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The Effects of Giving Probiotics in Dadiah Sampled on Increasing Immunoglobulin A (Iga) of Intestinal Tissue in Rats Model of Diabetic Nephropathy

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ABSTRACT

Dadiah is a traditional food of West Sumatra made from buffalo milk fermented in a bamboo tube container. Dadiah is a functional food because it is made through a spontaneous fermentation by lactic acid bacteria in the buffalo milk and bamboo tubes of the "Aua" type. This study aims to examine the effect of giving dadiah to white male mice, Rattus norvegicus Wistar strain, induced by alloxan to diabetic nephropathy, on increasing the microflora of LAB (Lactic Acid Bacteria) in the intestine and increase the value of IgA. This research used an experimental method tested on five white rats with a total sample of 25 rats, including the control group. The diabetic nephropathy model is characterized by blood sugar levels at >200 mg/dl and proteinuria. During eight weeks of treatment, the P1 treatment group was given 3 ml/day of dadiah, while P2 and P3 groups were given 1 ml and 2 ml/ day Lactobacillus fermentum DA3 isolates. This research indicated that giving dadiah and Lactobacillus fermentum DA3 isolates increased the total LAB and level of Immunoglobulin A (IgA) intestinal tissue, as seen in the experimental compared to the control group. The best result of this study was seen in the P1 group, which was given by 3 ml dadiah, where the treatment for this group was able to increase the total number of LAB colonies and increase IgA immunity in the mouse model induced by diabetic nephropathy.

Keywords: *Lactic Acid Bacteria, Dadiah, IgA, Diabetic Nephropathy*

1. INTRODUCTION

Indonesia is an archipelagic state with various customs and cultures. Diversity can also be seen in the multiple types of plants, animals, and even foods. Each place has unique culinary delights with different characteristics and benefits. West Sumatra is one of the many provinces in Indonesia with traditional food cooked by the fermentation method, better known as "Dadiah." Dadiah is a traditional food made from buffalo milk that is fermented spontaneously in a bamboo tube container. Previous research has proven that Lactic Acid Bacteria (LAB) in dadiah has the potential as a probiotic,namely L. fermentum, isolated from dadiah Lintau [1]. Probiotic used as functional food has unique and diverseproperties in each genus and even the bacterial strains [2]. Criteria of probiotics must be selected in choosing probiotic strains, including the resistance to acid and tolerance to bile, survival via the gastrointestinal tract, ability to colonize the surface of the intestine, antimicrobial activity against pathogenic bacteria, and impact on the health of consumers [3]. The functional properties of probiotics include hypocholesterolaemia activity by lowering plasma cholesterol [4], preventing and treating diarrhoea [5], and changing the immune system [6][7]. The mechanism of probiotics that give beneficial effects on the host includes the reduction in luminal pH, competition with pathogens for adhesion sites and nutrient sources, secretion of antimicrobial agents, inactivation of toxins, and enhancement of body immunity. Lactic acid/probiotic bacteria have high binding activity to mucosal epithelial cell membranes, can act as antigen carriers, and bind to target tissues, activating macrophages to generate mucosal immune responses, which can be identified by the emergence of IgA [8].

Immunoglobulin A (IgA) is the main immunoglobulin found in the mucosa, so IgA is also known as secretory immunoglobulin A (IgA). The

formed IgA will enter and exit the lumen or circulatory system, which in turn will stimulate the formation of IgG and IgM. Probiotic bacteria produce IgA about 80% in the intestinal mucosa/intestinal lamina propria [9]. Immunoglobulin A (IgA) is a protein produced by B lymphocytes and the main immunoglobulin is found in about 80% of the digestive tract mucosa, while the rest is found in the blood circulation. The IgA content in the small intestine can be used as an indicator of the health of the digestive tract. IgA production in the digestive tract serves to prevent the attachment of pathogenic microorganisms to intestinal epithelial cells [10], which can protect the intestine from pathogens attack. Probiotic bacteria will stimulate the activation of immunocompetent cells, both macrophages and dendrite cells, thus the lymphoid tissue in lamina propria will trigger plasma cells to produce IgA that plays a role in the mucosal immune system. Therefore, probiotic bacteria can be used as immunomodulators by producing IgA in the mucosa. Antibodies are immunoglobulin (Ig) that can react specifically with antigens that stimulate their production. Antibodies make up 20% of plasma protein [11]. Ig is formed by plasma cells derived from the proliferation of B cells due to contact with antigens. The digestive epithelium and lamina propria play the main function in the transport system and identification/carrier of antigens in the immune system. Macrophages and dendritic cells will then present antigens to T-CD4 + cells and stimulation of b cells take place, then migration of B cells and T cells into the lymphatic cycle occurs (Bosi, 2000). This study aims to determine the effects of giving dadiah and L. fermentum bacteria in white rats with Diabetic Nephropathy on immunoglobulin (IgA) and total LAB in the intestines of rats.

