellitus (DM) is one of the most significant health problems worldwide. According to the projections, the number of adult diabetic patients will exceed 430 million in 2030. Diabetic nephropathy (DN) is one of the most microvascular complications and is now the leading cause of end-stage renal disease (ESRD) [1-4].The prevalence of DM is increasing and is an essential cause of microvascular diseases such as DN [5]. DN is a serious microvascular complication of DM, and according to data in the United States, it is estimated to be suffered by 44% (30 - 40%) DM patients [3].

The main criteria to diagnose DN is the presence of an increased urinary albumin excretion (UAE), which is divided into microalbuminuria and macroalbuminuria, which is associated with an increased risk of decline in glomerular filtration rate (GFR) and a high risk of kidney failure [6]. Natural-history studies show the occurrence of proteinuria, eventually develops in 30-50% of diabetic persons [7,8]. Many pathways involving DN, such as hyperglycaemia, oxidative stress (OS), and protein kinase C (PKC) activation, have been postulated. As a significant mediator for DN development and progression, the upregulation of AGE receptors (RAGE) [9]. Renal fibrosis, characterized by extracellular matrix (ECM) protein accumulation, leads to CKD, including DN. It found that the process of signalling transformation of the growth factor (TGFB-1) plays a crucial role in mediating renal fibrosis. Signalling TGF-B1 antagonizing may be useful for the treatment of kidney disease [3].

Sirtuin-1 (SIRT-1) is a nicotine-amide adenine dinucleotide-dependent deacetylase. SIRT-1 is a crucial molecule in glucose, lipid, and energy metabolism. The renal protective effect of SIRT-1 is found in renal disorders with metabolic impairment, such as DN. Protective effects include the maintenance of glomerular barrier function, anti-fibrosis effects, anti-oxidative stress effects, and regulation of mitochondria function and energy

metabolism [10]. Oxidative stress is mainly due to the continuous production of free radicals, reactive oxidative stress (ROS), that imbalances with free radicals and antioxidant system production. It is negatively associated with cell viability, energy metabolism, aging, and metabolic and degenerative diseases. SIRT-1 is involved in several cellular functions, including chromosomal stability, cell cycle, apoptosis, DNA repair, metabolism, and aging by deacetylation of various transcription factors (NF-kB, P53, FOXO), histone and non-histone proteins [11].

SIRT-1 deficiency under stress conditions such as metabolic or oxidative stress is implicated in the pathophysiology of cardiovascular diseases, diabetes, neurodegenerative disorders, and renal disease. SIRT-1 may inhibit renal cell apoptosis, inflammation, and fibrosis in the kidneys. The activation of SIRT-1 in the kidney may be a new therapeutic target to increase resistance to many causal factors in developing renal diseases, including DN [12]. Since SIRT-1 is an essential metabolic sensor, its activity is regulated dynamically to allow for adaption and alteration to the cellular metabolic state. Nutritional, hormonal, and environmental signals, as well as the NAD+ level and SIRT-1 interacting proteins responding to those signals, compose the regulation network of SIRT-1. With a high-glucose and high-fat diet, SIRT-1 expression decreases, while during starvation and nutrient deprivation, SIRT-1 expression increases. During the stress response, SIRT-1 links chromatin dynamics/ gene expression to environmental stimuli [13]. SIRT-1 controls cellular transcription and metabolism, with a consequent crucial role in adaptation to oxidative, gen-toxic, or metabolic stresses [14].

Furthermore, scientific data indicates that the inflammatory factors tumor necrosis factor (TNF-α) and interleukin (IL)-6 are well reported to contribute to renal impairment in diabetes [15]. Probiotics appear to reduce inflammation and oxidative stress markers [16]. Additionally, Diabetes and obesity are both metabolism disorders associated with a low-grade inflammatory state. TNF-α may be a factor in the glomerular and interstitial tubule damage seen in diabetes [17]. According to a recent study, inhibiting TNF-α is a possible therapeutic method for experimental diabetic rats. These cytokines can be produced in diabetic kidneys by invading macrophage cells or by renal cells that are inherent to the kidney, like as endothelial cells, mesangial cells, glomerular cells, and tubular cells [18]. Therefore, antioxidative stress and anti-inflammation activity of some natural substances are essential approaches for preventing and treating renal fibrosis in DN.

Dadiah is considered a traditional food in the Minangkabau region, West Sumatra, Indonesia. Its benefits as a probiotic are supported by evidence regarding health and well-being. In addition, this *dadiah* is an important halal product for the Muslim population in the region. Therefore, biochemical and microbiological composition in *dadiah* is fundamental to learning to know the basic properties of health and disease prevention developments. *Dadiah Lintau* has been identified and has probiotic characteristics rich in lactic acid bacteria (LAB) with lactic acid bacterial composition 7.1 x 10^{10}. Based on molecular identification results using 16S rRNA methods and BLAST analysis, it has a similarity of 99.99% with *Lactobacillus fermentum* [19]. Another study has founded *L. plantarum* in *dadiah* sampled from Agam Bukittinggi West Sumatra [20].

Many studies are conducted by local and national researchers on the nutritional components and antimicrobial activity of dadiah. However, not many are clinically studied and scientifically proven their effects on various diseases. In addition, *dadiah* is also known to have characteristics of a probiotic with peptide components as antioxidants that can stimulate endogenous antioxidants in the host body [21]. Therefore, the use of antioxidants in the case of DM should be considered to prevent the development of DM into DN. Therefore, finding "probiotic products" to treat DN is necessary. This study aims to prove that *dadiah* has the potential as an activator of SIRT-1 to prevent the progressivity of DN through the repair of kidney tissue.

2. Materials and Methods

2.1. Research Design

This research consists of three continuing stages: *In vitro, in silico, and in vivo*. *In vivo* study is an experimental study base on animal trials with a post-test-only control-group design**.** This study has been approved by the Ethics Committee of Medical Faculty of Baiturrahmah University (No: 001/ETIK-FKUNBRAH/03/03/21).

2.1.1. Preparation of *Dadiah*

Dadiah's samples were taken from buffalo milk the village of Tanjung Bonai, Tanah Datar Regency, West Sumatra. Identification of specimen *dadiah* is carried out in the laboratory of animal husbandry biotechnology/Technology animal product. The *dadiah* was obtained from Lintau, West Sumatra. 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 [22]. The density (ρ) of *dadiah* was 1.04 g/mL, with the formula:

Density = mass (g) / volume (mL)

Mass = 1.04 g/mL x 100 mL = 104 g of *dadiah*

Thus, the recommended *dadiah* dosage: 104 - 208 g/70 kg of human.

From the Laurence table (2008), the conversion value of 70 kg of human weight to 200 g of rat weight is 0.018, thus the calculation of *dadiah* dosage for rat (1), (2), (3):

Dadiah dosage for rat = conversion value x *dadiah* dosage for human (1)

= 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

Dadiah dosage (g/mL) for treatment 1: K $= \frac{9.35\ \text{g/kg b. w x } 0.2\ \text{Kg}}{\text{mL}} = 0.935\ \text{g/mL}$ (2)

The weight of male white rat (*Rattus norvegicus*): ± 300 g = 0.3 Kg

Administered volume (mL) = 9.35 g/kg b.w x 0.3 Kg = 3 ml/ day (3)

0.935 g/m

Dadiah solution containing 1 g/mL was made by suspending *dadiah* with *aquadest*.

2.1.2. Preparation of Lactic Acid Bacteria (LAB)

Isolate *L. fermentum* is rejuvenated first, then propagated in the medium Mann Rugose Sharpe (MRS) broth at a temperature of 37°C for 24 hours and calculated the number of bacterial cells by diluting up to 10^8 CFU / ml. Dilution results are calculated on the MRS medium so that it is incluted at a temperature of 37°C for 2x24 hours in the incubator, to find out the number of LAB to be induced.

2.1.3. Preparation of a Cell-Free Supernatant (Bacteriocin)

The LAB of *dadiah* were cultivated in MRS broth (1000 ml) seeded with 10% inoculum of overnight culture and incubated at 37°C for 24 hour. Following incubation, the entire broth was centrifuged for 16 minutes at 10,000 X g for 16 minutes and the cell-free supernatant was used as crude bacteriocin [23].

2.2. In Vitro study

This research was conducted as a preliminary study to prove that *dadiah* has characteristics of a probiotic. The results obtained are macroscopic identification, microscopic identification, biochemical tests, acid and bile salt resistance assays, antimicrobial tests and identification LAB with 16S rRNA [19].

2.2.1. Macroscopic Identification

Media dilution that is used is de MRS broth. Results of dilution BAL done with spread method, at inoculation and stored in anaerobic jar after its incubation in incubator for 48 hours at a temperature of 37°C. Single colony that characterize BAL is round, smooth white yellowish colour were then transferred to de Mann ROGOSA Sharpe MRS media for purification of colony by streak method and incubated for 24 hours at a temperature of 37°C [24].

2.2.2. Microscopic Identification

Bacterial culture was taken in a Petri dish using an inoculation needle, then put into a glass preparation. Added drops of crystal violet. Wait for one minute, then rinsed with distilled water and dried, then drops of iodine was added, and wait 1 minute, Rinse with distilled water and dried, then dipped in ethanol for ± 20 minutes. One drop of safranin is added. Wait 30 seconds, rinse and dry and observe the shape of bacteria under the microscope [25].

2.2.3. Biochemical Properties

By adding LAB isolates into 5 ml of MRS BRC MERCK (Merck), the gas test was performed. Then, invert the Durham tube and incubate at 37 °C for 48 hours, observing for the presence or absence of air bubbles in the Durham tube. Next, the catalase test is performed by scraping the isolation to the glass preparation and dropping 3 percent (v/v) hydrogen peroxide (H_2O_2) on a microscope slide for the bacterial review [26].

2.2.4. Acid Resistance Test

1 mL bacterial culture was added to 9 mL MRS Broth media and incubated at 37°C for 24 hours. Then, up to 1 mL of bacterial culture was added to a reaction tube containing 9 mL MRS Broth without pH control (control) or MRS Broth pH 3 (pH regulated with HCl 5N) and incubated for 90 minutes. Finally, pH three and control cultures were diluted to 10-6 and spread onto MRS media for 48 hours at 37°C. The colony forming unit (CFU) determined the maximum number of bacteria that can survive. Cell viability has been selected by comparing their numbers before and after incubation [21].

2.2.5. Bile Salt Resistance Test

1 ml of bacterial culture was added to 9 ml MRS Broth medium and incubated at 37°C for 4 hours with ox gal settings of 0.5 percent (w/v). The culture was then diluted to 10^{-6} and inoculated on MRS media using the

spread method. It was then incubated for 48 hours at 37°C. The number of bacteria capable of survival was determined using the cup count method with the CFU [27].

2.2.6. Antimicrobial Test

The disk diffusion method was used to determine antimicrobial activity against *Escherichia coli* O157, *Listeria monocytogenes*, and *Staphylococcus aureus* ATCC 25923 microorganisms. A 1 mL LAB culture was placed in sterile Eppendorf tubes and of the LAB supernatant [20].

2.2.7. Identification LAB by 16S rRNA [28].

2.3. In Silico Study

The in silico method is used in the bioinformatics test to determine the number of things; The probiotic *L. fermentum* produces other metabolites and substances; Discovering the pathways associated with the target protein being studied in a laboratory that influence the development of DN; Determining which protein interactions have the most impact on biological processes that occur in DN. The test were Analysis of pathways with KEGG on STRING, Prediction of Target Proteins with SEA, Interaction Proteins with DB STRING and Analysis of Metabolite compounds using WAY2Drug PASS server.

2.3.1. Prediction of Target Proteins with SEA

The similarity ensemble approach search tool (SEArch), one of the most widely used drug discovery tool based on the similarity of binding ligands for *in silico* target prediction, was used to predict target proteins for each compound from *Lactobacillus fermentum*'s metabolite and other compounds. The SMILE string of the listed compounds was inputted into SEArch (http://sea.bkslab.org/search/). The selected results of target proteins were associated to DN. The potential hit was evaluated by E-values and **Tanimoto coefficient** (Tc). The suggested threshold of E-values and Max Tc was 10^{-4} and 0.57. The result of E values greater than the limit was not considered into the study, as they did not indicate great statistical significance [29,30].

2.3.2. Interaction Proteins with DB STRING

STRING DB (https://string-db.org/) was used to predict protein-protein interaction. Protein-protein interaction was used to understand physiology and to determine their efficacy. Homo sapiens database and high confidence score 0.7 used as minimum required interaction score. False Discovery Rate usedto describes how significant the enrichment is. Shown are p-values corrected for multiple testing within each category using the Benjamín-Hochberg procedure. Smallest P-value are the most significant [31].

2.3.3. Analysis of Metabolite compounds using WAY2Drug PASS server

Secondary metabolites in LAB literature study results analyzed its potential using WAY2DRUG PASS prediction. (http://www. pharmaexpert. ru/ passonline/predict.php) as diabetic treatment. Previously, each compound needed to be searched smile structure (simplified molecular-input line-entry system) obtained from pub-chem database (https://pubchem.ncbi.nlm.nih.gov/). Then the compound analyzed its potential using WAY2DRUG PASS prediction to find out its potential in DN. The Pa (Probability to be Active) value describes the potential of the compound being test. Determination of value is comparing the structure of compounds with compounds that have proved as a specific treatment. Potential Analysis has done using Way2Drug Pass Server. The Pa value is 0.7 more indicates that the compound is predicted to have a high potential as anti-diabetic. The high similarity with the compounds in the database has been proven as such treatments. Whereas the value of Pa is more than 0.3 but less than 0.7, then the compound computationally has low similarity to the compound that has been proven as the treatment [32].

2.4. In Vivo Study

2.4.1. The Nurture of Experimental Animal

The Wistar-strain male white rats (*Rattus norvegicus*) were first adapted for 2 weeks before being treated. Rats were placed in a cage with husk mat to absorb dirt. The cage was placed in a sufficiently ventilated room at a temperature of 20-26°C. The cage was cleaned every day. 30-40 g of standard foods was given each day for each animal and the drink was added with *ad libitium.*

2.4.2. The Treatment Phase

Before the experiment, we measured all rats' blood glucose levels by cutting 1 mm off their tails. Blood was then dropped on a glucometer (OneTouch Merck; accuracy ISO 15197:2003) and UriScan Test Strips for proteinuria (Biosys Laboratories, INC). After collecting all the data, we performed the first experiment that caused hyperglycemia (>200 mg/dL) and proteinuria in rats. Experimental rats were made diabetic by injecting i.p (intraperitoneal) alloxan 100 mg/kg [33].

A preliminary study found that Alloxan's dosage could cause DN in rats eight days after injection. On the eighth day, mice were injected with Alloxan to check blood sugar and urine protein levels with UriScan. Trial

mice with blood glucose levels above 200 mg/dl were randomly grouped in this study. Six groups of diabetic rats were created. One group received only aquadest (Control Positive), while the other received *dadiah* 3 gr a day in aqua solution (P1) and LAB 1 ml and 2 ml once a day for P2 and P3 groups. In addition, P4 and P5 received 1 and 2 ml bacteriocin. Thus, on day 8 of treatment, P1-P5 mice will receive it. Control groups (C- and C+) were given water and food ad libitum. Eight weeks of *dadiah*, LAB, and Bacteriocin were administered. Dissection was performed after 8 weeks of treatment is given.

2.4.3. Animal Termination Phase

Male white rats (*Ratus norvegicus*) were sacrificed by means of Anasthesia 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. Identification and nephrectomy were carried out, then directly put into a 10% BNF solution. After the kidney organ was removed, neck pressure was done to kill it while pulling it anteriorly (*dislocasio atlanto-occipitalis*) [34]. Examine dependent variables based on immunohistochemistry and histopathological profiles.

2.4.4. Laboratory Examination phase

2.4.4.1. 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 haematoxylin-eosin and sirius red. Measurements were taken by photo-shooting haematoxylin-eosin preparations with Olympus BX 51 light microscope at 400x (objective 40x) and 1000x (objective 100x) magnifications. Photomicrographs were taken in representative areas.

The paraffin block was cut with a 4µm-thick rotary microtome, then placed on a slide. Deparaffinized it with Xylene for 5 minutes, twice (2 x 5 minutes). Rehydrated it with graded alcohol, started by 100%, 96%, and 70% ethanol, then distilled water, 5 minutes for each. The preparation was stained with hematoxylin for 8 minutes. Rinsed it with aquadest for 10 minutes. Dehydrated it with 70% alcohol for 5 minutes. Then with 96% alcohol for 5 minutes. Next, immersed the preparation in Eosin Y solution for 2 minutes. Rinsed it in 96% ethanol for 5 minutes. then, with 100% Ethanol for 5 minutes. Cleared it in Xylene for 5 minutes, twice. Mounted the deck glass with entellant.

2.4.4.2. Hematoxillin-Eosin Procedure

The paraffin block was cut with a 4µm-thick rotary microtome, then placed on a slide. Deparaffinized it with Xylene for 5 minutes, twice (2 x 5 minutes). Rehydrated it with graded alcohol, started by 100%, 96%, and 70% ethanol, then distilled water, 5 minutes for each. The preparation was stained with hematoxylin for 8 minutes. Rinsed it with aquadest for 10 minutes. Dehydrated it with 70% alcohol for 5 minutes. Then with 96% alcohol for 5 minutes. Next, immersed the preparation in Eosin Y solution for 2 minutes. Rinsed it in 96% ethanol for 5 minutes. then, with 100% Ethanol for 5 minutes. Cleared it in Xylene for 5 minutes, twice. Mounted the deck glass with entellant. (ScyTek Laboratories Procedure)

2.4.4.3. Immunohistochemistry Procedure (IHC)

The paraffin block was cut with a rotary microtome with a 4µm-thick rotary microtome, then placed on a glass coated with Poly-L-lysine. Deparaffinized it with Xylene, then rehydrated it with graded alcohol, started by 100%, 96%, and 70% ethanol, then distilled water, 5 minutes for each. The heat induced retrieval epitope used a microwave for 10 minutes inside Citrate buffer at pH 6. Washed it in phosphate buffer saline (PBS) at pH 7.4 for 5 minutes, three times. The endogenous peroxidase blocking was with 3% H_20_2 in PBS at pH 7.4 for 3 minutes, followed by 0.3% H_20_2 in PBS at pH 7.4 for 30 minutes. Washed it in PBS at pH 7.4 for 5 minutes, three times. The non-specific protein block was with 2% NGS (Normal Goat serum) in PBS at pH 7.4 for 20 minutes, at room temperature. Applied the primary antibody and incubated it in a 4°C humid chamber over-night. TNF-α dilution 1; 100, and Anti SIRT-1 antibody (EPR 18239) ab 189494, ABCAM; dilution 1; 50. Washed it in PBS at pH 7.4 for 5 minutes, three times. Incubated it with secondary antibodies at room tempera-ture for 30 minutes. Washed it in PBS at pH 7.4 for 5 minutes, three times. Incubated it with the avidin biotin complex at room temperature for 30 minutes (ScyTek Laboratories Procedure)

2.4.4.4. Assessing Fibrocollagen Matrix Deposition in Sirius Red Staining

The collagen matrix was stained red on the Sirius red staining. The area measurement was done by taking a photomicrograph at 400x magnification (40x objective) in 5 different fields. The red-stained area was measured using the ImageJ program (ImageJ v1.49 software, National Institute of Health, Bethesda, MD, USA) by isolating the red-stained area on the Sirius red staining, and then calculating the colored area proportion to the field of view area; the positive-colored area was reported in percentage (Kiernan JA. Sirius Red Staining Protocol for Collagen. MedEmoryEdu).

2.4.4.5. Assessing the Expressions of and SIRT-1 and TNF on the IHC Staining

The expressions of SIRT-1 and TNF-α appeared brown on the IHC staining. The staining pattern was mainly in the form of cytoplasmic staining. The SIRT-1 and TNF-α expression was calculated cell positive in percentage with ImageJ based on quantitative assessment methods. It has been shown using the Olympus BX51 light microscope at 400x magnification (40x objective). The area has been evaluated for intracytoplasmic brown staining. Rats tissue was observed from five different fields of view. In each field of view, the staining intensity was reported in 4 levels (negative, weak, moderate, and strong) (ABCAM Procedure Antibody Kit SIRT-1 and TNF-α)

2.5. Data Analyze

Comparison The test was conducted using the average difference test, namely the one-way ANOVA test (for more than 2 treatment groups). 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 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.

3. Results

The results of the normality test showed that each significance value of the variable fibril-collagen matrix deposition with Sirius red (interstitial fibrosis) was greater than 0.05, then a decision will be to accept H0, which means the data was normally distributed. The normally distributed data will be continued with the one-way ANOVA analysis. However, data for the variable Mn-SOD expression and SIRT-1 expression, were not normally distributed with each significance value of less than 0.05. Data that are not normally distributed were continued with Kruskal Wallis analysis.

The normally distributed data will be continued with the one-way ANOVA analysis. However, data for the variable SIRT-1 expression and TNF-α expression were not normally distributed with each significance value of less than 0.05. Data that are not normally distributed were continued with Kruskal Wallis analysis. The results of normality test can be seen in Table 1.

Table 1. The normality test

Variable	Statistic	Significance
TNF-α Expression	0.352	0.000
Sirtuin-1 Expression	0.169	0.004
Matrix deposition fibril-collagen with Sirius-red (Glomerular-sclerosis)	0.131	0.068*

3.1. In Vitro Study

This research was conducted as a preliminary study to prove that *dadiah* has characteristics of a probiotic. The results obtained are Macroscopic identification found Colony of LAB: white beige, round shape, size 1,8 mm, surface smooth and convex, total LAB count 7.1 x 10^{10} CFU/g. Gram staining revealed that LAB from *dadiah* contained rod-shaped and gram-positive bacteria. Biochemical test of *dadiah* were negative catalase, and homofermentative. Percentage acid resistance viability 57.1% and bile salt resistance viability 66.7%. *E. coli* possessed had the largest inhibition zone (23.28 mm), the inhibitory activity of *dadiah* LAB against *E. coli* is classified as very strong. The PCR results and BLAST analysis, the isolated bacteria from *dadiah* had 99.99% similarity with *L. fermentum* [19].

3.2. In Silico Study

The results study of *in vitro* above, identification of isolated LAB from *dadiah* using 16S rRNA, had 99.99% similarity with *L. fermentum*. Furthermore, the researcher conducted bioinformatics studies as the base on experimental test in the next stage. *L fermentum* is a species of lactic acid-producing bacteria and evidenced by many literature studies that show that these bacteria also have a variety of other metabolite compounds. *L fermentum* is a species of lactic acid-producing bacteria and evidenced by many literature studies that show that these bacteria also have a variety of other metabolite compounds such as; Glutathione (Keiser et al., 2007), Riboflavin (Thakur & Tomar, 2016), Vitamin K2 (menaquinone) by Lim et al., 2011, and according Hati et al., 2019 it has several compounds such as acetic acid, B9, B12 and butyric acid. The others study showed L fermentum also containing ferulic acid (Westfall & Lomis, 2016), Propionic acid,

Caproic acid, valerate, iso-butyrate, iso-and valerate (Pereira et al., 2003). Exopolysaccharide also finding in L fermentum (Santiago-López et al., 2018), and several compounds such as ethyl-pentadecanoate, linoleic acid and vaccenic acid (Yoon et al., 2020) and Matsuguchi et al., 2003.

3.2.1. Pathway DN Based on KEGG

Pathway analysis on diabetes complications with target proteins related to DN found three major pathways, namely AGE-RAGE signalling, FOXO signalling, and longevity regulating pathway.

3.2.2. Protein-Ligan Network Analysis

Based on the protein-protein interaction (PPI) approach, search target proteins are involved in the mechanism of diabetes nephropathy AGE-RAGE signalling pathway (NFKB1, TGF, TNF), FOXO signalling pathway (EP300, SOD, SIRT), and longevity signalling pathway (NFKB1, SIRT, SOD).

The resulting potential protein-ligand network in this study showed ferulic acid, caproic acid, linoleic acid, and vaccenic acid suggested metabolite compound in *L. fermentum* were selected results of target proteins associated with DN pathways. The potential hit was evaluated by E-values and Tanimoto coefficient (Tc). The suggested threshold of E-values and Max-Tc was 10^{-4} and 0.57, respectively [35]. The result of E-values greater than the limit was not considered into the study, as they did not indicate great statistical significance [36].

In Fig. 1, several target proteins have a high score of PPI String, which is related to the DN pathway of the metabolite compound *L. fermentum*. Target proteins directly related to DN pathways are described as being in outer circles such as NF-κB, JUN, EP 300, PPARA, F3, MMP2, MMP9, NFE2L2, TLR2, TLR4 and PPARG. While based on the highest score of protein interaction (PPI Score), proteins closely related to the target protein that can be studied through laboratory studies are TNF-α and SIRT-1 (the inner circle). This protein computationally has high confidence if conducted in vivo and in vitro testing with results that have affected the occurrence of diabetes complications (DN) through the pathways set in KEGG. Below is described the biological activity of target proteins in DN with a significant p-value rate (Benjamin-Hochberg). Some studies showed that SIRT-1 has a reno-protective impact on ND through deacetylation of transcription factors involved in renal disease pathogenesis. Recently, it has been found that specific overexpression of SIRT-1 by podocyte cells may decrease proteinuria and kidney injury in experimental mice with ND [37]. SIRT-1 is involved in several cellular functions, including chromosomal stability, cell cycle, apoptosis, DNA repair, metabolism, and aging by deacetylation of various transcription factors (NF-kB, P53, FOXO) histone and non-histone proteins [11]. This target protein is associated with metabolite compounds through its various biological activities, shown by the color shown in the picture and table above.

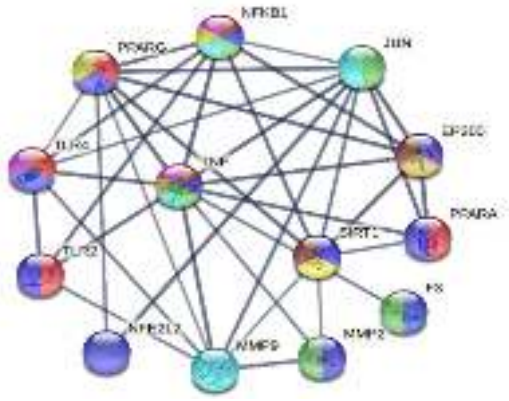

Fig. 1 High score PPI

In Table 2, the target protein is seen with pathways that play a role in DN. The lowest yield (p-value 0.00000000292) is the most significant seen in the path of "Regulation of response to stress" with the target protein, namely, NFKB1, EP300, PPARA, F3, MMP2, NFE2L2, TLR2, TLR4, PPARG, TNF, and SIRT-1 (blue coloring). Each target protein can have some biological activity, as seen in Fig. 2.

