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Antidiarrheal Effect of *Lactobacillus fermentum* Isolated from Dengke Naniura on *Escherichia coli*-*induced* Rats

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Abstract. Diarrhea is discharge of abnormal and liquid stool with a frequency of 3-4 times daily caused by pathogenic organisms, including Escherichia coli (E.coli). Dengke Naniura is a traditional Batak Toba food that can produce probiotics (*Lactobacillus fermentum*). Probiotics are defined as live microorganisms that confer a health benefit on the host. Nutritional supplements which are combination of probiotics and prebiotics are called synbiotics. The purpose of this study was to analyze the antidiarrheal effect of L.fermentum in rats given for 21 days. Antidiarrheal test was carried out by administering L. fermentum from day 1 to day 21. The rats were induced with *E.coli* on day 7th to day 14th of the experiment. Weight of the rats, stool consistency, onset, frequency, and duration of diarrhea were observed. This study found that weight of rats in the *L.fermentum* groups increased compared to those in group 1 ($p<0.05$). Consistency stool is soft (massless). Onset of diarrhea on day 1 after induced of E. coli in the group 1. Frequency of diarrhea increased to 9.25 ± 0.50 times on day 14 (group 1). And diarrhea occurs for 10 days. It could be concluded that *L.fermentum* showed an antidiarrheal effect in rats induced with *E.coli*.

Keyword: Antidiarrheal effect, *Lactobacillus fermentum, Escherichia coli,* Dengke Naniura, Probiotic, Prebiotic, Synbiotic

Abstrak. *Diare adalah keluarnya feses yang tidak normal dan cair dengan frekuensi 3-4 kali sehari yang disebabkan oleh organisme pathogen* termasuk Escherichia coli. *Dengke Naniura merupakan makanan tradisional Batak Toba yang dapat menghasilkan probiotik (Lactobacillus fermentum). Probiotik didefinisikan sebagai mikroorganisme hidup yang memberikan manfaat kesehatan pada inangnya. Nutrisi suplementasi yang merupakan kombinasi probiotik dan prebiotik disebut sinbiotik. Tujuan penelitian ini adalah untuk menganalisis efek pencegahan diare pada tikus yang diberi L.fermentum selama 21 hari. Uji efek preventif diare dilakukan dengan pemberian L.fermentum dari hari ke-1 sampai hari ke-21. Dari hari ke-7 sampai hari ke-14 tikus diinduksi dengan E.coli. Bobot tikus, konsistensi feses, onset diare, frekuensi diare, dan durasi diare diamati. Hasil pengamatan berat badan tikus pada kelompok L.fermentum meningkat dibandingkan dengan kelompok 1 ($p<0,05$). Konsistensi feses lunak (tidak bermassa). Onset diare pada hari 1 setelah induksi E.coli pada kelompok 1. Frekuensi diare meningkat menjadi 9,25 ± 0,50 kali pada hari ke 14 (kelompok 1). Diare terjadi selama 10 hari. Lactobacillus fermentum menunjukkan efek pencegahan diare pada tikus yang diinduksi E.coli.*

Kata Kunci: *Efek preventif, Lactobacillus fermentum, Escherichia coli, Dengke Naniura, Probiotik, Prebiotik, Sinbiotik*

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1. Introduction

Diarrhea is a public health problem in developing countries such as Indonesia. Diarrhea is an abnormal and liquid discharge with more frequency than usual. Diarrhea can be caused by malnutrition, delayed physical development, poor hygiene and sanitation. Diarrhea could also occurs due to bacterial, viral, and parasitic infections [1].

Every year, it is estimated that 2.5 billion cases of diarrhea occur in children under 5 years of age. The incidence of diarrhea causes a mortality rate of 9% in children worldwide. Diarrheal disease is the second highest cause of death in children under 5 years of age. Globally, from data on deaths in children that occur every day, the diarrheal disease has a percentage of 16% or equal to more than 1600 deaths in children under 5 years old [2].