2. MATERIALS AND METHOD

2.1. Materials

2.1.1. The Preparation of Dadiah

The dadiah was obtained from Lintau, West Sumatra, which has fulfilled the effective qualifications to inhibit pathogenic bacteria. From the examination results obtained information that dadiah Lintau contained LAB of 7.1 X 1010 (Amelia et al., 2020).

The Administered volume (mL) =

9.35 g/kg b.w x 0.3 Kg = 3 mL/day

0.935 g/mL

(Harnavi Harun et al., 2020)

Dadiah solution containing 1 g/mL was made by suspending dadiah with aquadest. EasyTouch kits were used for the estimation of blood glucose. Dadiah solution containing 1 g/mL was made by suspending dadiah with aquadest.

2.1.2. The Preparation of LAB

Isolate L. fermentum is rejuvenated first, then propagated in the medium MRS broth at a temperature of 37^0 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 37^0 C for 2x24 hours in the incubator, to find out the number of LAB to be induced.

2.2. Methods

This research is an experimental study based on animaltrials with a post-test only control-group design. The sampling method is Probability Sampling (Simple Randomized Sampling). This study used the Completely Randomized Design (CRD). Male Rattus Norvegicus strain Wistar rats were procured from Pharmacology Department, Andalas University West Sumatera. The research samples are part of the population that has criteria, healthy Wistar-strain male white rats (Rattus norvegicus), namely male white rats with glowing eyes and fur, as well as active and having good appetite, 3-4 months old and weighted 200-300 grams. All rats were maintained at 25 ^{0}C in 12-h dark/12-h light sicle, with both standard pellet diet and water ad libitum. After the acclimatization, except for the normal control group, all other groups were injected with alloxan 100 mg/ Kg b.w. and all rat groups were fed with standard pellet and treated by dadiah, LAB isolated from dadiah and bacteriocin of supernatant from dadiah Lintau West Sumatera for 3 groups treated. The experiment was conducted with 3 treatments groups and two control groups in the male rats of Rattus norvegicus. In this study, mice were divided into three treatment groups with the number of each group of five rats. The 15 diabetic nephropathy rats were to treat and five diabetic nephropathy rats without being treated (Positive control) and six normal rats (control negative) who did not have diabetes nephropathy (without alloxan injection and without treated). The number of samples was obtained by 25 mice.

2.2.1. Induction of diabetes and in vivo experimental

Before the experiment began, all the rats weighed weight and measured blood glucose levels by cutting off the rat's tail's 1 mm end. After that, the blood has 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 diabetic nephropathy for hyperglycaemia (>200 mg/dL) and proteinuria. Type II

DM mimicking human conditions in experimental rats was induced by alloxan 100 mg/Kg b.w. Hyperglycaemia and proteinuria was induced using Alloxan 100 mg/Kg b.w dissolved in buffer, pH 4.4 (0.1 M sodium citrate and 0.1 M citric acid), intraperitoneally. 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 5 rats per group and orally administered with dadiah, and LAB isolated sampled from dadiah for 28 consecutive days

Group I Normal Control rats (PN)

Group II Nephropathy Diabetic rats (P0)

Group III ND rats + dadiah 3 ml/ once daily (P1)

Group IV ND rats + LAB 1 ml/ once daily (P2)

Group V ND rats + LAB 2 ml/ once daily (P3)

2.2.2. The Dissection of Experimental Animals

Dissection was performed after 28 days of treatment is given. Male white rats (Rattus norvegicus) were killed by means of Anaesthesia 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 rats in a closed container, wait until it became immobile and its pupillary mydriasis and eyes were closed. If the rats lost consciousness then brought it outside the container then laparotomy, identification and nephrectomy were carried out, then directly put into a 10% BNF solution. After the small intestine (Ileum) organ was removed, neck pressure was done to kill it while pulling it anteriorly (dislocasio atlanto-occipitalis) (Harmita, 2008) .