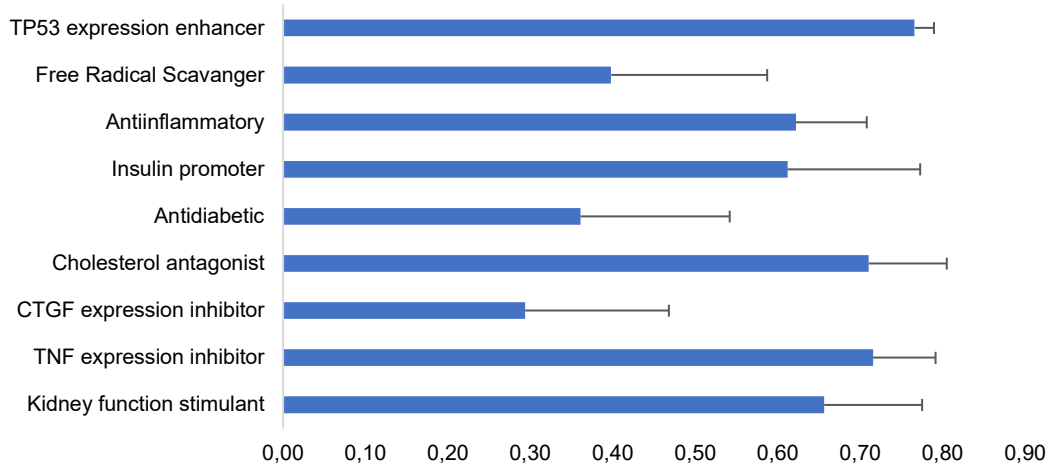

Fig. 2 Biological process of metabolites compound

Table 2. Role of Target Proteins in DN pathways by secondary compounds in *L. fermentum* (PPI STRING)

Pathway	False discovery rate Benjamini-Hochberg (p-value)	Color	Protein
Regulation of inflammatory response	0.00000017		**NFKB1 PPARA TLR2 TLR4 PPARG TNF**
Regulation of response to stress	0.00000000292		**NFKB1 EP300 PPARA F3 MMP2 NFE2L2 TLR2 TLR4 PPARG TNF SIRT1**
AGE-RAGE signaling pathway in diabetic complications	0.0000000061		**NFKB1 JUN F3 MMP2 TNF**
NF-kappa B signaling pathway	0.000230		**NFKB1 TLR4 TNF**
TGF signaling pathway	0.000180		**EP300 PPARG NFKB1**
TNF signaling pathway	0.0000103		**MMP9 TNF NFKB1 JUN**
FOXO signaling pathway	0.00047		**SIRT1 EP300 TNF**
Longevity signaling pathway	0.0002000		**SIRT1 PPARG NFKB1**

Secondary metabolites in *L. fermentum* literature study results analyzed its potential using WAY2DRUG PASS prediction. (http://www.pharmaexpert.ru/passonline/ predict.php) as diabetic treatment. Previously, each compound needed to be searched smile structure (simplified molecular-input line-entry system) obtained from pub-chem database (https://pubchem.ncbi.nlm.nih.gov/). Then the compound analyzed its potential using WAY2DRUG PASS prediction to find out its potential in DN. The Pa (probability to be active) value describes the potential of the compound being test. Determination of value is comparing the structure of compounds with compounds that have proved as a specific treatment. Potential Analysis has done using Way2Drug Pass Server. The Pa value is 0.7 more indicates that the compound is predicted to have a high potential as anti-diabetic. The high similarity with the compounds in the database has been proven as such treatments. Whereas the value of Pa is more than 0.3 but less than 0.7, then the compound computationally has low similarity to the compound that has been proven as the treatment.

3.3. In Vivo Study

3.3.1. The Expression of SIRT-1 by Immunohistochemistry in Kidneys of Experimental Animals

The expressions of SIRT-1 appeared brown on the IHC staining. The staining patterns was mainly in the form of cytoplasmic staining. The staining assessment used the semiquantitative assessment technique, using the IRS criteria. 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), 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). SIRT-1 immunohistochemical staining of experimental animal kidney tissue; negative control group (a, h), positive control (b, i), treatment with curd (c, j), low-dose LAB (d, k), high-dose (e, l), and low-dose bacteriocin (f, m) and high-dose

(b, i) (Fig. 3). SIRT-1 was stained brown, mainly with the matrix staining pattern around the glomerulus and tubules. There was a decrease in the SIRT-1 expression in the alloxan induction group. The treatment of *dadiah*, lactic acid bacteria, and bacteriocin showed a higher expression of SIRT-1 than the positive control. Immunoperoxidase, low magnification with 10x objective lens (top), and high magnification with 40x objective lens (bottom) 200µm scale. The number of SIRT-1 expression in each treatment can be seen in Table 3 and Fig. 4.

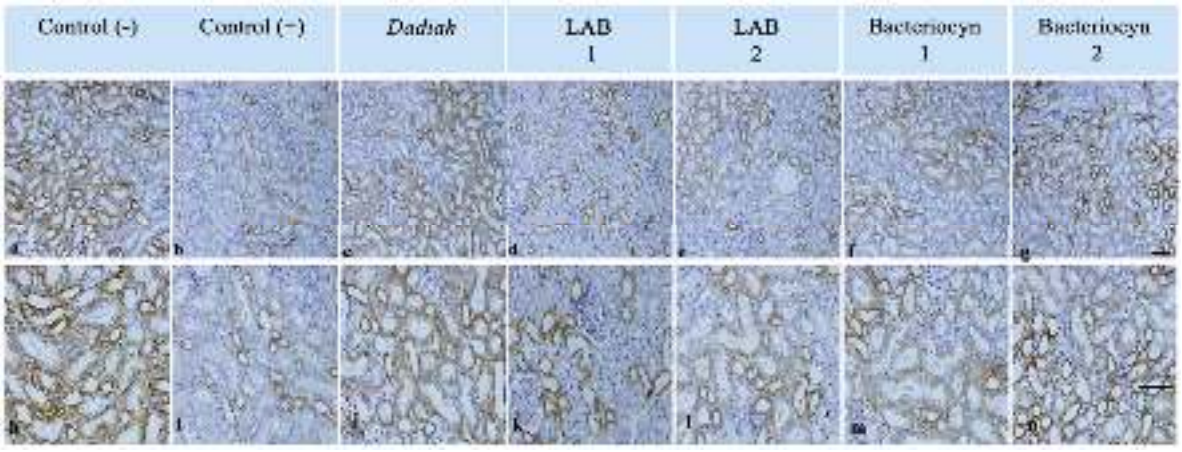

Fig. 3 The assessment of SIRT-1 expression by immunohistochemistry

Table 3. The average number of SIRT-1 expression in each treatment (% positive cells)

Samples	Average	Standard Deviation	Notation
Negative Control (C-)	80.0000	0.00000	d
Positive Control (C+)	36.6667	5.16398	a
P1	83.3333	5.16398	d
P2	51.6667	7.52773	b
P3	61.6667	20.41241	bc
P4	63.3333	19.66384	bc
P5	66.6667	12.11060	c
Chi-square count	= 26.131		
p-value	= 0.000		

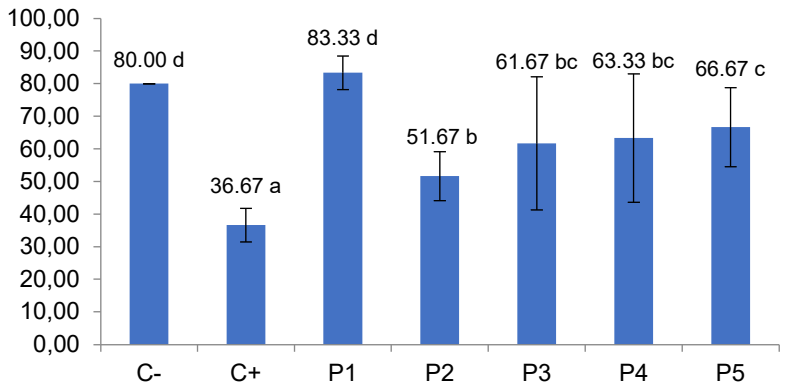

Fig. 4 SIRT-1 expression numbers in each treatment

Most studies have established the crucial effects of SIRT-1 deacetylase in protecting kidney cells from stress. SIRT-1 has been shown to protect podocytes and kidney tubular cells in a variety of kidney illness situations, including DN. Sirt-1 protects against DN in part by deacetylating disease-associated transcription factors such as p53, FOXO, p65, NF-kB, and STAT3. Recently, it was demonstrated that induction of SIRT-1 in podocytes significantly improved proteinuria and renal damage in an experimental DN model [37].

Due to the critical role of SIRT-1 as a metabolic sensor, its activity is dynamically regulated to allow for alteration to changes in the cellular metabolic state. SIRT-1's regulation network is comprised of nutritional,

hormonal, and environmental cues, as well as the NAD+ level and SIRT-1 interacting proteins that respond to these signals.

SIRT-1 expression is decreased in response to a high-glucose, high-fat diet, but it is raise in response to famine and food deprivation [38–40]. SIRT-1 establishes a connection between chromatin dynamics/gene expression and environmental cues during the stress response. SIRT-1 activation may assist the kidney in metabolic conditions such as diabetes mellitus. Wakino et al. [10] demonstrated that reduced SIRT-1 in the proximal tubules represents the initiation of DN using animal models of diabetes mellitus. Additionally, SIRT-1 is implicated in the pathogenesis of DN [13]. SIRT-1 a NAD+-dependent protein deacetylase, participates in various physiological activities, including hypoxia stress, DNA repair, cell aging, inflammatory, and mitochondria regulation; yet, its degradation is required for the formation of ND. SIRT-1 expression was significantly decreased in the renal of diabetic db/db rats in previous research. Recent research, however, indicates that SIRT-1 is involved in the endoplasmic reticulum stress response to hyperglycemia and hypoxia [41].

3.3.2. The Expression of TNF-α by Immunohistochemistry in Kidneys Tissues

The expressions of TNF-α, appeared brown on the IHC staining. The staining pattern was mainly in the form of cytoplasmic staining. 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 staining of TNF-α immunohistochemistry in the kidney tissue of animal model; the negative control group (a, h) and the positive control (b, i), the treatment with *dadiah* (c, j), the low-dosage LAB (d, k) and the high dosage (e, l), and the low-dosage bacteriocin (f, m) and the high dose (b, i) (Fig. 5). The TNF-α was stained brown in some tubular epithelial cells and some cells in the stroma, with a weak staining in the matrix around the glomeruli and tubules. There was an increase in the TNF-α expression in the alloxan induction group, both in epithelial and stromal cells. The administration of *dadiah*, lactic acid bacteria, and bacteriocin, showed lower TNF- α expression than the positive control. The average number of TNF expression in each treatment can be seen in Table 4.

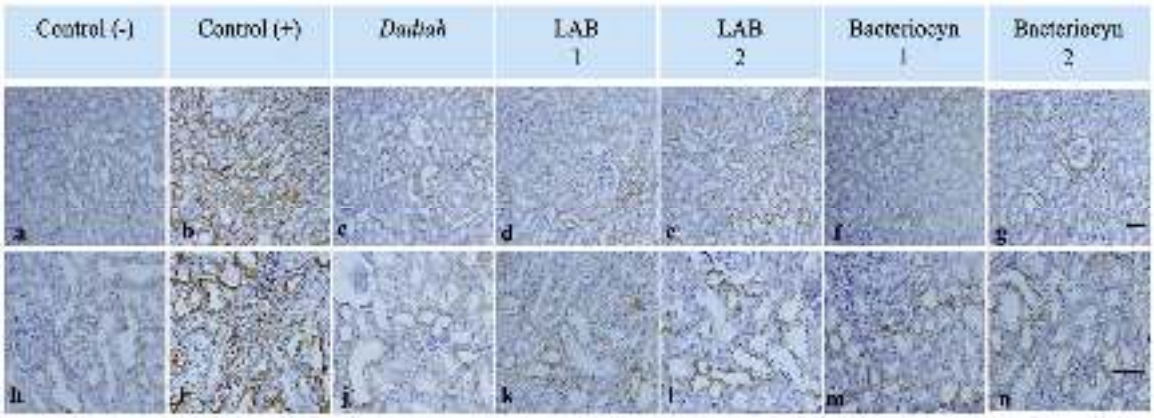

Fig. 5 The assessment of TNF-α expression with immunohistochemistry

Based on Fig. 6, it can be seen that the highest average of TNF expression in the C+ group (induced by alloxan + proteinuria) was 76.67±5.16, and the lowest average of TNF expression was in the C – group (not induced by alloxan and not given any treatment), which was equal to 16.67±5.16. To prove whether there was a statistically significant difference in the average number of TNF expression, the Kruskal Wallis statistical analysis would be carried out.

Table 4. The average number of TNF expression in each treatment

Sample	Average	Standard Deviation	Notation
C–	16.6667	5.16398	a
C+	76.6667	5.16398	d
P1	20.0000	6.32456	ab
P2	23.3333	12.11060	abc
P3	30.0000	0.00000	c
P4	26.6667	8.16497	bc
P5	23.3333	10.32796	abc
Chi-square count	= 24.362		

p-value	= 0.000

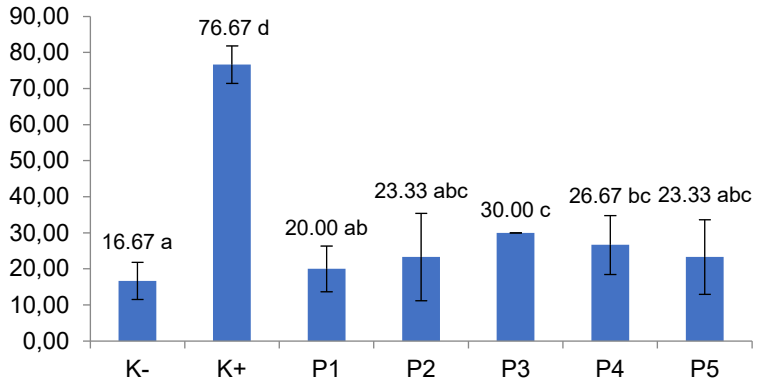

Fig. 6 TNF-α expression numbers in each treatment

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 is a significant difference in the average TNF expression number between treatments. To see the difference, further tests were carried out using the Mann Whitney test with the results notation in Table 5. It can be seen that:

The highest average of TNF expression in the C+ treatment was significantly different from the C-, P1, P2, P3, P4, and P5 treatments. The lowest average of TNF expression in C- groups was significantly different from C+, P3, and P4 treatment groups, but C- groups was not significantly different from P1, P2, and P5 treatment groups.

Table 5. Average of fibrosis interstitial fibrosis in the groups

Samples	Average	Standard Deviation	Notation
Negative Control (C-)	12.0667	0.78145	a
Positive Control (C+)	17.6667	0.90480	c
P1	14.9333	1.50687	b
P2	15.2833	1.95900	b
P3	15.8167	1.98133	b
P4	15.1833	1.79490	b
P5	15.0667	1.18434	b
F count	= 7.117		
p-value	= 0.000		

Additionally, scientific data indicates that the inflammatory factors TNF-α and IL-6 are well reported to contribute to renal impairment in diabetes [15]. Probiotics appear to reduce inflammation and oxidative stress markers, according to a growing body of studies [16]. Diabetes and obesity are both metabolism disorders associated with a low-grade inflammatory state. TNF-α is a marker inflammation cytokine that has been shown to phosphorylate the insulin receptor's serine residue substrate (IRS-1), inactivating it, while IL-1, TNF-α, and interferon (IFN) are known to function synergistically by invading the pancreas and generating-cell damage and apoptosis [42–44]. In STZ-induced diabetic rats, *Lactobacillus casei* strain Shirota significantly reduced pro-inflammatory cytokines IL-6, IL-4, and CRP. In a diabetes kidney rat model fed for 12 weeks, a probiotic mixture decreased TNF-α and increased IL-10. Similar studies have demonstrated the anti-inflammatory benefits of probiotic lactobacilli. Thus, our findings corroborate previous reports indicating that *L. fermentum* spp. had anti-inflammatory properties [45].

3.3.3. The Deposition of Fibro-Collagen Matrix with HE Sirius Red (Interstitial Fibrosis)

The connective tissue staining of the experimental animal kidneys with sirius red stained showed the interstitial and periglomerular connective tissue. The connective tissue matrix was stained with magenta. Negative control group (a, h), positive control (b, i), treatment with *dadiah* (c, q), low-dose LAB (d, k), high-dose (e, l), low-dose bacteriocin (f, m) and high-dose (b, i) (Fig. 7). The collagen deposition was measured using the ImageG program by extracting the red area, converting the image to black and white, and measuring the percentage area of the coloured area per unit area. The collagen deposition was lower in the experimental animals with *dadiah* treatment, lactic acid bacteria, and bacteriocin treatment, compared with the positive controls. The lowest collagen deposition was in the *dadiah* treatment, compared to other treatments.

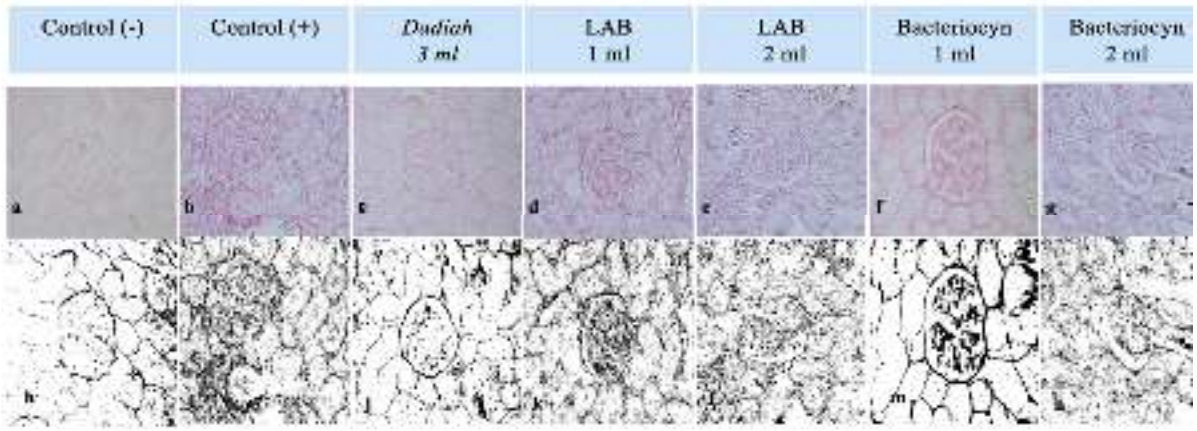

Fig. 7 Deposition of fibro-collagen matrix in kidneys with Sirius Red staining

Induction with alloxan administration showed an increase in collagen matrix deposition in the renal parenchyma as a sign of glomerulosclerosis [17,46].

Based on Fig. 8, it can be seen that the highest average of glomerular fibrosis rate in the C+ treatment (induced by alloxan + proteinuria) was 17.67 ± 0.90, and the lowest average of glomerular fibrosis was in the C– treatment (not induced by alloxan and not given a treatment), namely of 12.07 ± 0.78. The one-way ANOVA statistical analysis would be used to determine how a statistically significant difference in the average number of glomerular fibrosis existed. The one-way ANOVA test resulted in a p-value less than (0.000< 0.050), indicating a statistically significant difference in the average number of interstitial fibrosis between treatments. To demonstrate the distinction, more tests were conducted using the Duncan test and the notation results in Table 5. It can be seen that: The highest average of glomerular fibrosis in the C+ treatment was significantly different from the C-, P1, P2, P3, P4, and P5 treatments. The lowest average of kidney fibrosis in the C- treatment was significantly different from the C+, P1, P2, P3, P4, and P5 treatments.

The activation of metabolic, inflammatory, and hemodynamic pathways describes the pathophysiology of DKD. For example, chronic hyperglycaemia leads to increased PKC activity, alterations in polyol metabolism, increased secretion of profibrotic cytokines (such as TGF-B1), and non-enzymatic glycosylation glycation of glomerulosclerosis structures and the formation of AGEs. The accumulation of aberrant protein glycation markers is a significant factor in the onset and progression of DKD. AGEs increase in the mesangial and glomerular capillary walls in people with DN, according to immunohistochemistry findings. The kidney plays a crucial role in AGEs metabolism [9]. Renal fibrosis, myofibroblast, podocyte dysfunction, basement membrane thickening, and extracellular matrix protein build-up are all regarded to be standard features of DN. Podocytes are a type of high differentiation glomerular epithelial cell that has been linked to the early pathogenic mechanism of DN pathogenesis [47–51]. Furthermore, the increase in inflammation directly destroys renal function [49]. In diabetes, the deposition of advanced glycation end products (AGEs) plays a crucial role in the development of DN. Additionally, inflammation and peroxidation are associated with the onset and progression of DN, respectively. The main characteristics of DN include a thick basement membrane, mesangial expansion, podocyte loss in the glomerular, and increased urine microalbumin excretion. Further, ECM protein build-up plays a vital role in developing DN. DN is also characterized by renal fibrosis and glomerular sclerosis [3].

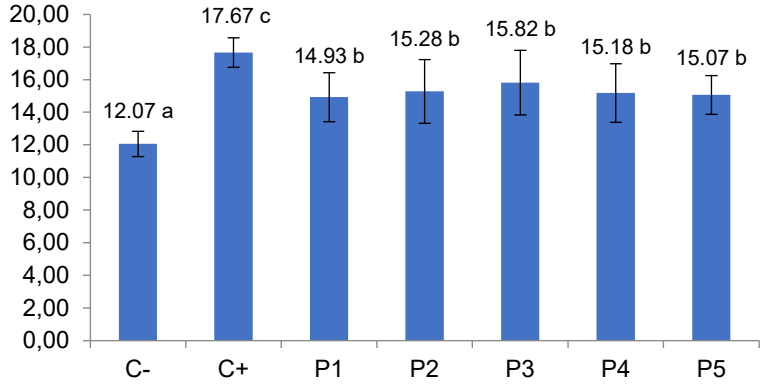

Fig. 8 The proportion of fibrocolagen matrix in the kidney tissue

4. Discussion

4.1. In Vitro Study

LAB in *dadiah* was 7.1 10^{10}. In comparison to probiotics from Prato cheese, which have a vibrant color [51]. According to Emmawati [52], the LAB isolate from *Mandai* is a fermented product made of *cempedak* (*Artocarpus champeden*) dami. *Mandai* samples has the total number of LAB as probiotic food. The other study, found 14 isolates LAB has the total colony result are the dilution of 10^{-7} is 1,25 x 10^{9} CFU/g and the dilution of 10^{-8} is 3,0 x 10^{8} CFU/g. The identification with macroscopic in MRS agar medium is seen the sign of colony is circle of the whole, broken white, round shape, convex, edge slick and small and big size [53]. Other study from fresh goat milk samples located in Western and North Western provinces of Sri Lanka, found the most of the isolated colonies were creamy circular in shape with wet surface, raised with entire margins [24]. Additionally, this study compared LAB research on kefir created from the fermentation of fresh milk with the addition of kefir grains as carrier components for probiotic organisms to an indigenous LAB source. The bacteria in kefir have a population density of between 6.4x10^{4} and 8,5x10^{8} CFU/g [54]. Microscopic identification showed The presence of LAB and its efficacy as probiotic sources in a traditional fermented foods was proved using *Cyprinus carpio*, *Dengke Naniura* of *Bataknese*, Indonesia. The identification of LAB morphology was found Gram-positive, bacilli, cocci, and bacilli cocci [56,57]. According to the catalase assay, the LAB isolate used in biochemical test does not produce catalase. The study reported no presence of bubbles and stated that the LAB from *dadiah* from Lintau Buo is homofermentative. The observation results are not seen there is a gel reservoir on the LAB, it is demonstrated that the catalase assay tool is significantly negative. According from Ibrahim [58] reported to the findings, LAB isolated from mango exhibited a negative catalase assay result.

Thus, acid resistance assays on *dadiah* LAB at pH 4 and 3 were performed. The control samples had a more significant number of colonies to grew (7 × 10^{8} CFU/L than the pH 3 (4 × 10^{8} CFU/ml), with a survival rate of 57.1%. The viability value changes according to the type of bacteria that can live at low pH and the strain of bacteria. Along with acid resistance, probiotics require LAB resistance to bile salts. The 0.5 percent concentration is sufficient to select for bile salt-resistant strains [59]. The LAB from dadiah demonstrated a significant antimicrobial effect of harmful microbiota. The results indicated that LAB from *dadiah* possessed an inhibitory effect on *E. coli* to kanamycin and ampicillin. According to Morales [60], the zone of inhibition is classified as weak (less than 5 mm), medium (5-10 mm), strong (>10-20 mm), and very strong (>20-30 mm). Thus, the inhibitory activity of *dadiah* LAB against *E. coli* is classified as very strong.

According to the PCR and BLAST analyses, the isolated bacteria from *dadiah* were 99.99 percent identical to *L. fermentum*.

Like other studies, Meekiri back-slopping, a traditional Sri Lankan food obtained from fermented buffalo milk products, also has several strains such as *L. fermentum, L. curvatus, and L. acidophilus, and L. plantarum*. In Sri Lanka, milk fermentation gel is obtained using a back-sloping technique that is a simple technique using a small inoculum derived from the previous coagulum as a culture starter in the selection of BAL strains [26].

Research about different isolations carried out by Syukur and Fachrial [61] obtained the *L. plantarum* bacteria isolated from *dadiah* from *Sijunjung*, in which the base length was 1525 bp. Similarly, according to the studies undertaken by Purwati et al. [53] the isolation and characterization of LAB from *dadiah* also resulted in *L. plantarum strain Dad-13*, which had a similarity value of 97–100 percent when BLAST analysis was used [54]. The research of Melia and Purwati [62] on buffalo milk samples from the *Agam* district (BMA 3.3) reported the classification LAB using BLAST analysis *as a strain of L. fermentum (L23)*. Sequencing results showed that 41.6 percent (5 isolates) were identified as *Lactococcus lactis* ssp. *lactis*, 25 percent (3 isolates) identified as *Lactobacillus plantarum* ssp. *plantarum*, 16.6 percent (2 isolates) identified as *L. lactis* ssp. *cremoris*, and 8.3 percent (1 isolate each) identified as *Pediococcus pentosaceus* and *Lactobacillus pentosus* [63]. This study is in line with research conducted by Sukma [64], wherein the LAB in *dadiah* was dominated by bacteria from the *Lactococcus*, *Lactobacillus*, and *Leuconostoc* groups.

4.2. In Silico Study

Pathway analysis on diabetes complications with target proteins related to DN found three major pathways, namely AGE-RAGE Signalling, FOXO Signalling, and Longevity Regulating Pathway, with results as seen in links: (AGE-RAGE Signalling pathway in diabetic complications); (FOXO Signalling pathway); (Longevity regulating pathway).

4.2.1. Protein-Ligan Network Analysis

The resulting potential protein-ligand network in this study showed ferulic acid, caproic acid, linoleic acid, and vaccenic acid suggested metabolite compound in *L. fermentum* were selected results of target proteins associated with DN pathways (Table 2). The potential hit was evaluated by E-values and Tanimoto coefficient (Tc). The suggested threshold of E-values and Max-Tc was 10^{-4} and 0.57, respectively [35]. The result of E- values greater than the limit was not considered into the study, as they did not indicate great statistical significance [36].