2.2.3. Laboratory Test

2.2.3.1.Calculation of Total Lactic Acid Bacteria

The media used for calculating the total LAB in rat intestines were de Mann Ragosa Sharpe (MRS) agar and MRS broth (Merck). The MRS broth was diluted up to 107 CFU/g. the spread plate method was used to incubate it an-aerobically at 37oC for 48 hours. And then, observations and calculation were conducted using the Quebec Colony Counter.

2.2.3.2. Immunoglobulin (IgA) Test

The test refers to the KIT ELISA (Bioassay Technology Laboratory) protocol. The next step is to prepare the reagents and samples in room temperature conditions then map the position of the sample. A total of 50 μl of standard solution was added to the marked wells then 40 μl of sample and 10 μl of Anti-IgA were added to the marked wells. In the standard wells and sample wells, 50 μl of streptavidin-HRP was added then covered with a cover plate, incubated for 1 hour in a 370C incubator. Then, the solution in the wells was discarded, and each well was washed with a 350 μl wash buffer and then let stand for 1 minute. This washing was carried out 5 times. Substrate solution of A 50 μl was inserted, then substrate B 50 μl, covered with a cover plate and incubated for 1 hour in a 370C incubator. A total of 50μl Stop Solution was added to all wells and waited for 10 minutes. Furthermore, the OD value was read with Elisa Reader Multiskan Ex (Thermo Scientific) at 450 nm.

2.2.4. Data Analyze

The design used in this study was a randomized block design with 5 groups and 3 replications. Statistical data analysis was conducted using the SPSS 26 statistics application.

3. RESULT AND DISCUSSION

3.1 Total Lactic Acid Bacteria

Based on the research conducted, it was found that the total colonies of anaerobic bacteria or LAB were found in the rat's intestines as seen in table 1.

Graph 1, showed an increase in LAB colonies in the ileum. The increase in total LAB Based on table 1, it can be seen that the total range of LAB in the study is in the range of 74 - 213 x10^7 CFU/g. The results showed that rats without diabetes nephropathy and treatment (P0) were not significantly different from ($P < 0.05$) mice with nephropathy diabetes (ND), as well as P1, P2 and P3 ($P < 0.05$), while PO and PN were significantly different from P1. Significant results can be seen in PO where P1, P2 and P3 were significantly different ($P > 0.01$). This shows that the LAB found in dadiah and Isolate L. fermentum DA3 isolates are able to colonize in the intestine. According to the statement of (Ren et al., 2018), one of the criteria for LAB as a probiotic candidate is that it must be able to colonize and inhibit the colonization of pathogenic bacteria in the intestine.

The highest result on total intestinal LAB was found at P1, namely by adding dadiah as much as 3 ml/day, although the results were not significantly different from treatment P2 and P3 ($P < 0.05$). This shows that alloxan-induced rats as diabetes will also be related to intestinal microflora and its impact on health.

This study is supported by the results of a study conducted by [12] . The study found that probiotic doses varied in several other studies ranging from 10^7-10^{10} CFU daily depending on the method used. However, the most common dosage is about 10^8–10^{10} CFU/day.