4.2.2. Protein-Protein Interaction by STRING DB

Target proteins directly related to DN pathways are described as being in outer circles such as NF-□B, JUN, EP 300, PPARA, F3, MMP2, MMP9, NFE2L2, TLR2, TLR4 and PPARG. While based on the highest score of protein interaction (PPI Score), proteins closely related to the target protein that can be studied through laboratory studies are TNF and SIRT-1 (the inner circle). This protein computationally has high confidence if conducted in vivo and in vitro testing with results that have affected the occurrence of diabetes complications (DN) through the pathways set in KEGG. Below is described the biological activity of target proteins in DN with a significant p-value rate (Benjamin-Hochberg). Some studies showed that SIRT-1 has a reno-protective impact on DN through deacetylation of transcription factors involved in renal disease pathogenesis. Recently, it has been found that specific overexpression of SIRT-1 by podocyte cells may decrease proteinuria and kidney injury in experimental mice with ND [36]. SIRT-1 is involved in several cellular functions, including chromosomal stability, cell cycle, apoptosis, DNA repair, metabolism, and aging by deacetylation of various transcription factors (NF-kB, P53, FOXO) histone and non-histone proteins [11]. The target protein is seen with pathways that play a role in DN. The lowest yield (p-value 0.00000000292) is the most significant seen in the path of "Regulation of response to stress" with the target protein, namely, NFKB1, EP300, PPARA, F3, MMP2, NFE2L2, TLR2, TLR4, PPARG, TNF, and SIRT-1 (Table 3).

4.2.3. Bioactive and Metabolites Compounds Potential *L. fermentum* as DN Treatment

Secondary metabolites in *L. fermentum* literature study results analyzed its potential using WAY2DRUG PASS prediction. (http://www.pharmaexpert.ru/ passonline/ predict.php) as diabetic treatment. Previously, each compound needed to be searched smile structure (simplified molecular-input line-entry system) obtained from pub-chem database (https://pubchem.ncbi.nlm.nih.gov/). Then the compound analyzed its potential using WAY2DRUG PASS prediction to find out its potential in DN. The Pa (Probability to be Active) value describes the potential of the compound being test. Determination of value is comparing the structure of compounds with compounds that have proved as a specific treatment. Potential analysis has done using Way2Drug Pass Server. The Pa value is 0.7 more indicates that the compound is predicted to have a high potential as anti-diabetic. The high similarity with the compounds in the database has been proven as such treatments. Whereas the value of Pa is more than 0.3 but less than 0.7, then the compound computationally has low similarity to the compound that has been proven as the treatment.

Fig. 2 have seen the potential of metabolite compound *L. fermentum* in the incidence of DN with a significant score of>0.7 will have high potential, while the score 0.5-0.7 has a moderate potential effect on DN computationally.

Suppose the average score of various metabolite compounds produced by *L. fermentum* in literature studies with biological processes occurs. In that case, the metabolite compounds of *L. fermentum* with a computational influence are lactic acid compounds with a score of 0.579 and ferulic acid compounds 0.580. While the most instrumental biological activity is TP53 expression enhancer (0.77) and TNF-α expression inhibitor (0.72), this is following several in vivo studies that state that inflammatory processes are an essential mechanism of dm progressivity into DN, so that by inhibiting TNF expression and increased expression of TP53, it can inhibit inflammatory processes in diabetes, so that microvascular complications will be inhibited. The study showed that high circulating TNF receptor levels might be e a new indicator of DN. TNF-α receptors 1 and 2 are critical, independent predictors for the production of macroalbuminuria in DN [9]. Inflammatory cytokines such as IL-1, IL-6, IL-18, TNF-α have been linked to the development and progression of DN [65].

4.3. In Vivo Study

SIRT-1 a NAD+-dependent protein deacetylase, participates in various physiological activities, including hypoxia stress, DNA repair, cell aging, inflammatory, and mitochondria regulation; yet, its degradation is required for the formation of ND. SIRT-1 expression was significantly decreased in the renal of diabetic db/db rats in previous research. Recent research, however, indicates that SIRT-1 is involved in the endoplasmic reticulum stress response to hyperglycemia and hypoxia [41]. Most studies have established the crucial effects of SIRT-1 deacetylase in protecting kidney cells from stress. SIRT-1 has been shown to protect podocytes and kidney tubular cells in a variety of kidney illness situations, including DN. Sirt-1 protects against DN in part by deacetylating disease-associated transcription factors such as p53, FOXO, p65, NF-kB, and STAT3. Recently, it was demonstrated that induction of SIRT-1 in podocytes significantly improved proteinuria and renal damage in an experimental DN model [37].

Additionally, scientific data indicates that the inflammatory factors tumor necrosis factor TNF-α and IL-6 are well reported to contribute to renal impairment in diabetes [41]. Probiotics appear to reduce inflammation and oxidative stress markers, according to a growing body of studies [16]. In STZ-induced diabetic rats, Lactobacillus casei strain Shirota significantly reduced pro-inflammatory cytokines IL-6, IL-4, and CRP. In a diabetes kidney rat model fed for 12 weeks, a probiotic mixture decreased TNF-α and increased IL-10.

Similar studies have demonstrated the anti-inflammatory benefits of probiotic lactobacilli. Thus, our findings corroborate previous reports indicating that *L. fermentum* spp. had anti-inflammatory properties [45].

The activation of metabolic, inflammatory, and hemodynamic pathways describes the pathophysiology of DN. For example, chronic hyperglycaemia leads to increased PKC activity, alterations in polyol metabolism, increased secretion of profibrotic cytokines (such as TGF-B1), and non-enzymatic glycosylation glycation of glomerulosclerosis structures and the formation of AGEs. The accumulation of aberrant protein glycation markers is a significant factor in the onset and progression of DKD. AGEs increase in the mesangial and glomerular capillary walls in people with DN, according to immunohistochemistry findings. The kidney plays a crucial role in AGEs metabolism [9]. Renal fibrosis, myofibroblast, podocyte dysfunction, basement membrane thickening, and ECM protein build-up are all regarded to be standard features of DN.

Furthermore, the increase in inflammation directly destroys renal function [5]. In diabetes, the deposition of AGEs plays a crucial role in the development of DN. Additionally, inflammation and peroxidation are associated with the onset and progression of DN, respectively. The main characteristics of DN include a thick basement membrane, mesangial expansion, podocyte loss in the glomerular, and increased urine microalbumin excretion. Further, ECM protein build-up plays a vital role in developing DN. DN is also characterized by renal fibrosis and glomerular sclerosis [3].

5. Conclusions

Oral administration of *dadiah* and probiotics and secondary metabolite compounds of LAB have been shown to increase the production of SIRT-1 and reducing the TNF-α expression that marker in stress oxidative and inflammatory processes caused by hyperglycaemia, and therefore alleviating renal fibrosis.

Administration of *dadiah* solution, isolate probiotic strain *L. fermentum*, and isolate *bacteriocin* from *dadiah* has been shown to ameliorate renal tissue fibrosis in DN mice when stained with Sirius-red. In addition, oral administration of *dadiah*, probiotics and secondary metabolite compounds of lactic acid bacteria showed to increase the expression of SIRT-1 and reduced TNF-α, which functions were to reduce stress oxidative and inflammatory processes caused by hyperglycaemia, and therefore alleviating renal fibrosis. The findings of this study could be to develop novel treatments for DN that aim to reduce the cascade of oxidative stress and inflammatory signals in kidney tissue.

5.1. Study Limitation

- Proteinuria examined in this study was measured qualitatively using UriScan. Urine measurements should be quantitative by Radioimmunoassay (RIA) to be statistically analyzed for their effect on the administration of dadiah and its metabolites.
- Bacteriocin isolated from probiotics *dadiah* is not pure bacteriocin but contains other metabolite components produced by lactic acid bacteria *dadiah* (free supernatant cell).
- In the experimental stage study, researchers only looked at the relationship between variable oxidative stress and inflammation to changes in the anatomical pathology structure of kidney tissue with DN. DN is a complex event partially mediated and modified by genetic factors, lifestyle, and environmental exposure (epigenetic).
- No examination of other metabolite compounds contained in *dadiah* with *Spectrophotometer* method.

5.2. Future Study

This research is still being done on experimental animals, so it is necessary to conduct further research for clinical trials in humans. Clinical trials are essential in proving the effect of *dadiah* on kidney function improvement in DN patients who are known to have damage and death of glomerular podocyte cells that cause proteinuria in DM. The examination of this clinical trial can be done using urine samples. The various examinations include macroalbuminuria and microalbuminuria, the number of podocyte cells in the urine (podosituria), angiotensinogen, and nephrin. In addition, serum creatinine examination can also do to show the glomerular filtration rate (e-LFG). This study only examined the potency of *dadiah* and its metabolites against inflammatory repair parameters and antioxidant effects on DN without comparing it to antidiabetic drugs. Further study experimental research with comparing to anti-diabetic drugs.

Statements and Declarations

Funding: This research was funded by Baiturrahmah University foundation.

Disclosure of potential conflicts of interest: The authors have no competing interests to declare that are relevant to the content of this article.

Acknowledgments: The authors would like to thank Prof. dr. Hj Endang Purwati, MS, Ph.D. from the Laboratory of Animal Husbandry Biotechnology/ Technology Animal Product, Andalas University Padang West Sumatra, Indonesia, for the preparation of research material and also The Baiturrahmah University foundation has helped finance research.

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of Medical Faculty of Baiturrahmah University (No: 001/ETIK-FKUNBRAH/03/03/21).

References

1. Chen J, Liu J, Yan C, et al. Sarcodon aspratus polysaccharides ameliorated obesity-induced metabolic disorders and modulated gut microbiota dysbiosis in mice fed a high-fat diet. Food Funct. 2020;11:2588–602; https://doi.org/10.1039/C9FO00963A
2. Guilbaud A, Howsam M, Niquet-Léridon C, et al. The effect of *Lactobacillus fermentum* ME-3 Treatment on glycation and diabetes complications. Mol Nutr Food Res. 2020;64:e1901018; https://doi.org/10.1002/mnfr.201901018
3. Kundu A, Dey P, Sarkar P, et al. Protective effects of croton hookeri on streptozotocin-induced diabetic nephropathy. Food Chem Toxicol. 2020;135:110873; https://doi.org/10.1016/j.fct.2019.110873
4. Zheng HX, Qi SS, He J, Hu CY, Han H, Jiang H, Li XS. Cyanidin-3-glucoside from black rice ameliorates diabetic nephropathy via reducing blood glucose, suppressing oxidative stress and inflammation, and regulating transforming growth factor B1/Smad expression. J Agric Food Chem. 2020;68:4399–410; https://doi.org/10.1021/acs.jafc.0c00680
5. Li YJ, Chen X, Kwan TK, et al. Dietary Fiber protects against diabetic nephropathy through short-chain fatty acid-mediated activation of g protein-coupled receptors GPR43 and GPR109A. J Am Soc Nephrol. 2020;31:1267–81; https://doi.org/10.1681/ASN.2019101029
6. Pérez-Morales RE, Del Pino MD, Valdivielso JM, Ortiz A, Mora-Fernández C, Navarro-González JF. Inflammation in diabetic kidney disease. Nephron. 2019;143:12–6; https://doi.org/10.1159/000493278
7. Nagarajrao R, Alharbi SA. Relationship between oxidant and antioxidant enzymes status in type 2 diabetic patients with nephropathy in Saudi population. Asian J Pharm Clin Res. 2018;11:363; https://doi.org/10.22159/ajpcr.2017.v11i1.22940
8. Seaquist ER, Goetz FC, Rich S, Barbosa J. Familial clustering of diabetic kidney disease. evidence for genetic susceptibility to diabetic nephropathy. N Engl J Med. 1989;320:1161–5; https://doi.org/10.1056/NEJM198905043201801
9. Macisaac RJ, Ekinci EI, Jerums G. Markers of and risk factors for the development and progression of diabetic kidney disease. Am J Kidney Dis. 2014;63:S39-62; https://doi.org/10.1053/j.ajkd.2013.10.048
10. Wakino S, Hasegawa K, Itoh H. Sirtuin and metabolic kidney disease. Kidney Int. 2015;88:691–8; https://doi.org/10.1038/ki.2015.157
11. Balaiya S. Sirtuins in the modulation of oxidative stress–a mini view. Ec Ophthalmol. 2017;3:73–7.
12. Kitada M, Kume S, Takeda-Watanabe A, Kanasaki K, Koya D. Sirtuins and renal diseases: relationship with aging and diabetic nephropathy. Clin Sci Lond Engl 1979. 2013;124:153–64; https://doi.org/10.1042/CS20120190

13. Guan Y, Hao C-M. SIRT1 and kidney function. Kidney Dis. 2016;1:258–65; https://doi.org/10.1159/000440967
14. Cencioni C, Spallotta F, Mai A, et al. Sirtuin function in aging heart and vessels. J Mol Cell Cardiol. 2015;83:55–61; https://doi.org/10.1016/j.yjmcc.2014.12.023
15. Song Y, Liu W, Tang K, Zang J, Li D, Gao H. Mangiferin Alleviates renal interstitial fibrosis in streptozotocin-induced diabetic mice through regulating the PTEN/PI3K/Akt signaling pathway. J Diabetes Res. 2020;2020:e9481720; https://doi.org/10.1155/2020/9481720
16. Mahmoodpoor F, Rahbar Saadat Y, Barzegari A, Ardalan M, Zununi Vahed S. The impact of gut microbiota on kidney function and pathogenesis. Biomed Pharmacother. 2017;93:412–9; https://doi.org/10.1016/j.biopha.2017.06.066
17. Navarro JF, Mora-Fernández C. The role of TNF-alpha in diabetic nephropathy: pathogenic and therapeutic implications. Cytokine Growth Factor Rev. 2006;17:441–50; https://doi.org/10.1016/j.cytogfr.2006.09.011
18. Sassy-Prigent C, Heudes D, Mandet C, et al. Early glomerular macrophage recruitment in streptozotocin-induced diabetic rats. Diabetes. 2000;49:466–75; https://doi.org/10.2337/diabetes.49.3.466
19. Amelia R, Philip K, Pratama YE, Purwati E. Characterization and probiotic potential of lactic acid bacteria isolated from dadiah sampled in West Sumatra. Food Sci Technol. 2020;41:746–52; https://doi.org/10.1590/fst.30020
20. Abdullah, D., Poddar, S., Rai, R. P., Purwati, E., Dewi, N. P., & Pratama, Y. E. Molecular Identification of Lactic Acid Bacteria Approach to Sustainable food Security. *Journal of Public Health Research*, *February 2022*. https://doi.org/10.4081/jphr.2021.2508
21. Harun H, Wirasti Y, Purwanto B, Purwati E. Characterization of lactic acid bacteria and determination of antimicrobial activity in dadih from air dingin Alahan Panjang District, Solok Regency-West Sumatera. Syst Rev Pharm. 2020;11:583–6.
22. Harun H, Yanwirasti Y, Purwanto B, Rahayuningsih EP. The effect of giving dadih on malondialdehyde levels and renal interstitial fibrosis at aging kidney. Open Access Maced J Med Sci. 2020;8:293–6; https://doi.org/10.3889/oamjms.2020.4285
23. Pato U, Yusmarini Y, Fitriani S, Jonnaidi NN, Wahyuni MS, Feruni JA, Jaswir I. Antimicrobial activity of lactic acid bacteria strains isolated from dadih against *Escherichia coli*. IOP Conf Ser Earth Environ Sci. 2021;709:012019; https://doi.org/10.1088/1755-1315/709/1/012019
24. Abdullah D, Poddar S, Rai RP, Purwati E, Dewi NP, Pratama YE. Molecular identification of lactic acid bacteria an approach to sustainable food security. J Public Health Res. 2021;10; https://doi.org/10.4081/jphr.2021.2508
25. Premasiri DLDS, Rajawardana DU, Muddannayake DC, Hewajulige IGN. Isolation, characterization and identification of industrially beneficial probiotic lactic acid bacteria from goat milk. J Agric Sci – Sri Lanka. 2021;16:369–82; https://doi.org/10.4038/jas.v16i2.9341
26. Adikari AMMU, Priyashantha H, Disanayaka JNK, et al. Isolation, identification and characterization of lactobacillus species diversity from Meekiri: traditional fermented buffalo milk gels in Sri Lanka. Heliyon. 2021;7:e08136; https://doi.org/10.1016/j.heliyon.2021.e08136
27. Son S-H, Jeon H-L, Yang S-J, et al. Probiotic lactic acid bacteria isolated from traditional Korean fermented foods based on β-glucosidase activity. Food Sci Biotechnol. 2018;27:123–9; https://doi.org/10.1007/s10068-017-0212-1

28. García-Cano I, Rocha-Mendoza D, Ortega-Anaya J, Wang K, Kosmerl E, Jiménez-Flores R. Lactic acid bacteria isolated from dairy products as potential producers of lipolytic, proteolytic and antibacterial proteins. Appl Microbiol Biotechnol. 2019;103:5243–57; https://doi.org/10.1007/s00253-019-09844-6
29. Rácz A, Bajusz D, Héberger K. Life beyond the Tanimoto coefficient: similarity measures for interaction fingerprints. J Cheminformatics. 2018;10:48; https://doi.org/10.1186/s13321-018-0302-y
30. Vogt M, Bajorath J. Ccbmlib – a python package for modeling Tanimoto similarity value distributions. F1000Research. 2020;9:100; doi:10.12688/f1000research.22292.1
31. Wilfinger WW, Miller R, Eghbalnia HR, Mackey K, Chomczynski P. Strategies for detecting and identifying biological signals amidst the variation commonly found in RNA sequencing data. BMC Genomics. 2021;22:322; https://doi.org/10.1186/s12864-021-07563-9
32. Druzhilovskiy DS, Rudik AV, Filimonov DA, et al. Computational platform Way2Drug: from the prediction of biological activity to drug repurposing. Russ Chem Bull. 2017;66:1832–41; https://doi.org/10.1007/s11172-017-1954-x
33. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res. 2001;50:537–46.
34. Nugroho RA. Mengenal mencit sebagai hewan laboratorium. Samarinda: Mulawarman University Press; 2018.
35. Keiser MJ, Roth BL, Armbruster BN, Ernsberger P, Irwin JJ, Shoichet BK. Relating protein pharmacology by ligand chemistry. Nat Biotechnol. 2007;25:197–206; https://doi.org/10.1038/nbt1284
36. Sá MS, de Menezes MN, Krettli AU, et al. Antimalarial activity of physalins B, D, F, and G. J. Nat Prod. 2011;74:2269–72; https://doi.org/10.1021/np200260f
37. Zhong Y, Lee K, He JC. SIRT1 is a potential drug target for treatment of diabetic kidney disease. Front Endocrinol. 2018;9:624; https://doi.org/10.3389/fendo.2018.00624
38. Kane AE, Sinclair DA. Sirtuins and NAD+ in the development and treatment of metabolic and cardiovascular diseases. Circ Res. 2018;123:868–85; https://doi.org/10.1161/CIRCRESAHA.118.312498
39. Liu G, Park S-H, Imbesi M, et al. Loss of NAD-dependent protein deacetylase sirtuin-2 alters mitochondrial protein acetylation and dysregulates mitophagy. Antioxid Redox Signal. 2017;26:849–63; https://doi.org/10.1089/ars.2016.6662
40. Yacoub R, Lee K, He JC. The role of SIRT1 in diabetic kidney disease. Front Endocrinol. 2014;5.
41. Zhang Q, Jia J, He L, Fan Y, Wang N. Sitagliptin ameliorates ER stress in diabetic kidney disease through upregulation of SIRT1. Diabet Nephrop. 2021;1:33–41; https://doi.org/10.2478/dine-2021-0007
42. Maciel FR, Punaro GR, Rodrigues AM, et al. Immunomodulation and nitric oxide restoration by a probiotic and its activity in gut and peritoneal macrophages in diabetic rats. Clin Nutr. 2016;35:1066–72; https://doi.org/10.1016/j.clnu.2015.07.018
43. Miraghajani M, Zaghian N, Mirlohi M, Feizi A, Ghiasvand R. The impact of probiotic soy milk consumption on oxidative stress among type 2 diabetic kidney disease patients: a randomized controlled clinical trial. J Ren Nutr. 2017;27:317–24; https://doi.org/10.1053/j.jrn.2017.04.004
44. Sharma S, Kandasamy S, Kavitake D, Shetty PH. Probiotic characterization and antioxidant properties of *Weissella confusa* KR780676, isolated from an Indian fermented food. LWT. 2018;97:53–60; https://doi.org/10.1016/j.lwt.2018.06.033

45. Archer AC, Muthukumar SP, Halami PM. *Lactobacillus fermentum* MCC2759 and MCC2760 alleviate inflammation and intestinal function in high-fat diet-fed and streptozotocin-induced diabetic rats. Probiotics Antimicrob Proteins. 2021;13:1068–80; https://doi.org/10.1007/s12602-021-09744-0
46. Lan HY. Diverse roles of TGF-β/Smads in renal fibrosis and inflammation. Int J Biol Sci. 2011;7:1056–67; https://doi.org/10.7150/ijbs.7.1056
47. Gondaliya P, P Dasare A, Jash K, Tekade RK, Srivastava A, Kalia K. MiR-29b attenuates histone deacetylase-4 mediated podocyte dysfunction and renal fibrosis in diabetic nephropathy. J Diabetes Metab Disord. 2020;19:13–27; https://doi.org/10.1007/s40200-019-00469-0
48. Ishii H, Kaneko S, Yanai K, et al. MicroRNAs in podocyte injury in diabetic nephropathy. Front Genet. 2020;11:993; https://doi.org/10.3389/fgene.2020.00993
49. Fisher L. Retraction: long non-coding RNA TUG1 alleviates high glucose induced podocyte inflammation, fibrosis and apoptosis in diabetic nephropathy via targeting the MiR-27a-3p/E2F3 axis. RSC Adv. 2021;11:5244; https://doi.org/10.1039/D1RA90045E
50. Wu L, Wang Q, Guo F, Ma X, Wang J, Zhao Y, Yan Y, Qin G. Involvement of MiR-27a-3p in diabetic nephropathy via affecting renal fibrosis, mitochondrial dysfunction, and endoplasmic reticulum stress. J Cell Physiol. 2021;236:1454–68; https://doi.org/10.1002/jcp.29951
51. Yao T, Zha D, Gao P, Shui H, Wu X. MiR-874 alleviates renal injury and inflammatory response in diabetic nephropathy through targeting Toll-like receptor-4. J Cell Physiol. 2018;234:871–9; https://doi.org/10.1002/jcp.26908
52. Silva HLA, Balthazar CF, Silva R, et al. Sodium reduction and flavor enhancer addition in probiotic prato cheese: contributions of quantitative descriptive analysis and temporal dominance of sensations for sensory profiling. J Dairy Sci. 2018;101:8837–46; https://doi.org/10.3168/jds.2018-14819
53. Emmawati A, Laksmi BS, Nuraida L, Syah, D. Characterization of lactic acid bacteria isolates from mandai function as probiotic. agriTECH. 2017;35:146–55; https://doi.org/10.22146/agritech.9400
54. Purwati E, Hellyward J, Yuherman, Purwanto H, Puji HR. Isolation, characterization and identification of DNA'S lactic acid bacteria (LAB) of dadih lintau (Tanah Datar District) which is antibiotic resistance. Int J Adv Sci Eng Technol. 2018;6:28–31.
55. Mitra S, Ghosh BC. Quality characteristics of kefir as a carrier for probiotic *Lactobacillus rhamnosus* GG. Int J Dairy Technol. 2020;73:384–91; https://doi.org/10.1111/1471-0307.12664
56. Hamid THAT, Amysya NF. Lactic acid bacterium with antimicrobial properties from selected Malay traditional fermented foods. Int J Life Sci Biotechnol. 2020; https://doi.org/10.38001/ijlsb.781522
57. Manik M, Kaban J, Silalahi J, Ginting M. Lactic acid bacteria (LAB) with probiotic potential from dengke naniura. Baghdad Sci J. 2021;18:35–40; https://doi.org/10.21123/bsj.2021.18.1.0035
58. Ibrahim A, Fridayanti A, Delvia F. Solasi dan identifikasi bakteri asam laktat (BAL) dari buah mangga (*Mangifera indica* L.). J Ilm Manuntung. 2017;1:159; https://doi.org/10.51352/jim.v1i2.29
59. Gilliland SE, Walker DK. Factors to consider when selecting a culture of *Lactobacillus acidophilus* as a dietary adjunct to produce a hypocholesterolemic effect in humans. J Dairy Sci. 1990;73:905–11; https://doi.org/10.3168/jds.S0022-0302(90)78747-4
60. Morales G, Sierra P, Mancilla A, Paredes A, Loyola LA, Gallardo O, Borquez J. Secondary metabolites from four medicinal plants from Northern Chile: antimicrobial activity and biotoxicity against *Artemia salina*. J Chil Chem Soc. 2003;48:13–8; https://doi.org/10.4067/S0717-97072003000200002

61. Syukur S, Fachrial E, Jamsari A. Isolation, antimicrobial activity and protein bacteriocin characterization of lactic acid bacteria isolated from dadih in Solok, West Sumatera. Res J Pharm Biol Chem Sci. 2014;5:1096–104.
62. Melia S, Yuherman J, Purwati E. Selection of buffalo milk lactic acid bacteria with probiotic potential. Asian J Pharm Clin Res. 2018;11:186–9.
63. Wirawati CU, Sudarwanto MB, Lukman DW, Wientarsih I, Srihanto EA. Diversity of lactic acid bacteria in dadih produced by either back-slopping or spontaneous fermentation from two different regions of West Sumatra, Indonesia. Vet World. 2019;12:823–9; https://doi.org/10.14202/vetworld.2019.823-829
64. Sukma A, Toh H, Nguyen TTT, et al. Microbiota community structure in traditional fermented milk dadiah in Indonesia: insights from high-throughput 16S RRNA gene sequencing. Milk Sci Int. 2018;71:1–3.
65. Završnik M, Letonja J, Makuc J, Šeruga M, Cilenšek I, Petrovič D. Interleukin-4 (IL4) -590C/T (Rs2243250) gene polymorphism is not associated with diabetic nephropathy (DN) in Caucasians with type 2 diabetes mellitus (T2DM). Bosn J Basic Med Sci. 2018;18:347–51; https://doi.org/10.17305/bjbms.2018.2688

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

Commented [-2]: Fifty percent

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.

Commented [-3]: DM

Commented [-4]: T2DM

Commented [-5]: Weak English statements, need re-editing.

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).

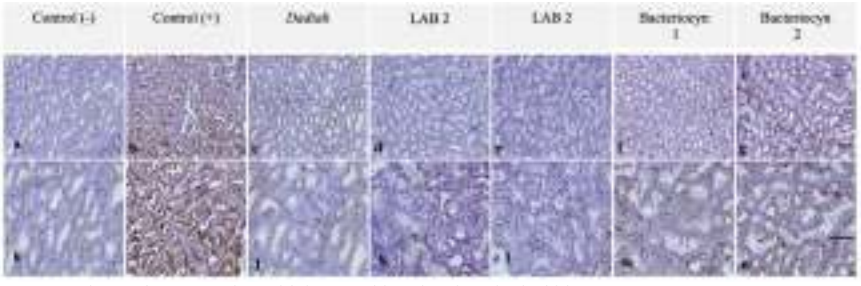

Figure 1 The NF-kB expression with immunohistochemistry in the kidney

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.

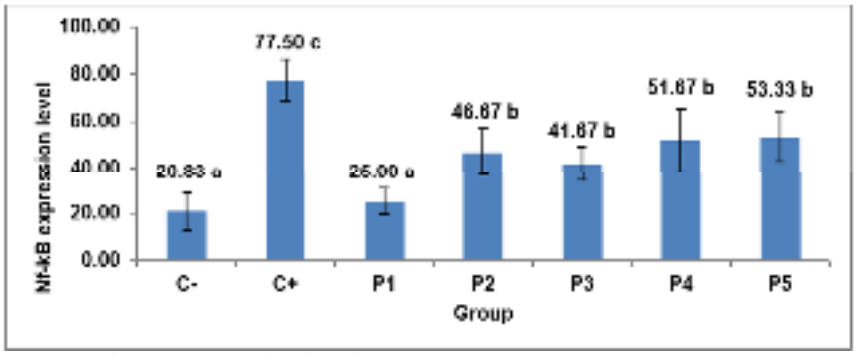

Figure 2 NF-kB expression numbers in each group

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**).

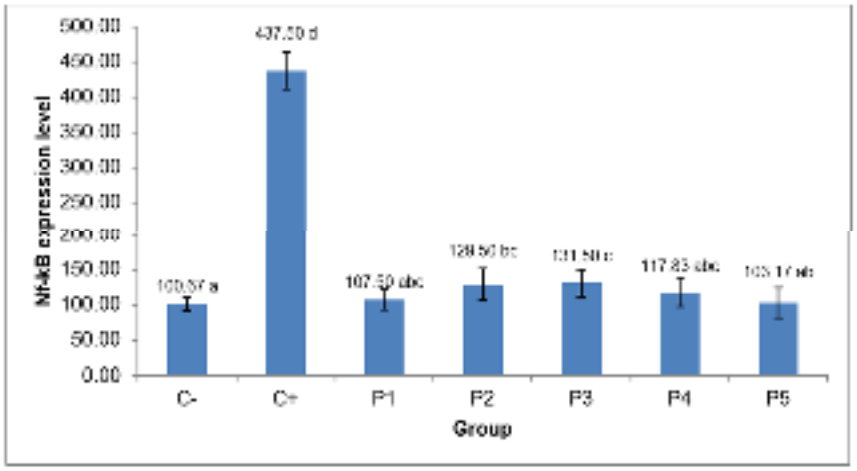

Figure 3 Blood glucose levels in each treatment

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.

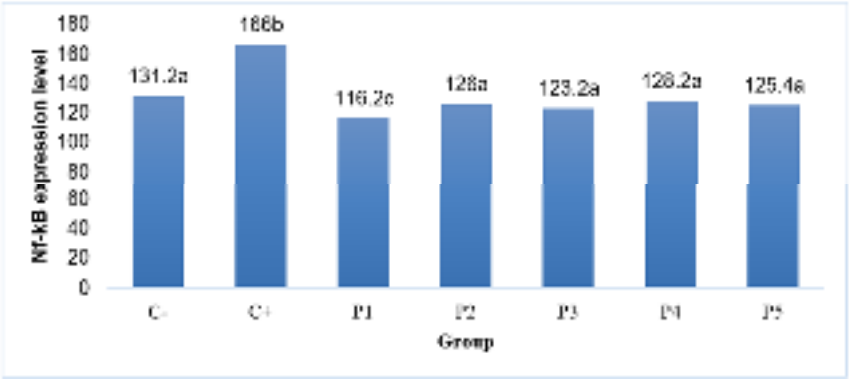

Figure 4. Serum cholesterol levels in each group

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

The authors would like to thank Prof. drh. Hj. Endang Purwati, MS, Ph. D from the Laboratory of Animal Husbandry Biotechnology/Technology Animal Product, Universitas Andalas, Padang, West Sumatra, Indonesia. Thank dr. Tofrizal Sp. PA., Ph. D from the Department of Pathological Anatomy, Universitas Andalas, Padang, West Sumatra, Indonesia. for assistance on histopathological analysis. Thanks to Universitas Baiturrahmah, Padang, West Sumatra, Indonesia foundation has helped finance research.

References

[1] A. T. Kharroubi, "Diabetes mellitus: The epidemic of the century," *World J. Diabetes*, vol. 6, no. 6, p. 850, 2015, doi: 10.4239/wjd.v6.i6.850.

[2] Y. Zheng, S. H. Ley, and F. B. Hu, "Global etiology and epidemiology of type 2 diabetes mellitus and its complications," *Nat. Rev. Endocrinol.*, vol. 14, no. 2, pp. 88–98, Feb. 2018, doi: 10.1038/nrendo. 2017.151.

[3] E. C. Westman, "Type 2 Diabetes Mellitus: A Pathophysiologic Perspective," *Front. Nutr.*, vol. 8, p. 707371, Aug. 2021, doi: 10.3389/fnut.2021.707371.

[4] M. Li, Q. Guo, H. Cai, H. Wang, Z. Ma, and X. Zhang, "miR-218 regulates diabetic nephropathy via targeting IKK-β and modulating NK-κB-mediated inflammation," *J. Cell. Physiol.*, vol. 235, no. 4, pp. 3362–3371, Apr. 2020, doi: 10.1002/jcp.29224.

[5] W. Yu *et al.*, "The gut microbiome in the differential diagnosis of diabetic kidney disease and membranous nephropathy," *Ren. Fail.*, vol. 42, no. 1, pp. 1100–1110, Jan. 2020, doi: 10.1080/0886022X.2020.1837869.

[6] H. Sawaf, G. Thomas, J. J. Taliercio, G. Nakhoul, T. J. Vachharajani, and A. Mehdi, "Therapeutic Advances in Diabetic Nephropathy," *J. Clin. Med.*, vol. 11, no. 2, p. 378, Jan. 2022, doi: 10.3390/jcm11020378.

[7] M. Ruiz-Ortega, R. R. Rodrigues-Diez, C. Lavoz, and S. Rayego-Mateos, "Special Issue 'Diabetic Nephropathy: Diagnosis, Prevention and Treatment,'" *J. Clin. Med.*, vol. 9, no. 3, p. 813, Mar. 2020, doi: 10.3390/jcm9030813.

[8] T. Kocher, J. König, W. S. Borgnakke, C. Pink, and P. Meisel, "Periodontal complications of hyperglycemia/diabetes mellitus: Epidemiologic complexity and clinical challenge," *Periodontol. 2000*, vol. 78, no. 1, pp. 59–97, Oct. 2018, doi: 10.1111/prd.12235.

[9] R. J. Galindo, M. Fayfman, and G. E. Umpierrez, "Perioperative Management of Hyperglycemia and Diabetes in Cardiac Surgery Patients," *Endocrinol. Metab. Clin. North Am.*, vol. 47, no. 1, pp. 203–222, Mar. 2018, doi: 10.1016/j.ecl.2017.10.005.

[10] G. Davì *et al.*, "Diabetes Mellitus, Hypercholesterolemia, and Hypertension but Not Vascular Disease Per Se Are Associated with Persistent Platelet Activation In Vivo: Evidence Derived from the Study of Peripheral Arterial Disease," *Circulation*, vol. 96, no. 1, pp. 69–75, Jul. 1997, doi: 10.1161/01.CIR.96.1.69.

[11] E. B.-M. Daliri, Y. Kim, Y. Do, R. Chelliah, and D.-H. Oh, "In Vitro and In Vivo Cholesterol Reducing Ability and Safety of Probiotic Candidates Isolated from Korean Fermented Soya Beans," *Probiotics Antimicrob. Proteins*, vol. 14, no. 1, pp. 87–98, Feb. 2022, doi: 10.1007/s12602-021-09798-0.

[12] T. Rittiphairoj, K. Pongpirul, K. Janchot, N. T. Mueller, and T. Li, "Probiotics Contribute to Glycemic Control in Patients with Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis," *Adv. Nutr.*, vol. 12, no. 3, pp. 722–734, May 2021, doi: 10.1093/advances/nmaa133.

[13] D. Xavier-Santos, R. Bedani, E. D. Lima, and S. M. I. Saad, "Impact of probiotics and prebiotics targeting metabolic syndrome," *J. Funct. Foods*, vol. 64, p. 103666, Jan. 2020, doi: 10.1016/j.jff.2019.103666.

[14] M. Shimizu, M. Hashiguchi, T. Shiga, H. Tamura, and M. Mochizuki, "Meta-Analysis: Effects of Probiotic Supplementation on Lipid Profiles in Normal to Mildly Hypercholesterolemic Individuals," *PLOS ONE*, vol. 10, no. 10, p. e0139795, Oct. 2015, doi: 10.1371/journal.pone.0139795.

[15] Z. Guo *et al.*, "Influence of consumption of probiotics on the plasma lipid profile: A meta-analysis of randomized controlled trials," *Nutr. Metab. Cardiovasc. Dis.*, vol. 21, no. 11, pp. 844–850, Nov. 2011, doi: 10.1016/j.numecd.2011.04.008.

[16] A. Ataie-Jafari, B. Larijani, H. Alavi Majd, and F. Tahbaz, "Cholesterol-Lowering Effect of Probiotic Yogurt in Comparison with Ordinary Yogurt in Mildly to Moderately Hypercholesterolemic

Commented [-6]: delete

Commented [-7]: delete

Commented [-8]: delete

Commented [-9]: delete

Commented [-10]: delete

Commented [-11]: delete

Commented [-12]: need 6 authors then et al.

Subjects," *Ann. Nutr. Metab.*, vol. 54, no. 1, pp. 22–27, 2009, doi: 10.1159/000203284.
[17] S. Usmiati and Risfaheri, "Pengembangan Dadih Sebagai Pangan Fungsional Probiotik Asli Sumatera Barat," *J. Litbang Pert*. Vol. 32 N0. 32, pp. 20-29, 2013.
[18] E. Purwati, S. N. Aritonang, S. Melia, I. Juliyarsi, and H. Purwanto, *Manfaat Probiotik Bakteri Asam Laktat Dadiah Menunjang Kesehatan Masyarakat*. Lembaga Pengembangan Teknologi Informasi dan Komunikasi (LPTIK) Universitas Andalas, 2017. doi: 10.25077/car.9.9.
[19] A. Kundu *et al.*, "Protective effects of Croton hookeri on streptozotocin-induced diabetic nephropathy," *Food Chem. Toxicol.*, vol. 135, p. 110873, Jan. 2020, doi: 10.1016/j.fct.2019.110873.
[20] R. Amelia *et al.*, "Characterization and probiotic potential of lactic acid bacteria isolated from Dadiah sampled in West Sumatra," *Food Sci. Technol.*, vol. 41 (Suppl., pp. 746–752, Dec. 2021, doi: 10.1590/fst.30020.
[21] Y. Wang, D. Dilidaxi, Y. Wu, J. Sailike, X. Sun, and X. Hua Nabi, "Composite probiotics alleviate type 2 diabetes by regulating intestinal microbiota and inducing GLP-1 secretion in db/db mice," *Biomed. Pharmacother.*, vol. 125, no. January, p. 109914, 2020, doi: 10.1016/j.biopha.2020.109914.
[22] X. Guo *et al.*, "NFκB promotes oxidative stress-induced necrosis and ischemia/reperfusion injury by inhibiting Nrf2-ARE pathway," *Free Radic. Biol. Med.*, vol. 159, pp. 125–135, 2020, doi: 10.1016/j.freadadbiomed.2020.07.031.
[23] H. T. Zeng *et al.*, "SIRT1 prevents atherosclerosis via liver-X-receptor and NF-κB signaling in a U937 cell model," *Mol. Med. Rep.*, vol. 8, no. 1, pp. 23–28, 2013, doi: 10.3892/mmr.2013.1460.
[24] L. Kong *et al.*, "Sirtuin 1: A Target for Kidney Diseases," vol. 1, pp. 87–97, 2015, doi: 10.2119/molmed.2014.00211.
[25] Harnavi Harun, Yanwirasti Yanwirasti, Bambang Purwanto, and Endang Purwati Rahayuningsih, "The Effect of Giving Dadih on Malondialdehyde Levels and Renal Interstitial Fibrosis at Aging Kidney," *Open Access Maced. J. Med. Sci.*, vol. 8, no. A, pp. 293–296, 2020, doi: 10.3889/oamjms.2020.4285.
[26] A. Guilbaud *et al.*, "The Effect of Lactobacillus fermentum ME-3 Treatment on Glycation and Diabetes Complications," *Mol. Nutr. Food Res.*, vol. 64, no. 6, pp. 1–13, 2020, doi: 10.1002/mnfr.201901018.
[27] A. A. Dumas, A. Lapointe, M. Dugrenier, V. Provencher, B. Lamarche, and S. Desroches, "A systematic review of the effect of yogurt consumption on chronic diseases risk markers in adults," *Eur. J. Nutr.*, vol. 56, no. 4, pp. 1375–1392, 2017, doi: 10.1007/s00394-016-1341-7.
[28] H. Zhong *et al.*, "Probiotics-fermented blueberry juice prevents obesity and hyperglycemia in high fat diet-fed mice in association with modulating the gut microbiota," *Food Funct.*, pp. 0–52, 2020, doi: 10.1039/d0fo00334d.
[29] E. Amirani, Z. Asemi, and M. Taghizadeh, "The effects of selenium plus probiotics supplementation on glycemic status and serum lipoproteins in patients with gestational diabetes mellitus: A randomized, double-blind, placebo-controlled trial," *Clin. Nutr. ESPEN*, no. xxxx, 2022, doi: 10.1016/j.clnesp.2022.02.010.
[30] L. Wang *et al.*, "The effects of probiotics on total cholesterol," *Med. U. S.*, vol. 97, no. 5, 2018, doi: 10.1097/MD.0000000000009679.
[31] S. M. Lim, J. J. Jeong, K. H. Woo, M. J. Han, and D. H. Kim, "Lactobacillus sakei OK67 ameliorates high-fat diet-induced blood glucose intolerance and obesity in mice by inhibiting gut microbiota lipopolysaccharide production and inducing colon tight junction protein expression," *Nutr. Res.*, vol. 36, no. 4, pp. 337–348, 2016, doi: 10.1016/j.nutres.2015.12.001.
[32] L. Wu *et al.*, "Involvement of miR-27a-3p in diabetic nephropathy via affecting renal fibrosis, mitochondrial dysfunction, and endoplasmic reticulum stress," *J. Cell. Physiol.*, no. July 2020, doi: 10.1002/jcp.29951.
[33] B. S. Sivamaruthi, P. Kesika, and C. Chaiyasut, "A mini-review of human studies on cholesterol-lowering properties of probiotics," *Scientia Pharmaceutica*, vol. 87, no. 4. 2019. doi: 10.3390/scipharm87040026.
[34] A. Khare and S. Gaur, "Cholesterol-Lowering Effects of Lactobacillus Species,"*Curr. Microbiol.*, vol. 77, no. 4, pp. 638–644, 2020, doi: 10.1007/s00284-020-01903-w.
[35] H. J. Zheng *et al.*, "The effect of probiotic and synbiotic supplementation on biomarkers of inflammation and oxidative stress in diabetic patients: A systematic review and meta-analysis of randomized controlled trials," *Pharmacol. Res.*, vol. 142, no. February, pp. 303–313, 2019, doi: 10.1016/j.phrs.2019.02.016.
[36] B. Bhat and B. K. Bajaj, "Multifarious cholesterol-lowering potential of lactic acid bacteria equipped with desired probiotic functional attributes," *3 Biotech*, vol. 10, no. 5, pp. 1–16, 2020, doi:

10.1007/s13205-020-02183-8.
[37] L. Merone and R. McDermott, “Nutritional anti-inflammatories in the treatment and prevention of type 2 diabetes mellitus and the metabolic syndrome,” *Diabetes Res. Clin. Pract.*, vol. 127, pp. 238–253, 2017, doi: 10.1016/j.diabres.2017.02.019.
[38] B. Xue *et al.*, “Microbial diversity analysis of vineyard on the eastern foothills of the Helan Mountain region using high-throughput sequencing,” *Food Sci. Technol.*, vol. 42, pp. 1–11, 2022, doi: 10.1590/fst.66320.

TRENDS IN SCIENCES 20xx; 20(xx): xxxxx RESEARCH ARTICLE
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 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

Commented [LC1]: Should check the latest data

Commented [LC2]: DM is not only of two types

Commented [LC3]: Recheck the fact

Commented [LC4]: Abbreviated wrongly

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].

Commented [LC5]: Should be properly sequenced

Commented [LC6]: Should be sequenced properly

Commented [LC7]: A few more details should be added - as it Is the primary factor of research

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.

Commented [LC8]: Standard reference should be added

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.

Commented [LC9]: Check the temperature

Commented [LC10]: Reference should be added

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.

Commented [LC11]: Basis for determining the sample number?

Commented [LC12]: Alloxan induces type 1 DM but the expt is on type 2 , Justification is required?

Commented [LC13]: Basis for determining the control group

Commented [LC14]: Standard reference?

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.

Commented [LC15]: Why tail is required to cut? When the blood sample can be collected to retro orbital

Commented [LC16]: ND - means?

Commented [LC17]: reference

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).

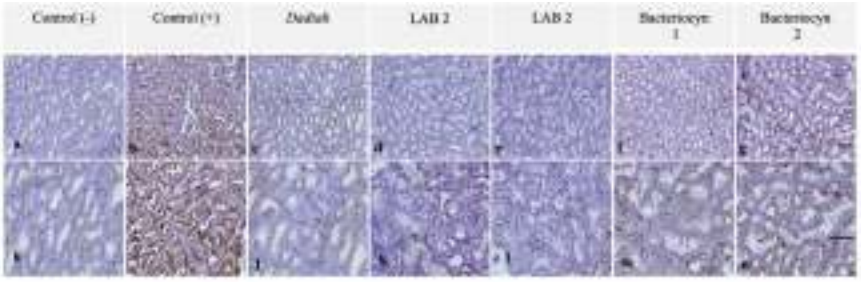

Figure 1 The NF-kB expression with immunohistochemistry in the kidney

Commented [LC18]: reference

Commented [LC19]: reference

Commented [LC20]: reference

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.

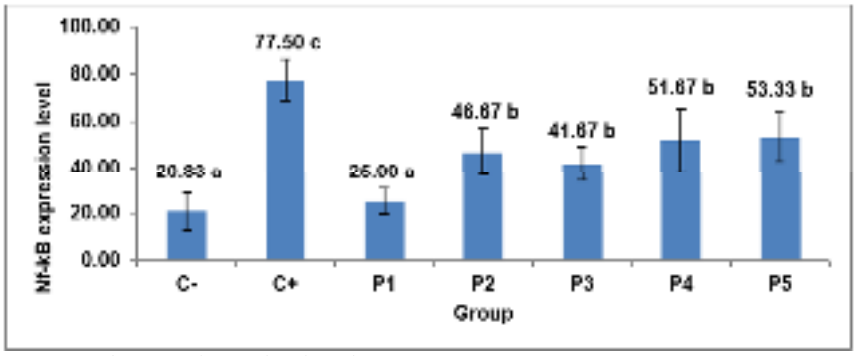

Figure 2 NF-kB expression numbers in each group

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**).

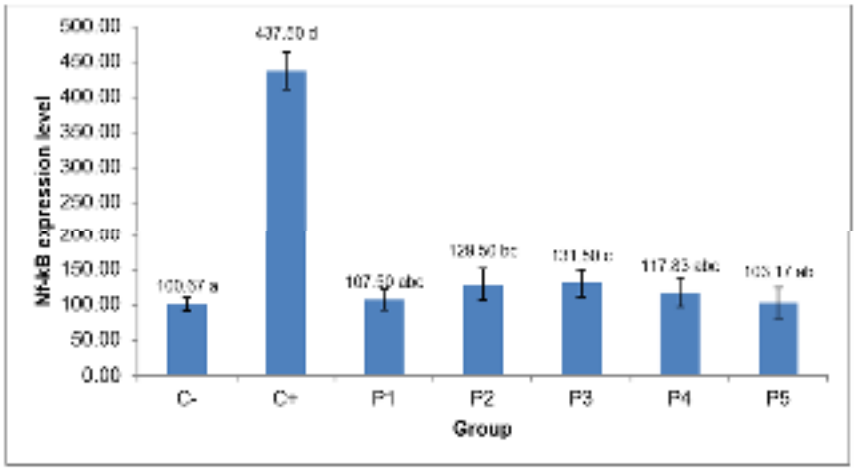

Figure 3 Blood glucose levels in each treatment

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.

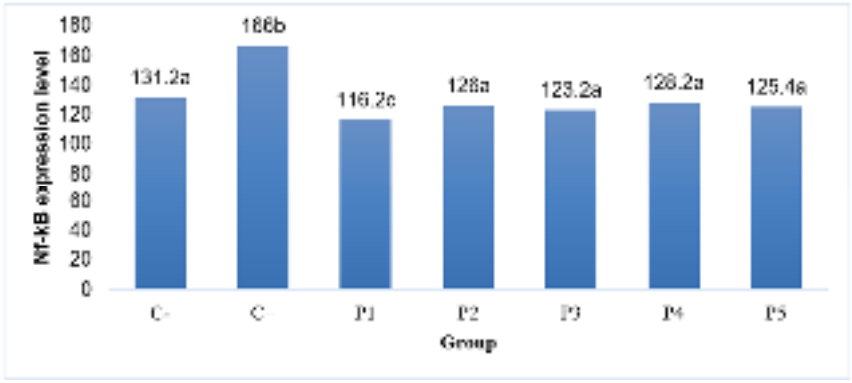

Figure 4. Serum cholesterol levels in each group

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.

Acknowledgments

The authors would like to thank Prof. drh. Hj. Endang Purwati, MS, Ph. D from the Laboratory of Animal Husbandry Biotechnology/Technology Animal Product, Universitas Andalas, Padang, West Sumatra, Indonesia. Thank dr. Tofrizal Sp. PA., Ph. D from the Department of Pathological Anatomy, Universitas Andalas, Padang, West Sumatra, Indonesia. for assistance on histopathological analysis. Thanks to Universitas Baiturrahmah, Padang, West Sumatra, Indonesia foundation has helped finance research.

References

[1] A. T. Kharroubi, "Diabetes mellitus: The epidemic of the century," *World J. Diabetes*, vol. 6, no. 6, p. 850, 2015, doi: 10.4239/wjd.v6.i6.850.

[2] Y. Zheng, S. H. Ley, and F. B. Hu, "Global etiology and epidemiology of type 2 diabetes mellitus and its complications," *Nat. Rev. Endocrinol.*, vol. 14, no. 2, pp. 88–98, Feb. 2018, doi: 10.1038/nrendo. 2017.151.

[3] E. C. Westman, "Type 2 Diabetes Mellitus: A Pathophysiologic Perspective," *Front. Nutr.*, vol. 8, p. 707371, Aug. 2021, doi: 10.3389/fnut.2021.707371.

[4] M. Li, Q. Guo, H. Cai, H. Wang, Z. Ma, and X. Zhang, "miR-218 regulates diabetic nephropathy via targeting IKK-β and modulating NK-κB-mediated inflammation," *J. Cell. Physiol.*, vol. 235, no. 4, pp. 3362–3371, Apr. 2020, doi: 10.1002/jcp.29224.

[5] W. Yu *et al.*, "The gut microbiome in the differential diagnosis of diabetic kidney disease and membranous nephropathy," *Ren. Fail.*, vol. 42, no. 1, pp. 1100–1110, Jan. 2020, doi: 10.1080/0886022X.2020.1837869.

[6] H. Sawaf, G. Thomas, J. J. Taliercio, G. Nakhoul, T. J. Vachharajani, and A. Mehdi, "Therapeutic Advances in Diabetic Nephropathy," *J. Clin. Med.*, vol. 11, no. 2, p. 378, Jan. 2022, doi: 10.3390/jcm11020378.

[7] M. Ruiz-Ortega, R. R. Rodrigues-Diez, C. Lavoz, and S. Rayego-Mateos, "Special Issue 'Diabetic Nephropathy: Diagnosis, Prevention and Treatment,'" *J. Clin. Med.*, vol. 9, no. 3, p. 813, Mar. 2020, doi: 10.3390/jcm9030813.

[8] T. Kocher, J. König, W. S. Borgnakke, C. Pink, and P. Meisel, "Periodontal complications of hyperglycemia/diabetes mellitus: Epidemiologic complexity and clinical challenge," *Periodontol. 2000*, vol. 78, no. 1, pp. 59–97, Oct. 2018, doi: 10.1111/prd.12235.

[9] R. J. Galindo, M. Fayfman, and G. E. Umpierrez, "Perioperative Management of Hyperglycemia and Diabetes in Cardiac Surgery Patients," *Endocrinol. Metab. Clin. North Am.*, vol. 47, no. 1, pp. 203–222, Mar. 2018, doi: 10.1016/j.ecl.2017.10.005.

[10] G. Davì *et al.*, "Diabetes Mellitus, Hypercholesterolemia, and Hypertension but Not Vascular Disease Per Se Are Associated with Persistent Platelet Activation In Vivo: Evidence Derived fromthe Study of Peripheral Arterial Disease," *Circulation*, vol. 96, no. 1, pp. 69–75, Jul. 1997, doi: 10.1161/01.CIR.96.1.69.

[11] E. B.-M. Daliri, Y. Kim, Y. Do, R. Chelliah, and D.-H. Oh, "In Vitro and In Vivo Cholesterol Reducing Ability and Safety of Probiotic Candidates Isolated from Korean Fermented Soya Beans," *Probiotics Antimicrob. Proteins*, vol. 14, no. 1, pp. 87–98, Feb. 2022, doi: 10.1007/s12602-021-09798-0.

[12] T. Rittiphairoj, K. Pongpirul, K. Janchot, N. T. Mueller, and T. Li, "Probiotics Contribute to Glycemic Control in Patients with Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis," *Adv. Nutr.*, vol. 12, no. 3, pp. 722–734, May 2021, doi: 10.1093/advances/nmaa133.

[13] D. Xavier-Santos, R. Bedani, E. D. Lima, and S. M. I. Saad, "Impact of probiotics and prebiotics targeting metabolic syndrome," *J. Funct. Foods*, vol. 64, p. 103666, Jan. 2020, doi: 10.1016/j.jff.2019.103666.

[14] M. Shimizu, M. Hashiguchi, T. Shiga, H. Tamura, and M. Mochizuki, "Meta-Analysis: Effects of Probiotic Supplementation on Lipid Profiles in Normal to Mildly Hypercholesterolemic Individuals," *PLOS ONE*, vol. 10, no. 10, p. e0139795, Oct. 2015, doi: 10.1371/journal.pone.0139795.

[15] Z. Guo *et al.*, "Influence of consumption of probiotics on the plasma lipid profile: A meta-analysis of randomized controlled trials," *Nutr. Metab. Cardiovasc. Dis.*, vol. 21, no. 11, pp. 844–850, Nov. 2011, doi: 10.1016/j.numecd.2011.04.008.

[16] A. Ataie-Jafari, B. Larijani, H. Alavi Majd, and F. Tahbaz, "Cholesterol-Lowering Effect of Probiotic Yogurt in Comparison with Ordinary Yogurt in Mildly to Moderately Hypercholesterolemic Subjects," *Ann. Nutr. Metab.*, vol. 54, no. 1, pp. 22–27, 2009, doi: 10.1159/000203284.