3.2 Immunoglobulin A

IgA analysis from rat intestines can be seen in the graph below

IgA analysis from rat intestines can be seen in the graph 1. In this study, it can be seen that there is an increase in the IgA value of rats that were added with dadiah and L. fermentum DA3. The range of IgA values in this study was 25.27-76.58. This is in line with the increase in total LAB (Table 1), where the increase in total LAB affects the IgA value because oral administration of LAB can activate both specific and nonspecific immune systems. Masahata et al., 2014 showed the relationship between IgA-secreted cells and microbiota composition. This study interprets the importance of the relationship between microbiota composition and lymphoid tissue in IgA to secreting cell generation from bacteria-free rats. This study found a decrease in IgA-secreting cells in the large intestine, as well a decrease in fecal IgA levels [13]

Other studies, stated that L. fermentum ACA-DC 179 *in Vitro* had probiotic properties such as antimicrobial and immunomodulating activities, was successfully applied in vivo to a mouse model infected with Salmonella [14]. It was also significantly able to reduce colitis in a TNBS-colitis mouse model. also revealed that L. fermentum was able to stimulate the immune system andinhibit the attachment of pathogenic microorganisms in the digestive tract of rats (MacÍas-Rodríguez et al., 2009). The local immune response in the intestine is caused by the interaction between probiotics and epithelial cells. After the interaction with epithelial cells, probiotics are internalized. The first cells that will interact with probiotics are antigen-presenting cells (APC), macrophages, and dendritic cells associated with intestinal lamina propria, and induce the release of interleukin-6 (IL-6) and IL-10. Interleukin-6 stimulates the growth and differentiation of B cells into plasma cellsthat produce IgA, while IL-10 plays a role in controllingnon-specific immune reactions and cellular immunity.

An increase in the value of IgA added with dadiah and L. fermentum in diabetic nephropathy rats showed that the probiotics were contained in local food in West Sumatra, which is dadiah, and L. fermentum isolates from dadiah Lintau (Amelia et al., 2020) have the potential as probiotics and can increase body immunity. (Maldonado Galdeano et al., 2007) stated that a systematic immune response can be induced by the probiotic bacteria after the probiotics interact with immune cells in Peyer's Plaques, wherein this peyer's plaque, probiotics or their fragments are internalized by M cells or in the paracellular pathway through follicle-associated epithelial cells (FAE).

5. CONCLUSSION

The addition of dadiah Lintau and L. fermentum DA3 culture to rats with diabetes nephropathy for 4 weeks was able to increase the total LAB in the ileum and increase intestinal immunoglobulin (IgA). The best results in this study is in the P1 treatment, namely by adding dadiah as much as 3 ml/day/rat..

6. ETHICAL CLEARANCE

Animal experiments were approved by the local ethics committee and respected the principles of animal experimentation. Ethics clearance was approved by the Ethics Committee of Medical Faculty of Baiturrahmah University (No: 001/ETIK-FKUNBRAH/03/03/21)

7. FIGURES AND TABLES

Table 1 Total Average of LAB in Ileum

Treatment	Total LAB 10^7 CFU/g
PN (Healthy rats)	$111\pm11{,}68^{ab}$
PO (Diabetic rats) without treatment	74 ± 9.17^{a}
P1 (Diabetic rats + *dadiah* 3 ml/day)	213 ± 24.91^{d}
P2 (Diabetic rats + LAB low dose)	197 ± 24.25^{cd}
P3 (Diabetic rats + LAB high dose)	206 ± 24.27^{d}

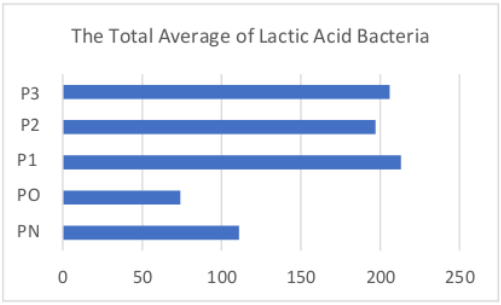

Figure 1 The Total Average of LAB

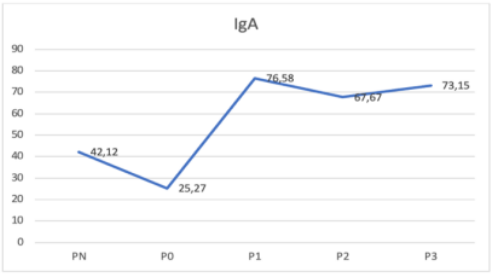

Figure 2 Immunoglobulin A (IgA) Values in the ileum tissue of rats

AUTHORS' CONTRIBUTIONS

All researchers have contributed both in the preparation of research proposals, laboratory research and the preparation of manuscripts to be published in proceedings

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