[17] S. Usmiati and Risfaheri, "Pengembangan Dadih Sebagai Pangan Fungsional Probiotik Asli Sumatera Barat," *J. Litbang Pert*. Vol. 32 N0. 32, pp. 20-29, 2013.
[18] E. Purwati, S. N. Aritonang, S. Melia, I. Juliyarsi, and H. Purwanto, *Manfaat Probiotik Bakteri Asam Laktat Dadiah Menunjang Kesehatan Masyarakat*. Lembaga Pengembangan Teknologi Informasi dan Komunikasi (LPTIK) Universitas Andalas, 2017. doi: 10.25077/car.9.9.
[19] A. Kundu *et al.*, "Protective effects of Croton hookeri on streptozotocin-induced diabetic nephropathy," *Food Chem. Toxicol.*, vol. 135, p. 110873, Jan. 2020, doi: 10.1016/j.fct.2019.110873.
[20] R. Amelia *et al.*, "Characterization and probiotic potential of lactic acid bacteria isolated from Dadiah sampled in West Sumatra," *Food Sci. Technol.*, vol. 41 (Suppl., pp. 746–752, Dec. 2021, doi: 10.1590/fst.30020.
[21] Y. Wang, D. Dilidaxi, Y. Wu, J. Sailike, X. Sun, and X. Hua Nabi, "Composite probiotics alleviate type 2 diabetes by regulating intestinal microbiota and inducing GLP-1 secretion in db/db mice," *Biomed. Pharmacother.*, vol. 125, no. January, p. 109914, 2020, doi: 10.1016/j.biopha.2020.109914.
[22] X. Guo *et al.*, "NFκB promotes oxidative stress-induced necrosis and ischemia/reperfusion injury by inhibiting Nrf2-ARE pathway," *Free Radic. Biol. Med.*, vol. 159, pp. 125–135, 2020, doi: 10.1016/j.freadadbiomed.2020.07.031.
[23] H. T. Zeng *et al.*, "SIRT1 prevents atherosclerosis via liver-X-receptor and NF-κB signaling in a U937 cell model," *Mol. Med. Rep.*, vol. 8, no. 1, pp. 23–28, 2013, doi: 10.3892/mmr.2013.1460.
[24] L. Kong *et al.*, "Sirtuin 1: A Target for Kidney Diseases," vol. 1, pp. 87–97, 2015, doi: 10.2119/molmed.2014.00211.
[25] Harnavi Harun, Yanwirasti Yanwirasti, Bambang Purwanto, and Endang Purwati Rahayuningsih, "The Effect of Giving Dadih on Malondialdehyde Levels and Renal Interstitial Fibrosis at Aging Kidney," *Open Access Maced. J. Med. Sci.*, vol. 8, no. A, pp. 293–296, 2020, doi: 10.3889/oamjms.2020.4285.
[26] A. Guilbaud *et al.*, "The Effect of Lactobacillus fermentum ME-3 Treatment on Glycation and Diabetes Complications," *Mol. Nutr. Food Res.*, vol. 64, no. 6, pp. 1–13, 2020, doi: 10.1002/mnfr.201901018.
[27] A. A. Dumas, A. Lapointe, M. Dugrenier, V. Provencher, B. Lamarche, and S. Desroches, "A systematic review of the effect of yogurt consumption on chronic diseases risk markers in adults," *Eur. J. Nutr.*, vol. 56, no. 4, pp. 1375–1392, 2017, doi: 10.1007/s00394-016-1341-7.
[28] H. Zhong *et al.*, "Probiotics-fermented blueberry juice prevents obesity and hyperglycemia in high fat diet-fed mice in association with modulating the gut microbiota," *Food Funct.*, pp. 0–52, 2020, doi: 10.1039/d0fo00334d.
[29] E. Amirani, Z. Asemi, and M. Taghizadeh, "The effects of selenium plus probiotics supplementation on glycemic status and serum lipoproteins in patients with gestational diabetes mellitus: A randomized, double-blind, placebo-controlled trial," *Clin. Nutr. ESPEN*, no. xxxx, 2022, doi: 10.1016/j.clnesp.2022.02.010.
[30] L. Wang *et al.*, "The effects of probiotics on total cholesterol," *Med. U. S.*, vol. 97, no. 5, 2018, doi: 10.1097/MD.0000000000009679.
[31] S. M. Lim, J. J. Jeong, K. H. Woo, M. J. Han, and D. H. Kim, "Lactobacillus sakei OK67 ameliorates high-fat diet-induced blood glucose intolerance and obesity in mice by inhibiting gut microbiota lipopolysaccharide production and inducing colon tight junction protein expression," *Nutr. Res.*, vol. 36, no. 4, pp. 337–348, 2016, doi: 10.1016/j.nutres.2015.12.001.
[32] L. Wu *et al.*, "Involvement of miR-27a-3p in diabetic nephropathy via affecting renal fibrosis, mitochondrial dysfunction, and endoplasmic reticulum stress," *J. Cell. Physiol.*, no. July 2020, doi: 10.1002/jcp.29951.
[33] B. S. Sivamaruthi, P. Kesika, and C. Chaiyasut, "A mini-review of human studies on cholesterol-lowering properties of probiotics," *Scientia Pharmaceutica*, vol. 87, no. 4. 2019. doi: 10.3390/scipharm87040026.
[34] A. Khare and S. Gaur, "Cholesterol-Lowering Effects of Lactobacillus Species,"*Curr. Microbiol.*, vol. 77, no. 4, pp. 638–644, 2020, doi: 10.1007/s00284-020-01903-w.
[35] H. J. Zheng *et al.*, "The effect of probiotic and synbiotic supplementation on biomarkers of inflammation and oxidative stress in diabetic patients: A systematic review and meta-analysis of randomized controlled trials," *Pharmacol. Res.*, vol. 142, no. February, pp. 303–313, 2019, doi: 10.1016/j.phrs.2019.02.016.
[36] B. Bhat and B. K. Bajaj, "Multifarious cholesterol-lowering potential of lactic acid bacteria equipped with desired probiotic functional attributes," *3 Biotech*, vol. 10, no. 5, pp. 1–16, 2020, doi: 10.1007/s13205-020-02183-8.

[37] L. Merone and R. McDermott, "Nutritional anti-inflammatories in the treatment and prevention of type 2 diabetes mellitus and the metabolic syndrome," *Diabetes Res. Clin. Pract.*, vol. 127, pp. 238–253, 2017, doi: 10.1016/j.diabres.2017.02.019.

[38] B. Xue *et al.*, "Microbial diversity analysis of vineyard on the eastern foothills of the Helan Mountain region using high-throughput sequencing," *Food Sci. Technol.*, vol. 42, pp. 1–11, 2022, doi: 10.1590/fst.66320.

https://doi.org/

The Potential of West Sumatr an *Dadiah* as The Novelto Alleviate Hyperglycemia , Hypercholesterolemia, and Reducing NF-kB ExpressioninNephropathyDiabetesRat Model

Highlights

Dadiahis 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 reducedhyperglycemiaand 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 micro vascular 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- kBin kidney tissue due to chronic hyperglycemia and hypercholesterolemia. Hyperglycemia isrelated to change sin 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 naturallyfermented buffalo milk product in bamboo tubes. Dadiah comes from West Sumatra, Indonesia, there has not been much research on the clinica land biomolecular effects of various metabolic diseases .Even though this probiotic has health benefits,its mechanism as an antidiabetic is not widely known . This study aim store duce 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 ondiabetic rats induced by intra peritoneal Alloxan 100 mg/kg b. w. ND rats were treated with low and high doses of Dadiah ,LAB (Lactic Acid Bacteria),and Bacterioc in for eight weeks. Next ,we tested their effect on blood glucose levels ,serum cholesterol, and NF-kB antibody expression in kidney tissue using immune his to chemistry 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 micro vascular complications and hypercholesterolemia.

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

Introduction

DiabetesMellitus(DM)isacauseofprematuredeath,blindness,heartdisease,andkidneyfailureforsufferers . 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 millionpeoplein2035. DM isagroupofmetabolicdiseasesthatcausechronichyperglycemia[1].DMconsistsof2 types, namely, type 1 DM (T1DM) as a result of an autoimmune reaction to pancreatic cell proteins, andtype 2 DM (T2DM) as a result of a combination of genetic factors and environmental factors, such asobesity,overeating,lackoffood,exercise,stress,andaging[2].Generally,patientswithT2DMexperiencecomp lications, 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].

Diabeticnephropathy(ND)isaconditionofdecreasedkidneyfunctionandthemaincauseofend-stagekidneydisease[6].DNistriggeredbygenetic,environmental,cellular,andmolecularmechanismsthatplaya roleinkidneydamageindiabetes[7].DNisaclinicalsyndromecharacterizedbypersistentalbuminuriaandprogres sivedeclineinkidneyfunction.50%ofpatientswithDNwillexperienceend-

stagekidneydisease(ESKD)requiringtreatmentwithdialysisorkidneytransplantationwhichisassociatedwithsignificantlyincreasedcardiovascularmorbidityand mortality.Themainrisk factorsforthedevelopmentof DN are chronic hyperglycemia, hypercholesterolemia, and reduced expression of NF kappa B (NF-kB).ChronichyperglycemiainDMsufferersisfollowedbydamage,andimpairedfunctionoftheeyes,kidneys,nerves,heart,andbloodvessels.Thediagnosisofdiabetesmellitusismadebasedonthehighlevelofglucoseintheblood plasma[8-9].
Hypercholesterolemiacanleadtoatherosclerosis,coronaryheartdisease,pancreatitis,thyroiddisorders,liverdisease&disease[10].NF-kBplaysaroleinthedevelopmentandvariouscomplicationsofDMforsufferers, such as diabetic cardio myopathy, retinopathy ,nephropathy ,and DM neuropathy. Many therapeutic approaches for DM sufferers have been developed, such as the useofseveralantioxidants,flavonoids,andprobiotics.Probioticsarepromisingcandidatesforimprovingglycemicmanagement,inflammatorysystems,andlipidprofilesinindividualswithtype2diabetes.Probioticsupplementationimprovesglycemiccontrolandcardiometabolicriskmarkers.Onepossiblemechanismforthehypocholesterolemiaimpactofprobioticshasbeensuggestedbydirectcholesterolinteractionorassimilationbyprobiotics[11].
Theeffectivenessandsafetyofprobioticsforglycemic
controlin patientswithimpairedglucosecontrol,includingprediabetesandtype2diabetesmellitus[12].

Many studieshaveusedexperimentalmodelstoevaluatetheimpactofsupplementationwithprobiotics and prebiotics on various risk factors for metabolic syndrome [13]. The search for safer non-pharmacologicaltherapieswithcholesterol-loweringeffectscontinues tobecarriedoutby utilizingbacteria. Probiotic bacteria from the lactic acid group and Bifidobacterium can regulate serum cholesterolpotential [14]. The results of the study showed that Gaio was able to lower cholesterol. Gaio is a yogurtproduct 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 atraditional food.

Dadiah is a type of traditional fermented milk and has the potential to be developed as a functionalfood 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 existin nature as anino culantor with out
astarter. 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].
TheuseofantioxidantsinDMcasesneedstobeconsideredtopreventthedevelopmentof 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 toreducebloodsugarlevels,cholesterol,and theinflammatorymarkerNF-kB.

Materials and methods

Instruments of research

The instruments of research digital scale (ACS) with 0.01gram accuracy to weigh rats. Experimentalanimalcages,foodandwatercontainersforexperimentalanimals,sondetoinjectDadiahandLABisolatedsampled from Dadiah 1 ml/day and 2 ml/day. The tools used in this research are a luminometer, pipettes,microscope,microtome,slide glass, razorblades/scissors, aluminum foil, metal basket, rotary tissueprocessor,refrigerator,waterheater,processorcassette,autoclave(Hirayama),incubator(Fisher),hotplate, Eppendrof, bunsen, vortex, Erlenmeyer tubes, glucometer (Glucose blood level and cholesterol) and urineproteinstick(UriScan).

Experimentalanimals

Wistar-strain male white rats (*Rattusnorvegicus*) aged 2-3 months with a weight of ± 300-gram,standard feed as daily food, and Ad libitium drinking water. Dadiah. Lactic Acid Bacteria danbacteriocinisolated sampled in Dadiah from TanjungBonai, Lintau Tanah Datar, West Sumatra. The examinationresultsobtained informationthatDadiahcontainedLacticAcidBacteriaof7,1X1010CFU/g[20].

PreparationofDadiah

Dadiahdosageforrats=conversionvaluexDadiahdosageforhumans.Thedosageofadministration,basedon therecommendeddosageoffermentedmilkinhumanswithabodyweightof70kg,was100-200mLperday. Thedensity(ρ)ofDadiahwas1.04g/mL.TherecommendedDadiahdosageis104g/70kgofhuman.TheLaurencet able(2008),theconversion valueof70 kgofHuman weightto200gofRatweightis0,018.Thecalculation ofDadiahdosagefortheratisDadiah dosageforrat=conversionvaluexDadiahdosage 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 ofDadiah/200gofRatweight=9.35g/kgb.w.Theweightofthemalewhiterat(*Rattusnorvegicus*)is±300g=0,3kg Dadiahsolutioncontaining1g/mLwasmadebysuspendingDadiahwithaquadest.The material in this experimentalstudyisDadiah3mL.

PreparationofLAB

Isolate *L. fermentum*is rejuvenated first, then propagated in the medium MRS broth at a temperatureof 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 x24hoursintheincubator tofindoutthenumberofLABtobeinduced.Followingprevious*invitro*research obtained for1gDadiah, thereisaLABcolonyof7.1x10^{10} CFU/mL.

Preparationof Bacteriocin(ProductionofcrudeBacteriocin)

The LAB of Dadiah was cultivated in MRS broth (1000 ml) seeded with 10% inoculum of overnightculture and incubated at 37ºC for 24 hours. Following incubation, the entire broth was centrifuged for 16minutes at 10.000 X g find the cell-free supernatant was used as crude Bacteriocin [21]. The amount ofLABandBacteriocinusedinthis studywas1mL and2mLperday.

Methods

Thisresearchisanexperimentalstudybaseonanimaltrialswithapost-test-onlycontrol-groupdesign.Male*Rattusnorvegicus*strainwistarratswereprocuredfromPharmacologyDepartment,Universit asAndalas,Padang,WestSumatra,Indonesia.Theresearchsampleshavethecriteria,healthywithglowingeyes,ac tiveandhavingagoodappetite,2-3monthsold,andweigh200-300grams.Allratsweremaintainedat23-25ºC,withbothastandardpelletdietandwateradlibitum.Afteracclimatizationfortwoweeks,exceptforthenegati vecontrolgroup,allothergroupswereinjectedwithalloxan100mg/Kgb.w.Allgroups of micewere fed withstandard pellets.Furthermore,the treatment group willbe givenDadiah,LAB, and Bacteriocin. The experiment was conducted with five treatment groups and two control groups.In this study,ratsweredividedintofivegroups withthenumberofeachgroupofsixrats.Sothatsix DNratsweretreatedwithDadiah3mL/day(P1group)andisolatedsamplesofLABandBacteriocinfromDadiah 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 alloxaninjection).Thenumberofsamplesobtainedby42rats.

Induction of diabetesand *in vivo* experimental

Before the experiment began, all the rats were weighed, and measured blood glucose levels were cutofftherat'stail's1mmend.Afterthat,theblooddroppedontheglucosestickoftheglucometer(OneTouchMerck; accuracyISO 15197:2003)andthetestofproteinuriabyUriScanTestStrips(Biosys Laboratories,INC). After all the data have recorded, we had the first experiment that made rats into clinically markedND for hyperglycemia (> 200 mg/dL) and proteinuria. In a preliminary study, rats kept on fasting for 12hours received a single injection of freshly dissolved alloxan in 1.0 mL of sodium citrate buffer (0.1 M pH4.5)intraperitoneally(i.p),atarateof100mg/kgb.w.Thebloodwaswithdrawnfromthetailveinofrats,thenthem easurementoffastingbloodglucoseconcentrationandcholesterolserumeverytwoweeksalongthe experimental protocol (56 days/8 weeks). After 7 days of alloxan induction, animals with fastingglucose > 200 mg/dL and proteinuria were considered diabetic nephropathy and grouped accordingly withan average of 6 rats per group and orally administered with Dadiah, LAB, and Bacteriocin isolated fromDadiahforeightweeks or56consecutivedays.

The dissectionof experimentalanimals

Dissectionwasperformedafter56daysoftreatmentisgivenwheremalewhiterats(*Rattusnorvegicus*) were killed using Anesthesia with ether. The method was by mixing the concentrated ethersolution with 2% NaCl solvent or 10-25% in NaCl and a dose of 300 mg/kg or 1-1.25 g/kg placed on thebottom of the desiccator. Then put the rat in a closed container, wait until it became immobile, and itspupillary mydriasis and eyes were closed. If the rat lost consciousness, then broughtride inside thecontainer, then laparotomy and neck pressure were done to kill it while pulling it anteriorly (dislocationAtlanta-occipitalis. Identification and nephrectomy were carried out, then directly put into a 10% BNFsolution,afterthekidneyorganwasremoved.

Tissue processing

Rat renal tissue was processed into paraffin blocks and cut with a microtome with a thickness of 4mm. The preparations were stained with hematoxylin-eosin and Sirius red. Measurements were taken byphoto-shooting hematoxylin-eosin preparations with Olympus BX 51 light microscope at 400x (objective40x)and1000x(objective100x)magnifications.Photomicrographsweretakeninrepresentativeareas.

Thetechniquesofimmunohistochemicalpreparations

Kidneys were removed, trimmed, and weighed and the relative weight of the organ was calculated.Therelativeweightoftheorgan(%)wascalculatedasgram/100gramofbodyweight.Specimensfromt hekidneywerefixated immediatelyin10%buffered formalin forimmunohistochemicaltestingofNF-kB.

Data analyze

A comparison of the test was conducted using the average difference test, namely the one-wayANOVA 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 Wallistest .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 thetwo 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 andpeptides inhibiting NF-kB expression in rat kidney tissue modeled diabetic. Dadiah's clinical efficacy inlowering blood sugar and serum cholesterol indicates that it may be used as a future therapy to preventdiabeticprogression.

TheNF-kBexpressionwithimmunohistochemistryinthekidney

The expressions of NF-kB appeared brown on the IHC staining and the staining pattern was mainlyintheformofcytoplasmicstaining (**Figure1**).Themicroscopicassessmentused theOlympusBX51lightmicroscopeat400xmagnification(40xobjective)byassessingthepositiveintracytoplas micbrownstainingon the representative area. Each sample was observed in 5 different fields of view. In each field of view(40xobjective).

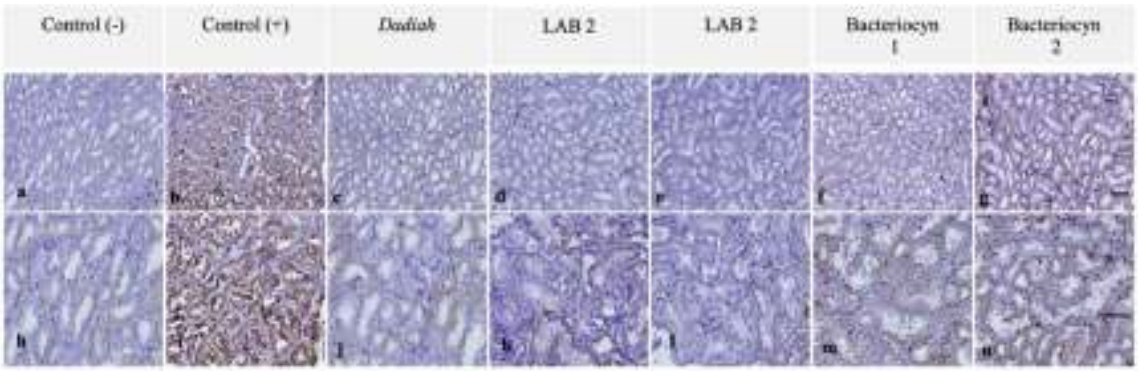

Figure1 TheNF-kBexpressionwith immunohistochemistryin thekidney

The proportion of epithelial cells with positive intracytoplasmic brown staining was calculated, thencompared 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 theanimal 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 inthecontrolanimalswithweaktomoderateexpressions,andsomecellsinthestromaandendothelium.Theinducti onwith alloxanshowedanincrease inthe NF-kB expressionwith mostof the tubularcellsexpressing moderate to strong. The treatment in the animal model showed a decrease in the NF-kBexpressioninthetissuescomparedtothepositivecontrol,bothadministeredbyDadiah,lacticacidbacteria,and Bcteriocin. The NF-kB expression appeared to be lower in the treatment with Dadiah compared toother treatments. The immune peroxidase, using the low magnification with 10x objective lens (top) andthehighmagnificationwith40xobjectivelens(bottom)at200µmscale.

NF-kBexpressionnumbersineachgroup

NF-κBisatranscriptionfactorthatregulatesthegeneexpressionofseveralproinflammatoryproteins.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(notinducedbyalloxanandnotgivenatreatment),namely20.83±8.01.Toprovewhethertherewasastati sticallysignificantdifferenceintheaveragenumberofNF-kBexpressions,theKruskalWallisstatisticalanalysiswouldbecarriedout.BasedontheresultsoftheKruskalWalli stest,thep-valuewassmallerthan(0.000<0.050),soitcanbeconcludedthattherewasasignificantdifference in the average NF-kB expression number between treatments. It was observed that the positivecontrol groups had significantly higher averages of NF-kB expression than the C-, P1, P2, P3, P4, and P5groups.

Conversely, the hostile control groups (C-) had considerably lower average NF-kB expression thanthe positive control groups (C+), P2, and P3 but were not significantly different from the P1, P4, and P5groups.NF-kBisacorenucleartranscriptionfactorintheinflammatoryresponse,increasingtheexpressionof various cytokines and chemical substances involved in the formation and development of ND, Inaddition, a more recent study found that antioxidants inhibited the activity of NF-kB and decreased theproductionofparticularpro-inflammatorymediators,especiallytheTumorNecrosisFactorandInterleukin-6(TNFandIL-6)[22, 23]. NF-kBisaubiquitouslydistributedtranscriptionfactorthataffectsinflammation,apoptosis,adhesion,angiogenesis,a ndcyclecells. Inflammationisoneofthekeymechanisms responsible for the development and progression of ND. Many inflammation-related proteinsareregulatedbyNF-kB[24].Dadiah isknowntocontainprobioticsandantioxidants,soithasbeenproventhat Dadiah can reduce oxidative stress and inflammation.

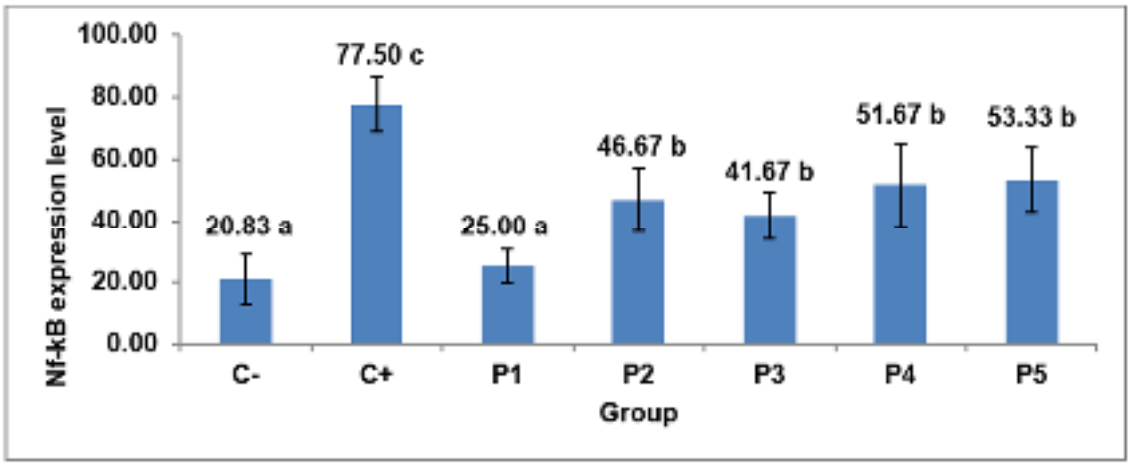

Figure2NF-kBexpressionnumbersin eachgroup

Even Dadiah itself contains a peptide that canstimulateendogenousantioxidantstoinhibittheproductionofNF-kB. AdministrationofDadiah,anisolateof lactic acid bacteria, and Bacteriocin has been shown to reduce macrophage activation in the productionof

proinflammatory cytokines. In addition, NF-kB expression shown in immunohistochemical examinationofkidneytissuedecreasessignificantlyclosetothenegativecontrol[25].

Bloodglucoselevels

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(**Figure3**).

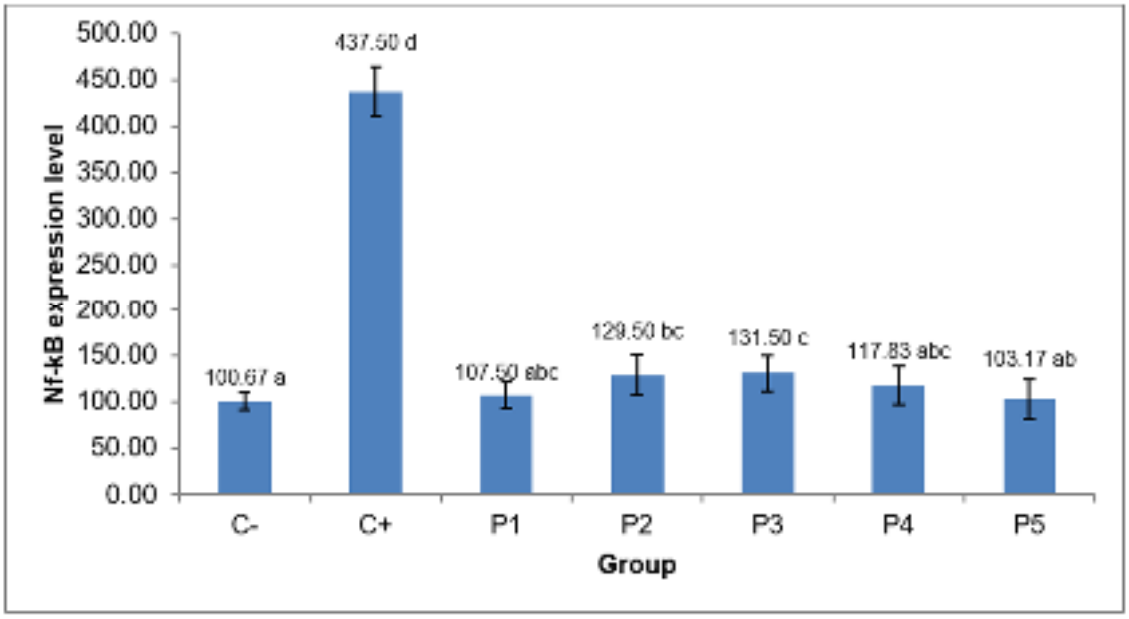

Figure 3 Blood glucose levels in each treatment

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 glucoselevelwasintheC– treatment(notinducedbyalloxanandnotgivenanytreatment),namely100.67±9.05.Thehighestaverageofblood glucoselevelsintheC+treatmentwassignificantlydifferentfromthe C-, P1, P2, P3, P4, and P5 treatments. The lowest average of blood glucose levels in the C- treatmentwas significantly different from the C+, P2, and P3 treatments, but the C- treatment was not significantlydifferentfromtheP1,P4,andP5treatments.Hyperglycemia-inducedoxidativestresshasbeenlinkedto various diabetes complications, including ND. There is significant evidence that oxidative stress and theinflammatory response have a role in DN development. Sustained hyperglycemia induces oxidative stressandgeneratessubstantialreactiveoxygenspecies(ROS)inrenaltissues,activatingthenucleartranscriptionfactorNF-kBandresultinginkidneyinflammation.Probiotic-based antidiabetic therapy has been proposed,anditsinfluenceonglycationisbeingexplored.*L.fermentum*ME-3maybeusedtherapeutically to inhibit the formation/accumulation of certain glycation products in the kidneys and toamelioratecertainfrequentdisease-relatedcomplications[26].

Probiotic-fermentedblueberryjuiceprotectsmicefedahigh-fatdietfromobesityandhyperglycemiabyalteringthegutflora.Inaddition,inHFD-fedmice,blueberryjuicesmarkedlyimprovedhyperlipidemiaand 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. Increasedblood 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 significantlyloweredfastingbloodglucose($p = 0.01$)andHbA1c($p = 0.05$)levelsandboostedtheactivitiesoferythrocytesuperoxide dismutase and glutathione peroxidase. Probiotic Yogurt made with *Lactobacillus acidophilus*and *Bifidobacteriumlactis*. These data imply that probiotic Yogurt is a functional food with potential anti-diabetic and antioxidant effects [24]. Furthermore, other research investigated whether giving probioticsandseleniumtoGDMpatientsforsix weeksimproved theirhyperglycemicstatusandlipidprofiles[29].

Severalstudiesdemonstratethattreatingdiabetespatientswith Voglibose(0.3mg/kg)andprobiotics(75mg

/kg)significantlydecreasedbloodglucoseandtotalcholesterollevelswhencomparedtothediabetesgrouptreated withonlyVoglibose(0.3mg/kg). Similarly,researchindicatesthatadministeringprobioticLsakei OK67 effectively prevents hyperglycemia development. the anti-diabetic effects of 14 probiotics indb/dbmiceresultedinimprovedintestinalbarrierfunctionandincreasedGLP-1production,indicatingthatthese probiotics may be suitable for preventing and treating diabetes. Other studies have discovered thatconsuming probiotic Yogurt can help lower fasting blood glucose levels. These findings suggest thatconsuming probiotic Yogurt regularly may have a beneficial effect on treating metabolic syndrome [30];[31, 32].

Serum cholesterol levels

The resultofserumcholesterollevelsshowedthatthe C+grouphasthe highest averagecholesterolof166.42,whiletheP1groupastreatedwithDadiahhasthelowestaveragecholesterolof116.2 4.66(**Figure4**).Toproveastatisticallysignificantdifferenceinaveragecholesterol,aKruskal Wallisstatistical analysis will be performed. Based on the Kruskal Wallis test results, we obtained a p-valuesmaller than α (0.003 < 0.050), so it can be concluded that there is a significant difference in averagecholesterolbetweentreatments.

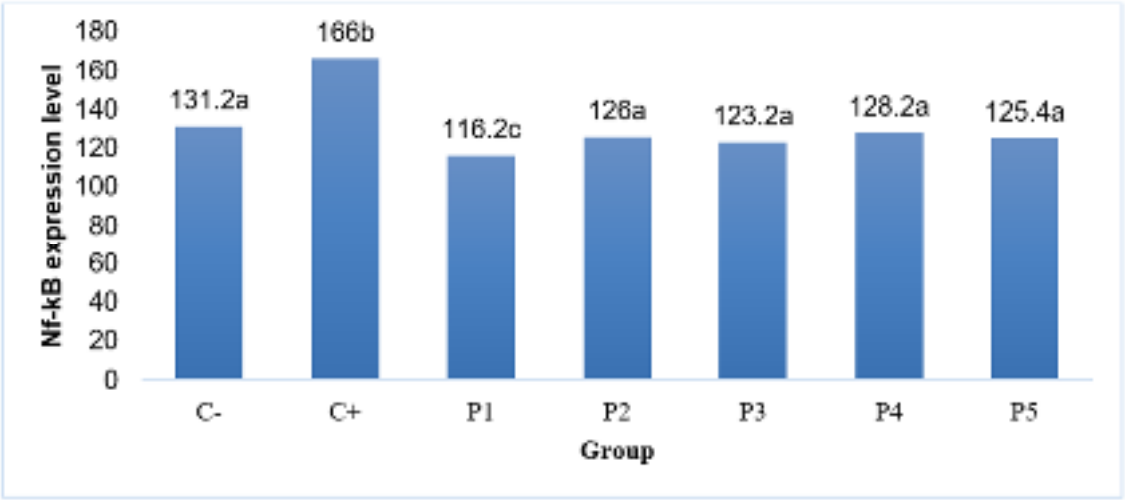

Figure 4. Serum cholesterol levels in each group

This study shows that the group of rats given the treatment of Dadiah can lower the cholesterol levels ofmice-modeled diabetic nephropathy compared to other groups. Lactobacillus species are the most oftenutilized bacteria in probiotic treatments, and studies have shown that they can decrease cholesterol levelsin humans. Consumption of probiotics may have a positive effect on managing cholesterol levels. Theconsumptionofprobioticyogurt(300gperday)containing*L.acidophilus*La5(~4.14×106CFU/g)and*B.lacti s*Bb12 (~3.61 × 106 CFU/g) for six weeks significantly improved the lipid profile of type 2 diabetesmellitus (T2D) patients. In addition, the results suggested that the regular consumption of probiotic yogurtcouldimprovethecholesterollevelofT2Dpatients.

The study concluded that probiotic consumption amended the glycemic control, inflammatorysystem, and lipid profile in T2D subjects[33-35]. In vitro studies have also shownthat 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, totalcholesterol, andox-LDL levels reduceddramatically,while HDL cholesterolshowedapotentialtoimprove. 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 lipidparametersandthemorphologicalchangesinthepancreas, liver,andkidney[38].

Conclusions

The use of Dadiah containing *L. fermentum*strains has been demonstrated to reduce inflammatoryreactions associated with diabetic complications (DN). This study can be observed in the lower expressionof NF-kB antibodies as proinflammatory biomarkers that rise with hyperglycemia. The

outcomes ofprovidingDadiahaloneagainstprobioticsaloneorLABmetabolitessuchasbacteriocinrevealedthesameimprovement in inflammation, blood glucose, and cholesterol. However, the gift of Dadiah had the mostsignificant impact on the control group. These results demonstrate that Dadiah with a comprehensivecompositionhas amoresubstantialeffectonbiomolecularandclinicaloutcomes.Forthis reason,probiotics, and new strategies from Dadiah need to prevent and treat metabolic diseases and prevent theprogressionofcomplicationsinDM.

Acknowledgments

The authors would like to thank Prof. drh. Hj. EndangPurwati, MS, Ph. D from the Laboratory ofAnimalHusbandryBiotechnology/TechnologyAnimalProduct,UniversitasAndalas,Padang,WestSumatra, Indonesia. Thank dr. Tofrizal Sp. PA., Ph. D from the Department of Pathological Anatomy,UniversitasAndalas,Padang,WestSumatra, Indonesia.forassistance on histopathological analysis.Thanks to UniversitasBaiturrahmah, Padang, West Sumatra, Indonesia foundation has helped financeresearch.

References

[1] A. T. Kharroubi, "Diabetes mellitus: The epidemic of the century," *World J. Diabetes*, vol. 6, no. 6,p.850, 2015, doi:10.4239/wjd.v6.i6.850.

[2] Y. Zheng, S. H. Ley, and F. B. Hu, "Global etiology and epidemiology of type 2 diabetes mellitusanditscomplications,"*Nat.Rev.Endocrinol.*,vol.14,no.2,pp.88–98,Feb.2018,doi:10.1038/nrendo.2017.151.

[3] E.C.Westman,"Type2DiabetesMellitus:APathophysiologicPerspective,"*Front.Nutr.*,vol.8,p.707371, Aug. 2021, doi:10.3389/fnut.2021.707371.

[4] M. Li, Q.Guo, H.Cai, H.Wang,Z.Ma,andX. Zhang,"miR-218regulatesdiabeticnephropathyviatargeting IKK-β and modulating NK-κB-mediated inflammation," *J. Cell. Physiol.*, vol. 235, no. 4,pp.3362–3371,Apr. 2020, doi:10.1002/jcp.29224.

[5] W.Yu*etal.*,"Thegutmicrobiomeinthe differentialdiagnosisofdiabetickidneydiseaseandmembranousnephropathy,"*Ren.Fail.*,vol.42,no.1,pp. 1100–1110,Jan.2020,doi:10.1080/0886022X.2020.1837869.

[6] H. Sawaf, G. Thomas, J. J. Taliercio, G. Nakhoul, T. J. Vachharajani, and A. Mehdi, "TherapeuticAdvancesinDiabeticNephropathy,"*J.Clin.Med.*,vol.11,no.2,p.378,Jan.2022,doi:10.3390/jcm11020378.

[7] M. Ruiz-Ortega, R. R. Rodrigues-Diez, C. Lavoz, and S. Rayego-Mateos, "Special Issue 'DiabeticNephropathy:Diagnosis,PreventionandTreatment,'"*J.Clin.Med.*,vol.9,no.3,p.813,Mar.2020, doi:10.3390/jcm9030813.

[8] T. Kocher, J. König, W. S. Borgnakke, C. Pink, and P. Meisel, "Periodontal complications ofhyperglycemia/diabetes mellitus: Epidemiologic complexity and clinical challenge," *Periodontol.2000*,vol.78, no.1,pp. 59–97, Oct.2018, doi:10.1111/prd.12235.

[9] R.J.Galindo,M.Fayfman,andG.E.Umpierrez,"PerioperativeManagementofHyperglycemiaandDiabetes in Cardiac Surgery Patients," *Endocrinol. Metab. Clin. North Am.*, vol. 47, no. 1, pp. 203–222,Mar. 2018,doi:10.1016/j.ecl.2017.10.005.

[10] G.Davì *et al.*, "Diabetes Mellitus, Hypercholesterolemia, and Hypertension but NotVascularDisease Per Se Are Associated with Persistent Platelet Activation In Vivo: Evidence Derived fromtheStudyofPeripheralArterialDisease,"*Circulation*,vol.96,no.1,pp.69–75,Jul.1997,doi: 10.1161/01.CIR.96.1.69.

[11] E. B.-M. Daliri, Y. Kim, Y. Do, R. Chelliah, and D.-H. Oh, "In Vitro and In Vivo CholesterolReducingAbilityandSafetyofProbioticCandidatesIsolatedfromKorean FermentedSoyaBeans,"*Probiotics Antimicrob. Proteins*, vol. 14, no. 1, pp. 87–98, Feb. 2022, doi: 10.1007/s12602-021-09798-0.

[12] T. Rittiphairoj, K. Pongpirul, K. Janchot, N. T. Mueller, and T. Li, "Probiotics Contribute toGlycemic Control in Patients with Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis,"*Adv.Nutr.*,vol.12,no.3,pp.722–734,May2021,doi:10.1093/advances/nmaa133.

[13] D. Xavier-Santos, R. Bedani, E. D. Lima, and S. M. I. Saad, "Impact of probiotics and prebioticstargetingmetabolicsyndrome,"*J.Funct.Foods*,vol.64,p.103666,Jan.2020,doi:10.1016/j.jff.2019.103666.

[14] M. Shimizu, M. Hashiguchi, T. Shiga, H. Tamura, and M. Mochizuki, "Meta-Analysis: Effects ofProbioticSupplementationonLipidProfilesinNormaltoMildlyHypercholesterolemicIndividuals,"*PL*

OSONE,vol.10,no.10,p.e0139795,Oct. 2015,doi:10.1371/journal.pone.0139795.
[15] Z. Guo*et al.*, "Influence of consumption of probiotics on the plasma lipid profile: A meta-analysisof randomized controlled trials," *Nutr. Metab. Cardiovasc. Dis.*, vol. 21, no. 11, pp. 844–850, Nov.2011,doi:10.1016/j.numecd.2011.04.008.
[16] A.Ataie-Jafari,B.Larijani,H.AlaviMajd,andF.Tahbaz,"Cholesterol-LoweringEffectofProbioticYogurtinComparisonwithOrdinaryYogurtinMildlytoModeratelyHypercholesterolemicSubjects,"*Ann.Nutr.Metab.*, vol.54,no. 1,pp.22–27,2009,doi:10.1159/000203284.
[17] S.Usmiati and Risfaheri, "PengembanganDadihSebagaiPanganFungsionalProbiotikAsliSumatera Barat,"*J. Litbang Pert*. Vol. 32 N0. 32, pp. 20-29, 2013.
[18] E. Purwati, S. N. Aritonang, S. Melia, I. Juliyarsi, and H. Purwanto, *ManfaatProbiotikBakteriAsamLaktatDadiahMenunjangKesehatanMasyarakat*.LembagaPengembanganTeknologiInformasidanKomunikasi (LPTIK) UniversitasAndalas, 2017. doi:10.25077/car.9.9.
[19] A.Kundu*etal.*,"ProtectiveeffectsofCrotonhookerionstreptozotocin-induceddiabeticnephropathy,"*FoodChem.Toxicol.*,vol.135,p.110873,Jan.2020,doi:10.1016/j.fct.2019.110873.
[20] R.Ameli*etal.*,"Characterizationandprobioticpotentialoflacticacidbacteriaisolatedfrom Dadiahsampled in West Sumatra," *Food Sci. Technol.*, vol. 41 (Suppl., pp. 746–752, Dec. 2021, doi:10.1590/fst.30020.
[21] Y. Wang, D. Dilidaxi, Y. Wu, J. Sailike, X. Sun, and X. HuaNabi, "Composite probiotics alleviatetype 2 diabetes by regulating intestinal microbiota and inducing GLP-1 secretion in db/db mice,"*Biomed.Pharmacother.*,vol.125,no.January,p.109914,2020,doi:10.1016/j.biopha.2020.109914.
[22] X. Guo*etal.*,"NFκBpromotesoxidativestress-inducednecrosisandischemia/reperfusioninjurybyinhibitingNrf2-AREpathway,"*FreeRadic.Biol.Med.*,vol.159,pp.125–135,2020,doi:10.1016/j.freadadbiomed.2020.07.031.
[23] H. T. Zeng *et al.*, "SIRT1 prevents atherosclerosis via liver-X-receptor and NF-κB signaling in aU937 cellmodel," *Mol.Med.Rep.*,vol.8,no.1,pp.23–28,2013,doi:10.3892/mmr.2013.1460.
[24] L.Kong*etal.*,"Sirtuin1:ATargetforKidneyDiseases,"vol.1,pp.87–97,2015,doi:10.2119/molmed.2014.00211.
[25] Harnavi Harun, YanwirastiYanwirasti, BambangPurwanto, and EndangPurwatiRahayuningsih,"The Effect of Giving Dadih on Malondialdehyde Levels and Renal Interstitial Fibrosis at AgingKidney,"*OpenAccessMaced.J.Med.Sci.*,vol.8,no.A,pp.293–296,2020,doi:10.3889/oamjms.2020.4285.
[26] A. Guilbaud*et al.*, "The Effect of Lactobacillus fermentum ME-3 Treatment on Glycation andDiabetesComplications,"*Mol.Nutr.FoodRes.*,vol.64,no.6,pp.1–13,2020,doi:10.1002/mnfr.201901018.
[27] A. A. Dumas, A. Lapointe, M. Dugrenier, V. Provencher, B. Lamarche, and S. Desroches, "Asystematic review of the effect of yogurt consumption on chronic diseases risk markers in adults,"*Eur.J.Nutr.*, vol.56, no.4,pp. 1375–1392,2017, doi:10.1007/s00394-016-1341-7.
[28] H. Zhong*et al.*, "Probiotics-fermented blueberry juice prevents obesity and hyperglycemia in highfat diet-fed mice in association with modulating the gut microbiota," *Food Funct.*, pp. 0–52, 2020,doi:10.1039/d0fo00334d.
[29] E.Amirani,Z.Asemi,andM.Taghizadeh,"Theeffectsofseleniumplusprobioticssupplementationonglycemicstatusandserumlipoproteinsinpatientswithgestationaldiabetesmellitus:Arandomized,double-blind,placebo-controlledtrial,"*Clin.Nutr.ESPEN*,no.xxxx,2022,doi:10.1016/j.clnesp.2022.02.010.
[30] L. Wang *et al.*, "The effects of probiotics on total cholesterol," *Med. U. S.*, vol. 97, no. 5, 2018, doi:10.1097/MD.0000000000009679.
[31] S.M.Lim,J.J.Jeong,K.H.Woo,M.J.Han,andD.H.Kim,"LactobacillussakeiOK67ameliorateshigh-fat diet-induced blood glucose intolerance and obesity in mice by inhibiting gut microbiotalipopolysaccharideproductionandinducingcolontightjunctionproteinexpression,"*Nutr.Res.*,vol.36,no.4, pp. 337–348,2016, doi:10.1016/j.nutres.2015.12.001.
[32] L. Wu *et al.*, "Involvement of miR-27a-3p in diabetic nephropathy via affecting renal fibrosis,mitochondrial dysfunction, and endoplasmic reticulum stress," *J. Cell. Physiol.*, no. July 2020, doi:10.1002/jcp.29951.
[33] B. S. Sivamaruthi, P. Kesika, and C. Chaiyasut, "A mini-review of human studies on cholesterol-loweringpropertiesofprobiotics,"*ScientiaPharmaceutica*,vol.87,no.4.2019.doi:10.3390/scipharm87040026.

[34] A. Khare and S. Gaur, "Cholesterol-Lowering Effects of Lactobacillus Species,"*Curr. Microbiol.*,vol.77, no.4,pp.638–644,2020,doi:10.1007/s00284-020-01903-w.

[35] H.J.Zheng *etal.*,"The effectofprobioticandsynbioticsupplementationonbiomarkersofinflammation and oxidative stress in diabetic patients: A systematic review and meta-analysis ofrandomized controlled trials," *Pharmacol. Res.*, vol. 142, no. February, pp. 303–313, 2019, doi:10.1016/j.phrs.2019.02.016.

[36] B.BhatandB.K.Bajaj,"Multifariouscholesterol-loweringpotentialoflacticacidbacteriaequippedwithdesiredprobioticfunctionalattributes,"*3Biotech*,vol.10,no.5,pp.1–16,2020,doi:10.1007/s13205-020-02183-8.

[37] L. Merone and R. McDermott, "Nutritional anti-inflammatories in the treatment and prevention oftype2diabetesmellitusandthemetabolicsyndrome,"*DiabetesRes.Clin.Pract.*, vol.127,pp.238–253,2017, doi:10.1016/j.diabres.2017.02.019.

[38] B. Xue*et al.*, "Microbial diversity analysis of vineyard on the eastern foothills of the HelanMountain region using high-throughput sequencing," *Food Sci. Technol.*, vol. 42, pp. 1–11, 2022,doi:10.1590/fst.66320.

TRENDS IN SCIENCES 20xx; 20(xx): xxxxx RESEARCH ARTICLE
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

Commented [as1]: interesting title and there is novelty.

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.

Commented [as2]: italic

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 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. probiotic metabolites on diabetic rats induced by intraperitoneal Alloxan 100 mg/kg b. w. 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.

Commented [as3]: word count of more than 250 words

Commented [as4]: using what intervention

Commented [as5]: what is the number of samples, how long is the duration, dosage, and frequency of the intervention, and when will it be evaluated?

Commented [as6]: analysis using what test?

Commented [as7]: what is the percentage decrease in blood sugar levels and others and what are the suggestions for future researchers?

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

Introduction

Commented [as8]: The background already describes the problems and phenomena that occur.

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

Commented [as9]: complete the research design, the number of samples used, the inclusion and exclusion criteria, as well as the ethical results of health research.

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).

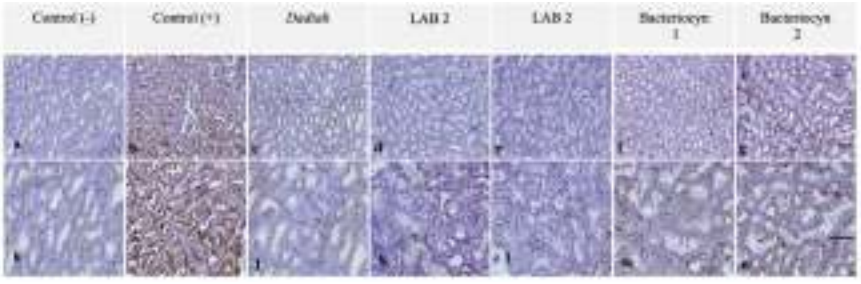

Figure 1 The NF-kB expression with immunohistochemistry in the kidney

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.

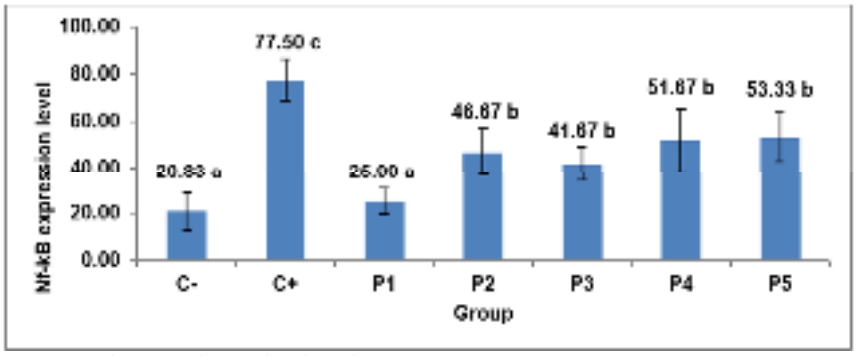

Figure 2 NF-kB expression numbers in each group

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**).

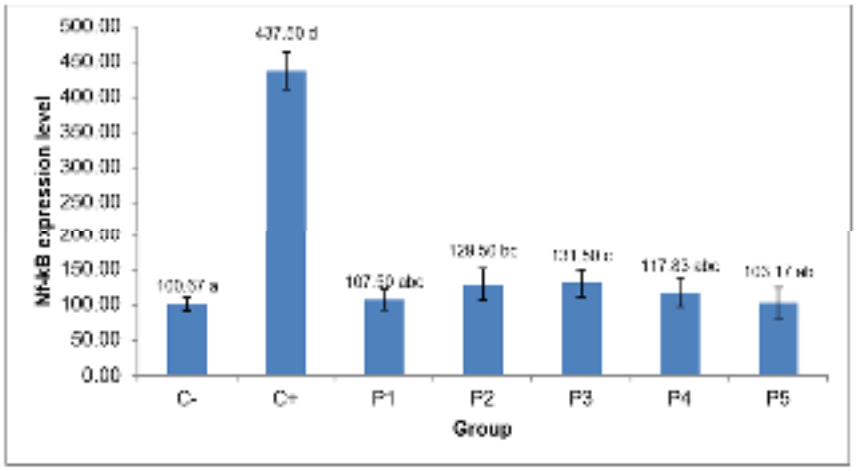

Figure 3 Blood glucose levels in each treatment

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.

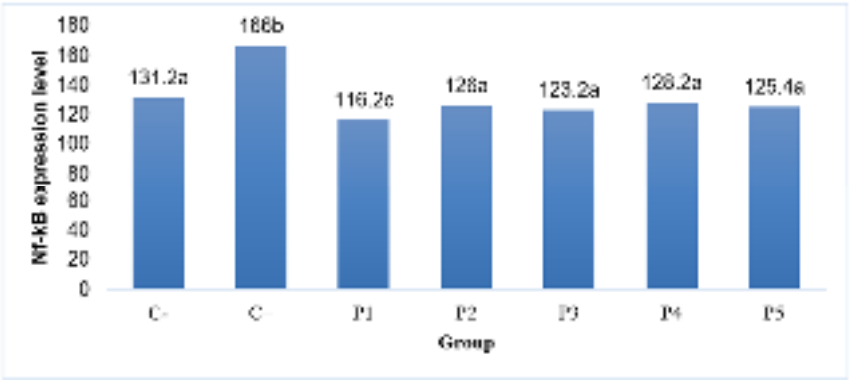

Figure 4. Serum cholesterol levels in each group

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

Commented [as10]: according to and answer the research objectives.

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

The authors would like to thank Prof. drh. Hj. Endang Purwati, MS, Ph. D from the Laboratory of Animal Husbandry Biotechnology/Technology Animal Product, Universitas Andalas, Padang, West Sumatra, Indonesia. Thank dr. Tofrizal Sp. PA., Ph. D from the Department of Pathological Anatomy, Universitas Andalas, Padang, West Sumatra, Indonesia. for assistance on histopathological analysis. Thanks to Universitas Baiturrahmah, Padang, West Sumatra, Indonesia foundation has helped finance research.

References

[1] A. T. Kharroubi, "Diabetes mellitus: The epidemic of the century," *World J. Diabetes*, vol. 6, no. 6, p. 850, 2015, doi: 10.4239/wjd.v6.i6.850.

[2] Y. Zheng, S. H. Ley, and F. B. Hu, "Global etiology and epidemiology of type 2 diabetes mellitus and its complications," *Nat. Rev. Endocrinol.*, vol. 14, no. 2, pp. 88–98, Feb. 2018, doi: 10.1038/nrendo. 2017.151.

[3] E. C. Westman, "Type 2 Diabetes Mellitus: A Pathophysiologic Perspective," *Front. Nutr.*, vol. 8, p. 707371, Aug. 2021, doi: 10.3389/fnut.2021.707371.

[4] M. Li, Q. Guo, H. Cai, H. Wang, Z. Ma, and X. Zhang, "miR-218 regulates diabetic nephropathy via targeting IKK-β and modulating NK-κB-mediated inflammation," *J. Cell. Physiol.*, vol. 235, no. 4, pp. 3362–3371, Apr. 2020, doi: 10.1002/jcp.29224.

[5] W. Yu *et al.*, "The gut microbiome in the differential diagnosis of diabetic kidney disease and membranous nephropathy," *Ren. Fail.*, vol. 42, no. 1, pp. 1100–1110, Jan. 2020, doi: 10.1080/0886022X.2020.1837869.

[6] H. Sawaf, G. Thomas, J. J. Taliercio, G. Nakhoul, T. J. Vachharajani, and A. Mehdi, "Therapeutic Advances in Diabetic Nephropathy," *J. Clin. Med.*, vol. 11, no. 2, p. 378, Jan. 2022, doi: 10.3390/jcm11020378.

[7] M. Ruiz-Ortega, R. R. Rodrigues-Diez, C. Lavoz, and S. Rayego-Mateos, "Special Issue 'Diabetic Nephropathy: Diagnosis, Prevention and Treatment,'" *J. Clin. Med.*, vol. 9, no. 3, p. 813, Mar. 2020, doi: 10.3390/jcm9030813.

[8] T. Kocher, J. König, W. S. Borgnakke, C. Pink, and P. Meisel, "Periodontal complications of hyperglycemia/diabetes mellitus: Epidemiologic complexity and clinical challenge," *Periodontol. 2000*, vol. 78, no. 1, pp. 59–97, Oct. 2018, doi: 10.1111/prd.12235.

[9] R. J. Galindo, M. Fayfman, and G. E. Umpierrez, "Perioperative Management of Hyperglycemia and Diabetes in Cardiac Surgery Patients," *Endocrinol. Metab. Clin. North Am.*, vol. 47, no. 1, pp. 203–222, Mar. 2018, doi: 10.1016/j.ecl.2017.10.005.

[10] G. Davì *et al.*, "Diabetes Mellitus, Hypercholesterolemia, and Hypertension but Not Vascular Disease Per Se Are Associated with Persistent Platelet Activation In Vivo: Evidence Derived fromthe Study of Peripheral Arterial Disease," *Circulation*, vol. 96, no. 1, pp. 69–75, Jul. 1997, doi: 10.1161/01.CIR.96.1.69.

[11] E. B.-M. Daliri, Y. Kim, Y. Do, R. Chelliah, and D.-H. Oh, "In Vitro and In Vivo Cholesterol Reducing Ability and Safety of Probiotic Candidates Isolated from Korean Fermented Soya Beans," *Probiotics Antimicrob. Proteins*, vol. 14, no. 1, pp. 87–98, Feb. 2022, doi: 10.1007/s12602-021-09798-0.

[12] T. Rittiphairoj, K. Pongpirul, K. Janchot, N. T. Mueller, and T. Li, "Probiotics Contribute to Glycemic Control in Patients with Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis," *Adv. Nutr.*, vol. 12, no. 3, pp. 722–734, May 2021, doi: 10.1093/advances/nmaa133.

[13] D. Xavier-Santos, R. Bedani, E. D. Lima, and S. M. I. Saad, "Impact of probiotics and prebiotics targeting metabolic syndrome," *J. Funct. Foods*, vol. 64, p. 103666, Jan. 2020, doi: 10.1016/j.jff.2019.103666.

[14] M. Shimizu, M. Hashiguchi, T. Shiga, H. Tamura, and M. Mochizuki, "Meta-Analysis: Effects of Probiotic Supplementation on Lipid Profiles in Normal to Mildly Hypercholesterolemic Individuals," *PLOS ONE*, vol. 10, no. 10, p. e0139795, Oct. 2015, doi: 10.1371/journal.pone.0139795.

[15] Z. Guo *et al.*, "Influence of consumption of probiotics on the plasma lipid profile: A meta-analysis of randomized controlled trials," *Nutr. Metab. Cardiovasc. Dis.*, vol. 21, no. 11, pp. 844–850, Nov. 2011, doi: 10.1016/j.numecd.2011.04.008.

[16] A. Ataie-Jafari, B. Larijani, H. Alavi Majd, and F. Tahbaz, "Cholesterol-Lowering Effect of Probiotic Yogurt in Comparison with Ordinary Yogurt in Mildly to Moderately Hypercholesterolemic Subjects," *Ann. Nutr. Metab.*, vol. 54, no. 1, pp. 22–27, 2009, doi: 10.1159/000203284.

[17] S. Usmiati and Risfaheri, "Pengembangan Dadih Sebagai Pangan Fungsional Probiotik Asli Sumatera Barat," *J. Litbang Pert*. Vol. 32 N0. 32, pp. 20-29, 2013.
[18] E. Purwati, S. N. Aritonang, S. Melia, I. Juliyarsi, and H. Purwanto, *Manfaat Probiotik Bakteri Asam Laktat Dadiah Menunjang Kesehatan Masyarakat*. Lembaga Pengembangan Teknologi Informasi dan Komunikasi (LPTIK) Universitas Andalas, 2017. doi: 10.25077/car.9.9.
[19] A. Kundu *et al.*, "Protective effects of Croton hookeri on streptozotocin-induced diabetic nephropathy," *Food Chem. Toxicol.*, vol. 135, p. 110873, Jan. 2020, doi: 10.1016/j.fct.2019.110873.
[20] R. Amelia *et al.*, "Characterization and probiotic potential of lactic acid bacteria isolated from Dadiah sampled in West Sumatra," *Food Sci. Technol.*, vol. 41 (Suppl., pp. 746–752, Dec. 2021, doi: 10.1590/fst.30020.
[21] Y. Wang, D. Dilidaxi, Y. Wu, J. Sailike, X. Sun, and X. Hua Nabi, "Composite probiotics alleviate type 2 diabetes by regulating intestinal microbiota and inducing GLP-1 secretion in db/db mice," *Biomed. Pharmacother.*, vol. 125, no. January, p. 109914, 2020, doi: 10.1016/j.biopha.2020.109914.
[22] X. Guo *et al.*, "NFκB promotes oxidative stress-induced necrosis and ischemia/reperfusion injury by inhibiting Nrf2-ARE pathway," *Free Radic. Biol. Med.*, vol. 159, pp. 125–135, 2020, doi: 10.1016/j.freadadbiomed.2020.07.031.
[23] H. T. Zeng *et al.*, "SIRT1 prevents atherosclerosis via liver-X-receptor and NF-κB signaling in a U937 cell model," *Mol. Med. Rep.*, vol. 8, no. 1, pp. 23–28, 2013, doi: 10.3892/mmr.2013.1460.
[24] L. Kong *et al.*, "Sirtuin 1: A Target for Kidney Diseases," vol. 1, pp. 87–97, 2015, doi: 10.2119/molmed.2014.00211.
[25] Harnavi Harun, Yanwirasti Yanwirasti, Bambang Purwanto, and Endang Purwati Rahayuningsih, "The Effect of Giving Dadih on Malondialdehyde Levels and Renal Interstitial Fibrosis at Aging Kidney," *Open Access Maced. J. Med. Sci.*, vol. 8, no. A, pp. 293–296, 2020, doi: 10.3889/oamjms.2020.4285.
[26] A. Guilbaud *et al.*, "The Effect of Lactobacillus fermentum ME-3 Treatment on Glycation and Diabetes Complications," *Mol. Nutr. Food Res.*, vol. 64, no. 6, pp. 1–13, 2020, doi: 10.1002/mnfr.201901018.
[27] A. A. Dumas, A. Lapointe, M. Dugrenier, V. Provencher, B. Lamarche, and S. Desroches, "A systematic review of the effect of yogurt consumption on chronic diseases risk markers in adults," *Eur. J. Nutr.*, vol. 56, no. 4, pp. 1375–1392, 2017, doi: 10.1007/s00394-016-1341-7.
[28] H. Zhong *et al.*, "Probiotics-fermented blueberry juice prevents obesity and hyperglycemia in high fat diet-fed mice in association with modulating the gut microbiota," *Food Funct.*, pp. 0–52, 2020, doi: 10.1039/d0fo00334d.
[29] E. Amirani, Z. Asemi, and M. Taghizadeh, "The effects of selenium plus probiotics supplementation on glycemic status and serum lipoproteins in patients with gestational diabetes mellitus: A randomized, double-blind, placebo-controlled trial," *Clin. Nutr. ESPEN*, no. xxxx, 2022, doi: 10.1016/j.clnesp.2022.02.010.
[30] L. Wang *et al.*, "The effects of probiotics on total cholesterol," *Med. U. S.*, vol. 97, no. 5, 2018, doi: 10.1097/MD.0000000000009679.
[31] S. M. Lim, J. J. Jeong, K. H. Woo, M. J. Han, and D. H. Kim, "Lactobacillus sakei OK67 ameliorates high-fat diet-induced blood glucose intolerance and obesity in mice by inhibiting gut microbiota lipopolysaccharide production and inducing colon tight junction protein expression," *Nutr. Res.*, vol. 36, no. 4, pp. 337–348, 2016, doi: 10.1016/j.nutres.2015.12.001.
[32] L. Wu *et al.*, "Involvement of miR-27a-3p in diabetic nephropathy via affecting renal fibrosis, mitochondrial dysfunction, and endoplasmic reticulum stress," *J. Cell. Physiol.*, no. July 2020, doi: 10.1002/jcp.29951.
[33] B. S. Sivamaruthi, P. Kesika, and C. Chaiyasut, "A mini-review of human studies on cholesterol-lowering properties of probiotics," *Scientia Pharmaceutica*, vol. 87, no. 4. 2019. doi: 10.3390/scipharm87040026.
[34] A. Khare and S. Gaur, "Cholesterol-Lowering Effects of Lactobacillus Species,"*Curr. Microbiol.*, vol. 77, no. 4, pp. 638–644, 2020, doi: 10.1007/s00284-020-01903-w.
[35] H. J. Zheng *et al.*, "The effect of probiotic and synbiotic supplementation on biomarkers of inflammation and oxidative stress in diabetic patients: A systematic review and meta-analysis of randomized controlled trials," *Pharmacol. Res.*, vol. 142, no. February, pp. 303–313, 2019, doi: 10.1016/j.phrs.2019.02.016.
[36] B. Bhat and B. K. Bajaj, "Multifarious cholesterol-lowering potential of lactic acid bacteria equipped with desired probiotic functional attributes," *3 Biotech*, vol. 10, no. 5, pp. 1–16, 2020, doi: 10.1007/s13205-020-02183-8.

[37] L. Merone and R. McDermott, "Nutritional anti-inflammatories in the treatment and prevention of type 2 diabetes mellitus and the metabolic syndrome," *Diabetes Res. Clin. Pract.*, vol. 127, pp. 238–253, 2017, doi: 10.1016/j.diabres.2017.02.019.

[38] B. Xue *et al.*, "Microbial diversity analysis of vineyard on the eastern foothills of the Helan Mountain region using high-throughput sequencing," *Food Sci. Technol.*, vol. 42, pp. 1–11, 2022, doi: 10.1590/fst.66320.

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 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).

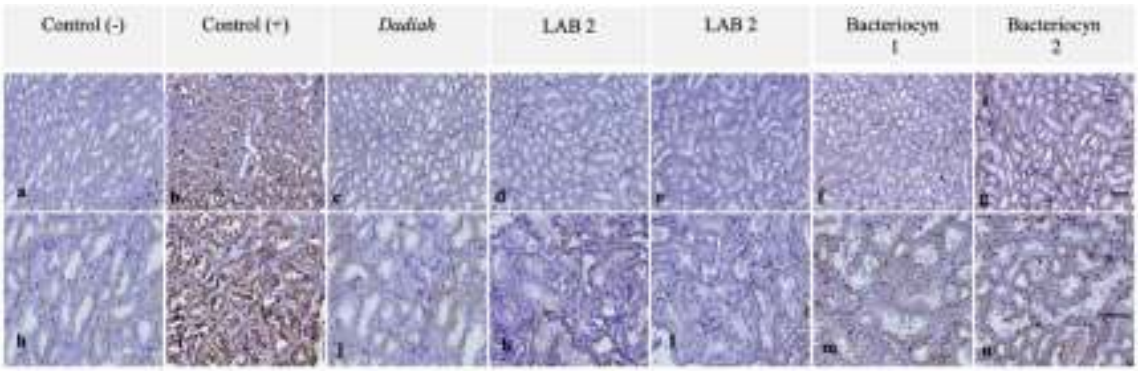

Figure 1 The NF-kB expression with immunohistochemistry in the kidney

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.

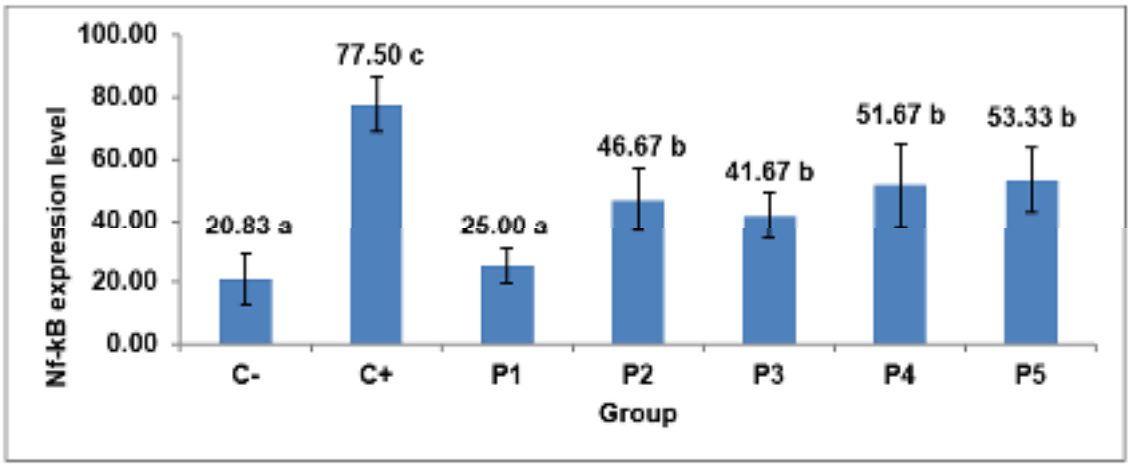

Figure 2 NF-kB expression numbers in each group

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**).

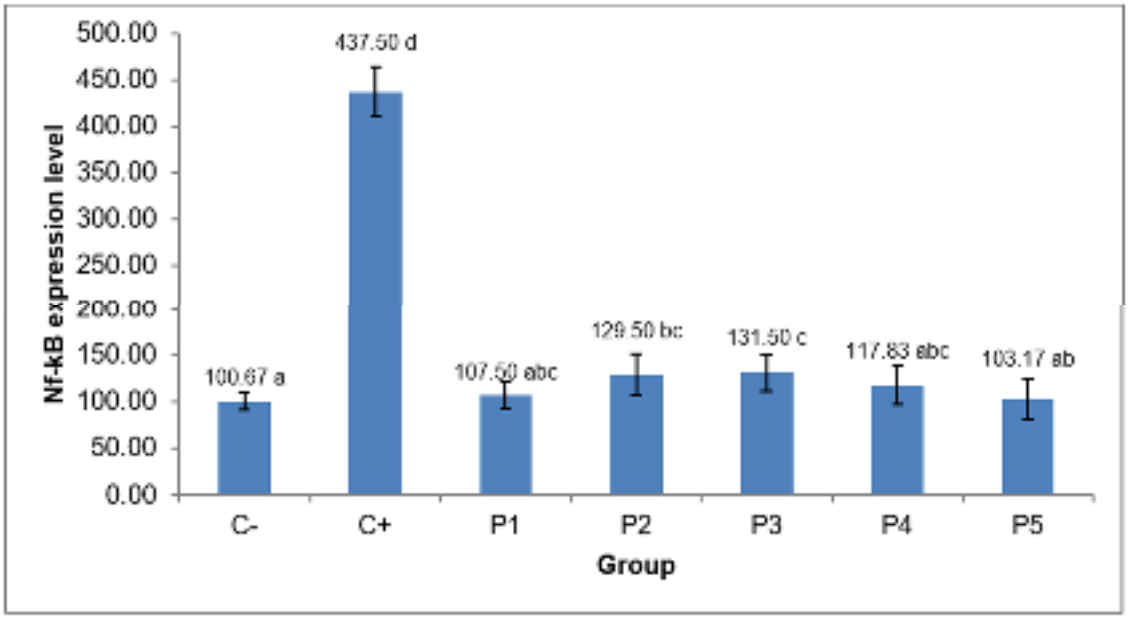

Figure 3 Blood glucose levels in each treatment

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.

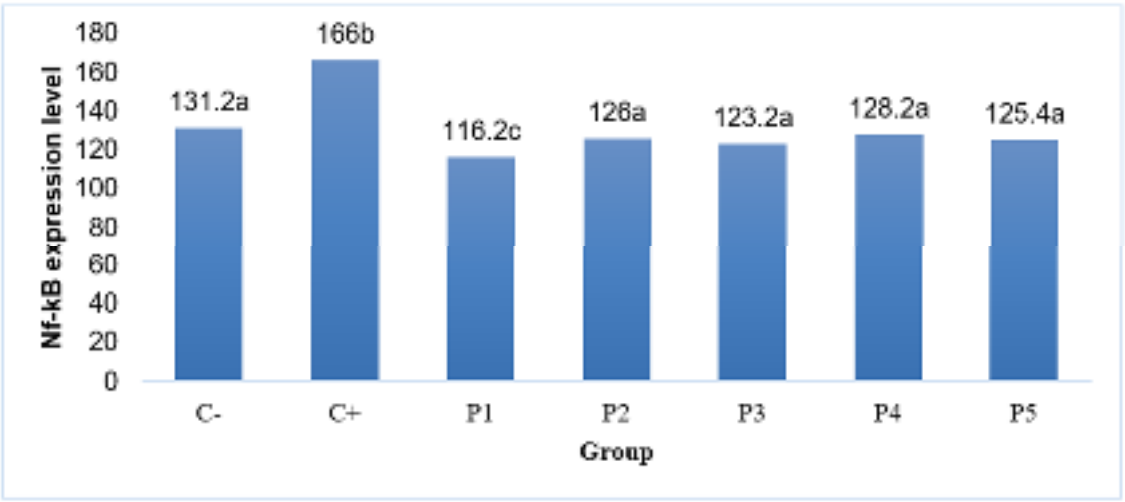

Figure 4. Serum cholesterol levels in each group

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.

Acknowledgments

The authors would like to thank Prof. drh. Hj. Endang Purwati, MS, Ph. D from the Laboratory of Animal Husbandry Biotechnology/Technology Animal Product, Universitas Andalas, Padang, West Sumatra, Indonesia. Thank dr. Tofrizal Sp. PA., Ph. D from the Department of Pathological Anatomy, Universitas Andalas, Padang, West Sumatra, Indonesia. for assistance on histopathological analysis. Thanks to Universitas Baiturrahmah, Padang, West Sumatra, Indonesia foundation has helped finance research.

References

[1] A. T. Kharroubi, "Diabetes mellitus: The epidemic of the century," *World J. Diabetes*, vol. 6, no. 6, p. 850, 2015, doi: 10.4239/wjd.v6.i6.850.

[2] Y. Zheng, S. H. Ley, and F. B. Hu, "Global etiology and epidemiology of type 2 diabetes mellitus and its complications," *Nat. Rev. Endocrinol.*, vol. 14, no. 2, pp. 88–98, Feb. 2018, doi: 10.1038/nrendo. 2017.151.

[3] E. C. Westman, "Type 2 Diabetes Mellitus: A Pathophysiologic Perspective," *Front. Nutr.*, vol. 8, p. 707371, Aug. 2021, doi: 10.3389/fnut.2021.707371.

[4] M. Li, Q. Guo, H. Cai, H. Wang, Z. Ma, and X. Zhang, "miR-218 regulates diabetic nephropathy via targeting IKK-β and modulating NK-κB-mediated inflammation," *J. Cell. Physiol.*, vol. 235, no. 4, pp. 3362–3371, Apr. 2020, doi: 10.1002/jcp.29224.

[5] W. Yu *et al.*, "The gut microbiome in the differential diagnosis of diabetic kidney disease and membranous nephropathy," *Ren. Fail.*, vol. 42, no. 1, pp. 1100–1110, Jan. 2020, doi: 10.1080/0886022X.2020.1837869.

[6] H. Sawaf, G. Thomas, J. J. Taliercio, G. Nakhoul, T. J. Vachharajani, and A. Mehdi, "Therapeutic Advances in Diabetic Nephropathy," *J. Clin. Med.*, vol. 11, no. 2, p. 378, Jan. 2022, doi: 10.3390/jcm11020378.

[7] M. Ruiz-Ortega, R. R. Rodrigues-Diez, C. Lavoz, and S. Rayego-Mateos, "Special Issue 'Diabetic Nephropathy: Diagnosis, Prevention and Treatment,'" *J. Clin. Med.*, vol. 9, no. 3, p. 813, Mar. 2020, doi: 10.3390/jcm9030813.

[8] T. Kocher, J. König, W. S. Borgnakke, C. Pink, and P. Meisel, "Periodontal complications of hyperglycemia/diabetes mellitus: Epidemiologic complexity and clinical challenge," *Periodontol. 2000*, vol. 78, no. 1, pp. 59–97, Oct. 2018, doi: 10.1111/prd.12235.

[9] R. J. Galindo, M. Fayfman, and G. E. Umpierrez, "Perioperative Management of Hyperglycemia and Diabetes in Cardiac Surgery Patients," *Endocrinol. Metab. Clin. North Am.*, vol. 47, no. 1, pp. 203–222, Mar. 2018, doi: 10.1016/j.ecl.2017.10.005.

[10] G. Davì *et al.*, "Diabetes Mellitus, Hypercholesterolemia, and Hypertension but Not Vascular Disease Per Se Are Associated with Persistent Platelet Activation In Vivo: Evidence Derived fromthe Study of Peripheral Arterial Disease," *Circulation*, vol. 96, no. 1, pp. 69–75, Jul. 1997, doi: 10.1161/01.CIR.96.1.69.

[11] E. B.-M. Daliri, Y. Kim, Y. Do, R. Chelliah, and D.-H. Oh, "In Vitro and In Vivo Cholesterol Reducing Ability and Safety of Probiotic Candidates Isolated from Korean Fermented Soya Beans," *Probiotics Antimicrob. Proteins*, vol. 14, no. 1, pp. 87–98, Feb. 2022, doi: 10.1007/s12602-021-09798-0.

[12] T. Rittiphairoj, K. Pongpirul, K. Janchot, N. T. Mueller, and T. Li, "Probiotics Contribute to Glycemic Control in Patients with Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis," *Adv. Nutr.*, vol. 12, no. 3, pp. 722–734, May 2021, doi: 10.1093/advances/nmaa133.

[13] D. Xavier-Santos, R. Bedani, E. D. Lima, and S. M. I. Saad, "Impact of probiotics and prebiotics targeting metabolic syndrome," *J. Funct. Foods*, vol. 64, p. 103666, Jan. 2020, doi: 10.1016/j.jff.2019.103666.

[14] M. Shimizu, M. Hashiguchi, T. Shiga, H. Tamura, and M. Mochizuki, "Meta-Analysis: Effects of Probiotic Supplementation on Lipid Profiles in Normal to Mildly Hypercholesterolemic Individuals," *PLOS ONE*, vol. 10, no. 10, p. e0139795, Oct. 2015, doi: 10.1371/journal.pone.0139795.

[15] Z. Guo *et al.*, "Influence of consumption of probiotics on the plasma lipid profile: A meta-analysis of randomized controlled trials," *Nutr. Metab. Cardiovasc. Dis.*, vol. 21, no. 11, pp. 844–850, Nov. 2011, doi: 10.1016/j.numecd.2011.04.008.

[16] A. Ataie-Jafari, B. Larijani, H. Alavi Majd, and F. Tahbaz, "Cholesterol-Lowering Effect of Probiotic Yogurt in Comparison with Ordinary Yogurt in Mildly to Moderately Hypercholesterolemic Subjects," *Ann. Nutr. Metab.*, vol. 54, no. 1, pp. 22–27, 2009, doi: 10.1159/000203284.

[17] S. Usmiati and Risfaheri, "Pengembangan Dadih Sebagai Pangan Fungsional Probiotik Asli Sumatera Barat," *J. Litbang Pert*. Vol. 32 N0. 32, pp. 20-29, 2013.
[18] E. Purwati, S. N. Aritonang, S. Melia, I. Juliyarsi, and H. Purwanto, *Manfaat Probiotik Bakteri Asam Laktat Dadiah Menunjang Kesehatan Masyarakat*. Lembaga Pengembangan Teknologi Informasi dan Komunikasi (LPTIK) Universitas Andalas, 2017. doi: 10.25077/car.9.9.
[19] A. Kundu *et al.*, "Protective effects of Croton hookeri on streptozotocin-induced diabetic nephropathy," *Food Chem. Toxicol.*, vol. 135, p. 110873, Jan. 2020, doi: 10.1016/j.fct.2019.110873.
[20] R. Amelia *et al.*, "Characterization and probiotic potential of lactic acid bacteria isolated from Dadiah sampled in West Sumatra," *Food Sci. Technol.*, vol. 41 (Suppl., pp. 746–752, Dec. 2021, doi: 10.1590/fst.30020.
[21] Y. Wang, D. Dilidaxi, Y. Wu, J. Sailike, X. Sun, and X. Hua Nabi, "Composite probiotics alleviate type 2 diabetes by regulating intestinal microbiota and inducing GLP-1 secretion in db/db mice," *Biomed. Pharmacother.*, vol. 125, no. January, p. 109914, 2020, doi: 10.1016/j.biopha.2020.109914.
[22] X. Guo *et al.*, "NFκB promotes oxidative stress-induced necrosis and ischemia/reperfusion injury by inhibiting Nrf2-ARE pathway," *Free Radic. Biol. Med.*, vol. 159, pp. 125–135, 2020, doi: 10.1016/j.freadadbiomed.2020.07.031.
[23] H. T. Zeng *et al.*, "SIRT1 prevents atherosclerosis via liver-X-receptor and NF-κB signaling in a U937 cell model," *Mol. Med. Rep.*, vol. 8, no. 1, pp. 23–28, 2013, doi: 10.3892/mmr.2013.1460.
[24] L. Kong *et al.*, "Sirtuin 1: A Target for Kidney Diseases," vol. 1, pp. 87–97, 2015, doi: 10.2119/molmed.2014.00211.
[25] Harnavi Harun, Yanwirasti Yanwirasti, Bambang Purwanto, and Endang Purwati Rahayuningsih, "The Effect of Giving Dadih on Malondialdehyde Levels and Renal Interstitial Fibrosis at Aging Kidney," *Open Access Maced. J. Med. Sci.*, vol. 8, no. A, pp. 293–296, 2020, doi: 10.3889/oamjms.2020.4285.
[26] A. Guilbaud *et al.*, "The Effect of Lactobacillus fermentum ME-3 Treatment on Glycation and Diabetes Complications," *Mol. Nutr. Food Res.*, vol. 64, no. 6, pp. 1–13, 2020, doi: 10.1002/mnfr.201901018.
[27] A. A. Dumas, A. Lapointe, M. Dugrenier, V. Provencher, B. Lamarche, and S. Desroches, "A systematic review of the effect of yogurt consumption on chronic diseases risk markers in adults," *Eur. J. Nutr.*, vol. 56, no. 4, pp. 1375–1392, 2017, doi: 10.1007/s00394-016-1341-7.
[28] H. Zhong *et al.*, "Probiotics-fermented blueberry juice prevents obesity and hyperglycemia in high fat diet-fed mice in association with modulating the gut microbiota," *Food Funct.*, pp. 0–52, 2020, doi: 10.1039/d0fo00334d.
[29] E. Amirani, Z. Asemi, and M. Taghizadeh, "The effects of selenium plus probiotics supplementation on glycemic status and serum lipoproteins in patients with gestational diabetes mellitus: A randomized, double-blind, placebo-controlled trial," *Clin. Nutr. ESPEN*, no. xxxx, 2022, doi: 10.1016/j.clnesp.2022.02.010.
[30] L. Wang *et al.*, "The effects of probiotics on total cholesterol," *Med. U. S.*, vol. 97, no. 5, 2018, doi: 10.1097/MD.0000000000009679.
[31] S. M. Lim, J. J. Jeong, K. H. Woo, M. J. Han, and D. H. Kim, "Lactobacillus sakei OK67 ameliorates high-fat diet-induced blood glucose intolerance and obesity in mice by inhibiting gut microbiota lipopolysaccharide production and inducing colon tight junction protein expression," *Nutr. Res.*, vol. 36, no. 4, pp. 337–348, 2016, doi: 10.1016/j.nutres.2015.12.001.
[32] L. Wu *et al.*, "Involvement of miR-27a-3p in diabetic nephropathy via affecting renal fibrosis, mitochondrial dysfunction, and endoplasmic reticulum stress," *J. Cell. Physiol.*, no. July 2020, doi: 10.1002/jcp.29951.
[33] B. S. Sivamaruthi, P. Kesika, and C. Chaiyasut, "A mini-review of human studies on cholesterol-lowering properties of probiotics," *Scientia Pharmaceutica*, vol. 87, no. 4. 2019. doi: 10.3390/scipharm87040026.
[34] A. Khare and S. Gaur, "Cholesterol-Lowering Effects of Lactobacillus Species,"*Curr. Microbiol.*, vol. 77, no. 4, pp. 638–644, 2020, doi: 10.1007/s00284-020-01903-w.
[35] H. J. Zheng *et al.*, "The effect of probiotic and synbiotic supplementation on biomarkers of inflammation and oxidative stress in diabetic patients: A systematic review and meta-analysis of randomized controlled trials," *Pharmacol. Res.*, vol. 142, no. February, pp. 303–313, 2019, doi: 10.1016/j.phrs.2019.02.016.
[36] B. Bhat and B. K. Bajaj, "Multifarious cholesterol-lowering potential of lactic acid bacteria equipped with desired probiotic functional attributes," *3 Biotech*, vol. 10, no. 5, pp. 1–16, 2020, doi: 10.1007/s13205-020-02183-8.

[37] L. Merone and R. McDermott, "Nutritional anti-inflammatories in the treatment and prevention of type 2 diabetes mellitus and the metabolic syndrome," *Diabetes Res. Clin. Pract.*, vol. 127, pp. 238–253, 2017, doi: 10.1016/j.diabres.2017.02.019.

[38] B. Xue *et al.*, "Microbial diversity analysis of vineyard on the eastern foothills of the Helan Mountain region using high-throughput sequencing," *Food Sci. Technol.*, vol. 42, pp. 1–11, 2022, doi: 10.1590/fst.66320.

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

Rinita Amelia[1,*], Faridah Mohd Said[2], Farzana Yasmin[3] and Harnavi Harun[4]

[1]Department of Histology, Faculty of Medicine, Universitas Baiturrahmah, Sumatera Barat 25586, Indonesia
[2]Postgraduate Nursing Studies, Faculty of Nursing, Lincoln University, Kelana Jaya 47300, Malaysia
[3]Faculty of Biotech, Lincoln University, Kelana Jaya 47300, Malaysia
[4]Department of Internal Medicine, Faculty of Medicine, Universitas Andalas, West Sumatra 25175, Indonesia

([*]Corresponding author's e-mail: rinitaamelia@fk.unbrah.ac.id)
Email of all authors: Faridah.msaid@lincoln.edu.my, farzanayasmin@lincoln.edu.my, hharnavi19@gmail.com

Received: 2 March 2023, Revised: 30 March 2023, Accepted: 24 April 2023, Published: xx July 2023

Abstract

Diabetic nephropathy (DN) is the most common microvascular complication in diabetes mellitus (DM) patients. The main mechanism for the development of DN 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. The therapeutic approach in DM sufferers using probiotics needs attention. *Dadiah* is a naturally fermented buffalo milk product in bamboo tubes. Even though this probiotic has health benefits, its mechanism as an antidiabetic is not widely known. This study aims to reduce blood sugar, cholesterol, and inflammatory marker NF-kB levels after Dadiah treatment. This study was an experimental animal study using 7 groups, each group of 6 rats, 8 weeks of intervention, and evaluation of serum glucose and cholesterol every 2 weeks. NF-kB expression in kidney tissue was examined after 8 weeks of the termination phase. We examined their effect on blood glucose levels, serum cholesterol, and NF-kB antibody expression in kidney tissue using immunohistochemical assays. probiotic metabolites in intraperitoneal Alloxan-induced diabetic rats 100 mg/kg b. w. The results showed the potential of *Dadiah* and its metabolites to significantly (one-way ANOVA test) 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 DN 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), there were approximately 451 million diabetic patients worldwide in 2017, which is expected to rise to 693 million by 2045. Diabetic nephropathy (DN) is a significant complication of diabetes; it is a leading cause of death and end-stage renal disease (ESRD) in diabetic patients. Around 30 % of people with diabetes have renal disease, and most of them develop DN [1]. DM consists of 3 main 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. Type 3 is Gestational diabetes, which is the third main form and occurs when pregnant women without a previous history of diabetes develop a high blood glucose level [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. Carbohydrate-restricted diets have been used effectively for over a century for the treatment of obesity and T2DM. Its effectiveness may be due to lowering the dietary contribution to glucose and insulin levels, which reduces hyperglycemia and hyperinsulinemia. Treatments for T2DM that enhance glycemic control

while decreasing blood insulin levels make sense from a pathophysiologic standpoint [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 (DN) 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 Nuclear Factor Kappa Betha (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 developmentand various complications of DM for sufferers, such as diabetic cardiomyopathy, retinopathy, nephropathy, and DM neuropathy. NF-kB is a ubiquitously expressed transcription factor that has a role in inflammation, apoptosis, adhesion, angiogenesis, and cell cycle. Inflammation has a significant role in the development and progression of ND. NF-κB controls numerous proteins involved in inflammation. Many therapeutic approaches for DM sufferers have been developed, such as the useof several antioxidants, flavonoids, and probiotics. Probiotics are promising candidates for improvingglycemic 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 T2DM [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 fermented milk originating from West Sumatra, Indonesia. *Dadiah* is made from natural fermented buffalo milk which involves lactic acid bacteria. *Dadiah* has the potential to be developed as a functional food source of probiotics. 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 0.01 g 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 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-g, 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 = 0.018×104 = 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 [21].

Preparation of LAB

Isolate *L. fermentum* is rejuvenated first, then propagated in the medium MRS broth at a temperature of 370 °C for 24 h, and calculated the number of bacterial cells is by diluting up to 10^8 CFU/mL. Dilution results are calculated on the MRS medium so that it is included at a temperature of 37 °C for 2×24 h 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 7.1×10^{10} CFU/mL.

Preparation of bacteriocin (production of crude bacteriocin)

The LAB of Dadiah was cultivated in MRS broth (1,000 mL) seeded with 10 % inoculum of overnight culture and incubated at 37 °C for 24 h. Following incubation, the entire broth was centrifuged for 16 min at 10.000 X g find the cell-free supernatant was used as crude Bacteriocin [22]. The amount of LAB and Bacteriocin used in this study was 1 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 g. All rats were maintainedat 23 - 25 °C, with both a standard pellet diet and water ad libitum. After acclimatization for 2 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 5 treatment groups and 2 control groups. In this study, rats were divided into 5 groups with the number of each group of 6 rats. So that 6 DN rats were treated with *Dadiah* 3 mL/day (P1 group) and isolated samples of LAB and Bacteriocin from*Dadiah* 1 and 2 mL/day (P2-P5 group). Control groups were 3 DN rats without being treated (Positive control/C+) and 3 normal rats (control negative/C−) who did not have DN (without alloxan injection). The minimum sample of this study was determined according to the formula Federer is (t-1) (n-1) ≥ 15, t is the number of treatment groups. This study was an experimental animal study using 7 groups, each group of 6 rats, 8 weeks of intervention, and evaluation of serum glucose and cholesterol every 2 weeks. NF-kB expression in kidney tissue was examined after 8 weeks of the termination phase. 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. Tail is required to follow up glucose serum level and cholesterol every week, retro orbital has collected in the final experimental before termination (8^{th} week). After that, the blood dropped on the glucose stick of the glucometer (OneTouchMerck; 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 DN for hyperglycemia (> 200 mg/dL) and proteinuria.

In a preliminary study, rats kept on fasting for 12 h 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 2 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 withan average of 6 rats per group and orally administered with *Dadiah*, LAB, and Bacteriocin isolated from *Dadiah* for eight weeks or 56 consecutive days [23].

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. The experimental protocol complied with the Helsinki Declaration as revised in 2013 and ethics clearance was approved by the Ethics Committee No:001/Ethics-A/03/03/2021.

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 1,000x (objective 100x) magnifications. Photomicrographs were taken in representative areas. The standard procedure followed the Pathology Anatomy Laboratory Andalas University protocol.

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 g 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 2 treatments is different, then the 2 treatments are significantly different. Meanwhile, if the notation between the 2 treatments is the same, then the 2 treatments are not significantly different test between treatments. The technique of IHC preparation in this study used ScyTek Laboratories procedure with UltraTech HRP Anti polyvalent (DAB)Staining Complete System

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).

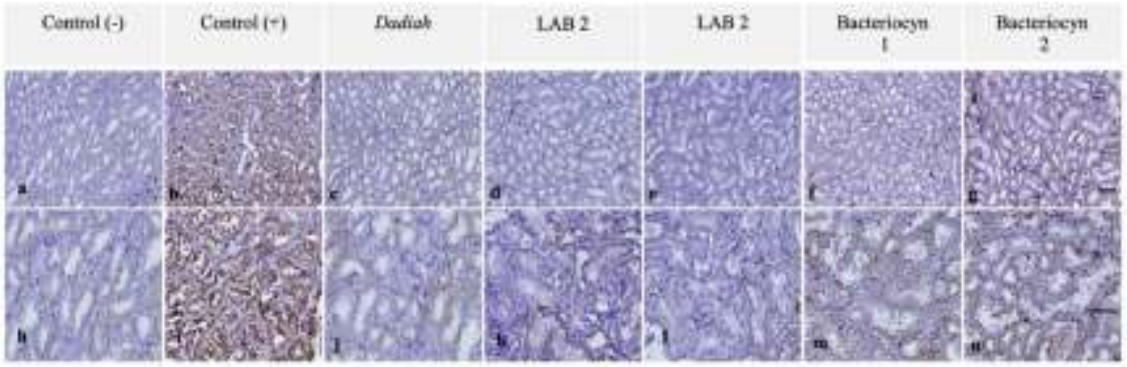

Figure 1 The NF-kB expression with immunohistochemistry in the kidney.

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 Bacteriocin. 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 DN, 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) [24,25]. 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 DN. Many inflammation-related proteinsare regulated by NF-kB. *Dadiah* is known to contain probiotics and antioxidants, so it has been proven that *Dadiah* can reduce oxidative stress and inflammation.

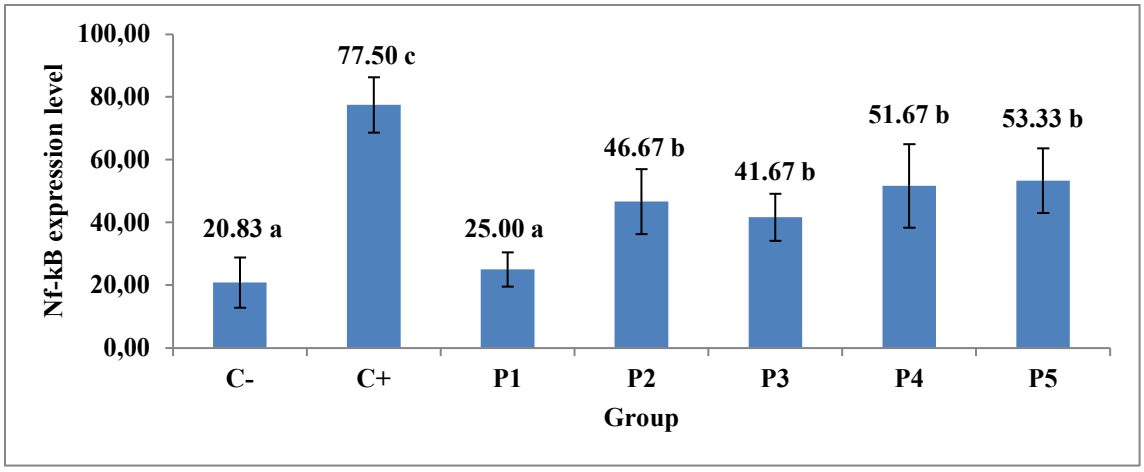

Figure 2 NF-kB expression numbers in each group.

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 [27].

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**).

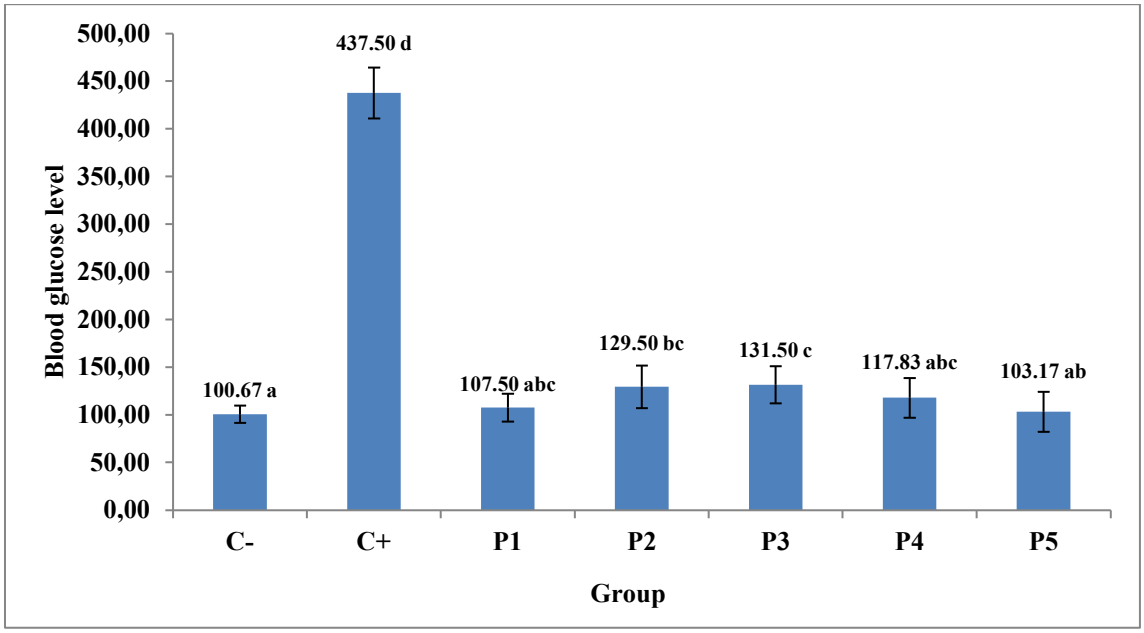

Figure 3 Blood glucose levels in each treatment.

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 DN. 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 [28].

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 [29-30]. 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. Furthermore, other research investigated whether giving probiotics and selenium to GDM patients for 6 weeks improved their hyperglycemic status and lipid profiles [31].

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 [32-31].

Serum cholesterol levels

The result of serum cholesterol levels showed that the C+ group has the highest average cholesterol of 166, while the P1 group as treated with *Dadiah* has the lowest average cholesterol of 116.2 (**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.

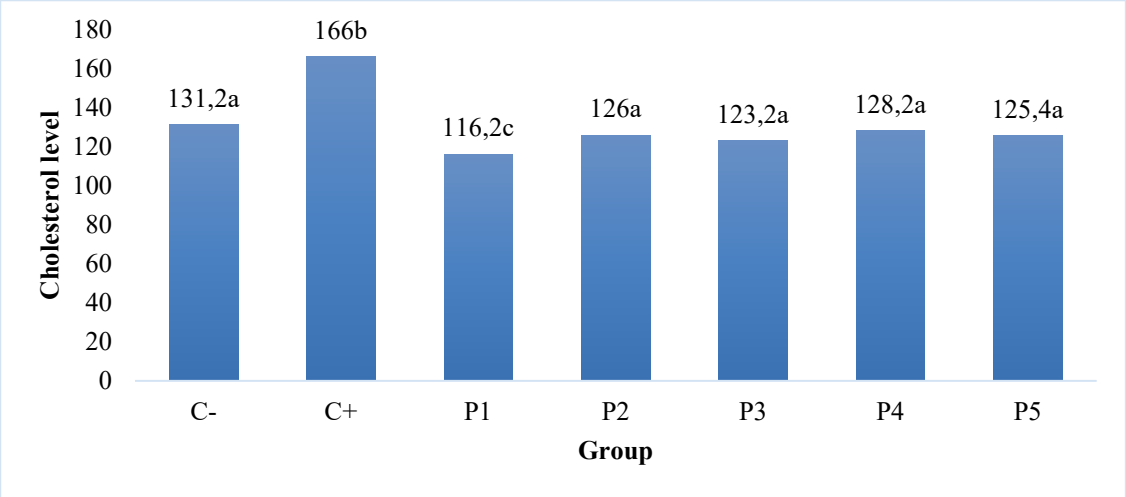

Figure 4 Serum cholesterol levels in each group.

This study shows that the group of rats given the treatment of *Dadiah* can lower the cholesterol levels of mice-modeled DN 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 (~4.14×10^6 CFU/g) and *B.lactis* Bb12 (~3.61×10^6 CFU/g) for 6 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 [34-36]. *In vitro* studies have also shown that *L. acidophilus* and *B. lactis* can lower cholesterol absorption. Similarly, in a study obtained, after 4 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 [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. We suggested a clinical study for humans in a future study.

Acknowledgments

The authors would like to thank Prof. drh. Hj. Endang Purwati, MS, Ph. D from the Laboratory of Animal Husbandry Biotechnology/Technology Animal Product, Universitas Andalas, Padang, West Sumatra, Indonesia. Thank dr. Tofrizal Sp. PA., Ph. D from the Department of Pathological Anatomy, Universitas Andalas, Padang, West Sumatra, Indonesia. for assistance on histopathological analysis. Thanks to Universitas Baiturrahmah, Padang, West Sumatra, Indonesia foundation has helped finance research.

References

[1] RE Pérez-Morales, MDD Pino, JM Valdivielso, A Ortiz, C Mora-Fernández and JF Navarro-González. Inflammation in diabetic kidney disease. *Nephron* 2019; **143**, 12-6.

[2] EC Westman. Type 2 diabetes mellitus: A pathophysiologic perspective. *Front. Nutr.* 2021; **8**, 707371.

[3] M Li, Q Guo, H Cai, H Wang, Z Ma and X Zhang. miR218 regulates diabetic nephropathy via targeting IKK-β and modulating NKκB-mediated inflammation. *J. Cell. Physiol.* 2020; **235**, 3362-71.

[4] W Yu, J Shang, R Guo, F Zhang, W Zhang, Y Zhang, F Wu, H Ren, C Liu, J Xiao and Z Zhaoa. The gut microbiome in differential diagnosis of diabetic kidney disease and membranous nephropathy. *Ren. Fail.* 2020; **42**, 1100-10.

[5] H Sawaf, G Thomas, JJ Taliercio, G Nakhoul, TJ Vachharajani and A Mehdi. Therapeutic advances in diabetic nephropathy. *J. Clin. Med.* 2022; **11**, 378.

[6] M Ruiz-Ortega, RR Rodrigues-Diez, C Lavoz and S Rayego-Mateos. Special issue 'diabetic nephropathy: Diagnosis, prevention and treatment. *J. Clin. Med.* 2020; **9**, 813.

[7] T Kocher, J König, WS Borgnakke, C Pink and P Meisel. Periodontal complications of hyperglycemia/diabetes mellitus: Epidemiologic complexity and clinical challenge. *Periodontology 2000* 2018; **78**, 59-97.

[8] RJ Galindo, M Fayfman and GE Umpierrez. Perioperative management of hyperglycemia and diabetes in cardiac surgery patients. *Endocrinol. Metabol. Clin. N. Am.* 2018; **47**, 203-22.

[9] G Davì, P Gresele, F Violi, S Basili, M Catalano, C Giammarresi, R Volpato, GG Nenci, G Ciabattoni and C Patrono. Diabetes mellitus, hypercholesterolemia, and hypertension but not vascular disease Per Se Are associated with persistent platelet activation *in vivo*: Evidence derived from the study of peripheral arterial disease. *Circulation* 1997; **96**, 69-75.
[10] EBM Daliri, Y Kim, Y Do, R Chelliah and DH Oh. *In vitro* and *in vivo* cholesterol reducing ability and safety of probiotic candidates isolated from korean fermented soya beans. *Probiotics Antimicrob. Protein.* 2022; **14**, 87-98.
[11] T Rittiphairoj, K Pongpirul, K Janchot, NT Mueller and T Li. Probiotics contribute to glycemic control in patients with type 2 diabetes mellitus: A systematic review and meta-analysis. *Adv. Nutr.* 2021; **12**, 722-34.
[12] D Xavier-Santos, R Bedani, ED Lima and SMI Saad. Impact of probiotics and prebiotics targeting metabolic syndrome. *J. Funct. Foods* 2020; **64**, 103666.
[13] M Shimizu, M Hashiguchi, T Shiga, H Tamura and M Mochizuki. Meta-analysis: Effects of probiotic supplementation on lipid profiles in normal to mildly hypercholesterolemic individuals. *PLos One* 2015; **10**, e0139795.
[14] Z Guo, XM Liu, QX Zhang, Z Shen, FW Tian, H Zhang, ZH Sun, HP Zhang and W Chen.Influence of consumption of probiotics on the plasma lipid profile: A meta-analysis of randomised controlled trials. *Nutr. Metabol. Cardiovasc. Dis.* 2011; **21**, 844-50.
[15] A Ataie-Jafari, B Larijani, HA Majd and F Tahbaz. Cholesterol-lowering effect of probiotic yogurt in comparison with ordinary yogurt in mildly to moderately hypercholesterolemic subjects. *Ann. Nutr. Metabol.* 2009; **54**, 22-7.
[16] S Usmiati and R Risfaheri. Pengembangan dadih sebagai pangan fungsional probiotik asli Sumatera Barat. *Jurnal Penelitian Pengembangan Pertanian* 2013; **32**, 20-9.
[17] E Purwati, SN Aritonang, S Melia, I Juliyarsi and H Purwanto. *Manfaat probiotik bakteri asam laktat dadiah menunjang kesehatan masyarakat*. Lembaga Pengembangan Teknologi Informasi dan Komunikasi, Padang, Indonesia, 2017.
[18] A Kundu, P Dey, P Sarkar, S Karmakar, IH Tae, KS Kim, JH Park, SH Lee, BM Lee, L Renthlei, Z Puia and HS Kim. Protective effects of Croton hookeri on streptozotocin-induced diabetic nephropathy. *Food Chem. Toxicol.* 2020; **135**, 110873.
[19] R Amelia, K Philip, YE Pratama and E Purwati. Characterization and probiotic potential of lactic acid bacteria isolated from dadiah sampled in West Sumatra. *Food Sci. Tech.* 2021; **41**, 746-52.
[20] Y Wang, D Dilidaxi, Y Wu, J Sailike, X Sun and X Nabi. Composite probiotics alleviate type 2 diabetes by regulating intestinal microbiota and inducing GLP-1 secretion in db/db mice. *Biomed. Pharmacother.* 2020; **125**, 109914.
[21] H Harun, Y Yanwirasti, B Purwanto and EP Rahayuningsih. The effect of giving dadih on malondialdehyde levels and renal interstitial fibrosis at aging kidney. *Open Access Macedonian J. Med. Sci.* 2020; **8**, 293-6.
[22] X Guo, S Hong, H He, Y Zheng, Y Chen, X Mo, J Li, L Li, R Steinmetz and Q Liu. NFκB promotes oxidative stress-induced necrosis and ischemia/reperfusion injury by inhibiting Nrf2-ARE pathway. *Free Radic. Biol. Med.* 2020; **159**, 125-35.
[23] M Miraghajani, N Zaghian, M Mirlohi, A Feizi and R Ghiasvand. The impact of probiotic soy milk consumption on oxidative stress among type 2 diabetic kidney disease patients: A randomized controlled clinical trial. *J. Ren. Nutr.* 2017; **27**, 317-24.
[24] Y Zheng, SH Ley and FB Hu. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat. Rev. Endocrinol.* 2018; **14**, 88-98.
[25] L Kong, H Wu, W Zhou, M Luo, Y Tan, L Miao and L Cai. Sirtuin 1: A target for kidney diseases. *Mol. Med.* 2015; **21**, 87-97.
[26] A Guilbaud, M Howsam, C Niquet-Leridon, F Delguste, M Fremont, S Lestavel, P Maboudou, A Garat, S Schraen, B Onraed, B Foligné, É Boulanger and FJ Tessier. The effect of *Lactobacillus fermentum* ME-3 treatment on glycation and diabetes complications. *Mol. Nutr. Food Res.* 2020; **64**, 1901018.
[27] AA Dumas, A Lapointe, M Dugrenier, V Provencher, B Lamarche and S Desroches. A systematic review of the effect of yogurt consumption on chronic diseases risk markers in adults. *Eur. J. Nutr.* 2017; **56**, 1375-92.
[28] H Zhong, Abdullah, L Deng, M Zhao, J Tang, T Liu, H Zhang and F Feng. Probiotic-fermented blueberry juice prevents obesity and hyperglycemia in high fat diet-fed mice in association with modulating the gut microbiota. *Food Funct.* 2020; **11**, 9192-207.
[29] E Amirani, Z Asemi and M Taghizadeh. The effects of selenium plus probiotics supplementation on

glycemic status and serum lipoproteins in patients with gestational diabetes mellitus: A randomized, double-blind, placebo-controlled trial. *Clin. Nutr. ESPEN* 2022; **48**, 56-62.
[30] L Wang, M Guo, Q Gao, J Yang, L Yang and X Juang. The effects of probiotics on total cholesterol: A meta-analysis of randomized controlled trials. *Medicine* 2018; **97**, e9679.
[31] SM Lim, JJ Jeong, KH Woo, MJ Han and DH Kim. *Lactobacillus sakei* OK67 ameliorates high-fat diet-induced blood glucose intolerance and obesity in mice by inhibiting gut microbiota lipopolysaccharide production and inducing colon tight junction protein expression. *Nutr. Res.* 2016; **36**, 337-48.
[32] L Wu, Q Wang, F Guo, X Ma, J Wang, Y Zhao, Y Yan and G Qin. Involvement of miR-27a-3p in diabetic nephropathy via affecting renal fibrosis, mitochondrial dysfunction, and endoplasmic reticulum stress. *J. Cell. Physiol.* 2021; **236**, 1454-68.
[33] A Khare and S Gaur. Cholesterol-lowering effects of lactobacillus species. *Curr. Microbiol.* 2020; **77**, 638-44.
[34] HJ Zheng, J Guo, Q Jia, YS Huang, H Wei-Jun, W Zhang, F Zhang, WJ Liu and Y Wang. The effect of probiotic and synbiotic supplementation on biomarkers of inflammation and oxidative stress in diabetic patients: A systematic review and meta-analysis of randomized controlled trials. *Pharmacol. Res.* 2019; **142**, 303-13.
[35] B Bhat and BK Bajaj. Multifarious cholesterol lowering potential of lactic acid bacteria equipped with desired probiotic functional attributes. *3 Biotech* 2020; **10**, 200.
[36] L Merone and R McDermott. Nutritional anti-inflammatories in the treatment and prevention of type 2 diabetes mellitus and the metabolic syndrome. *Diabetes Res. Clin. Pract.* 2017; **127**, 238-53.
[37] B Xue, J Yu, J Zhang, F Hao, X Zhang, J Dong, J Sun and J Xue. Microbial diversity analysis of vineyard son the eastern foothills of the Helan Mountain region using high-throughput sequencing. *Food Sci. Tech.* 2022; **42**, e66320.

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.

[TiS] Your article has now been published online

External Inbox

Trends in Sciences (TiS) 5 Sep
to me, Faridah, Farzana, Harnavi

Dear Author,

We are pleased to inform you that your article has now been published:

The Trends in Sciences

Title: The Potential of West Sumatran Dadiah as The Novel to Alleviate Hyperglycemia, Hypercholesterolemia, and Reducing NF-kB Expression in Nephropathy Diabetes Rat Model
Author(s): Rinita Amelia, Faridah Mohd Said, Farzana Yasmin, Harnavi Harun

We hope you will continue promoting your excellent paper in your academic social circles so that more researchers will be able to read it and consider our journal as a venue for future publications.

https://tis.wu.ac.th/index.php/tis/issue/archive

Thank you for publishing with the Trends in