Diarrhea can be caused by microorganisms, namely pathogenic bacteria such as *E.coli*, *Salmonella typhi*, *Shigella* sp, *Campylobacter jejuni*, and several other pathogenic bacteria. These bacteria enter the human body to the epithelial cells of the small intestine and form colonization causing infection. Consequently, fluids and food that enter the digestive tract are not absorbed and will accumulate in the small intestine and increase intestinal osmotic pressure and finally diarrhea occurs [3].

Dengke Naniura is a Batak Toba food in which its production uses a fast fermentation technique by soaking fish in jungga acid (it is a juice obtained from a type of Citrus species) until the meat becomes soft and can be directly eaten without cooking. Dengke naniura contains Lactobacillus sp bacteria that can be isolated and used as probiotics [4]. Hutahean [5] has isolated and identified Lactobacillus fermentum from Dengke naniura. Lactobacillus, Bifidobacterium, and Saccharomyces are the most commonly used microbes as probiotic strains for the prevention and treatment of diarrhea [6].

Probiotics are defined as live microorganisms that confer a health benefit on the host when administered in sufficient quantities. Probiotic intake with the right dose will provide great benefits to the host. Probiotics in the digestive tract will colonize and stick to the intestinal mucosa, especially the colon to maintain intestinal health after oral administration [7], [8]. Nutritional supplements that combine probiotics with prebiotics are called synbiotics. Synbiotics provide an effect that is considered to work together in increasing beneficial effects because both are synergistically more active in preventing digestive system disorders. In relation to the above description, this study aimed to analyze the diarrhea preventive effect of Lactobacillus fermentum on rats induced *E.coli* [9], [10].

2. Material and Methods

2.1 Material

Materials used in this study were *Lactobacillus fermentum* isolated from dengke naniura, *E.coli* obtained from Microbiology Laboratory, Faculty of Pharmacy, University of Sumatera Utara, Lacto-B sachet as a positive control, *deMann Rogosa and Sharpe Agar* (Himedia), *deMann Rogosa and Sharpe Broth* (Himedia), *Nutrient Agar* (Himedia), *Nutrient Broth* (Himedia), and male rats.

2.2 Preparation of Culture Stock and Inoculum of Lactobacillus fermentum

One colony of *Lactobacillus fermentum* was taken using a sterile ose and then cultured on *deMann Rogosa and Sharpe Agar* and incubated at 35±2ºC for 24 hours. After incubation, 1 colony of *Lactobacillus fermentum* was taken with a sterile ose and inserted into the *deMann Rogosa and Sharpe Broth* and incubated again at 35±2ºC for 24 hours. The inoculum turbidity was measured using a UV-VIS spectrophotometer until the number of colonies was 10^8 CFU/mL at wavelength of 580 nm [11].

2.3 Preparation of Culture Stock and Inoculum Escherichia coli

One colony of *E.coli* was taken using a sterile ose and then cultured on *Nutrient Agar* and incubated at 35±2ºC for 24 hours. After incubation, 1 colony of *E.coli* was taken with a sterile ose and inserted into the *Nutrient Broth* and incubated again at 35±2ºC for 24 hours. The inoculum turbidity was measured using a UV-VIS spectrophotometer until the number of colonies was 10^8 CFU/mL at wavelength 580nm [11].

2.4 Preparation of Rats

In this study, a total of 28 experimental male rats with weight of ± 200 grams were used and randomly divided into 7 groups. Groups 1 and 2 were used as a negative control and a normal group without treatment, respectively. Groups 3, 4, and 5 were treated with *Lactobacillus fermentum* at varied doses. Group 6 was provided synbiotics that consisted of *Lactobacillus fermentum* and cereal oats. Group 7 were given Lacto-B as a positive control.

2.5 The Diarrhea Preventive Effect Test

All rats were adapted for 7 days at constant temperature and humidity before the experiment was started. Groups 3, 4, and 5 were treated with 0.5 mL, 1 mL, and 1.5 mL of Lactobacillus fermentum, respectively. Group 6 was provided synbiotics that consisted of 0.5 mL of Lactobacillus fermentum and 0.5 mL of cereal oats. Group 7 were given Lacto-B. All groups were treated for 21 days (first day up to 21st day). After the 7th day of experiment, the rats in all groups were induced with *E.coli* for 14 days. The weight and feces consistency of the rats were observed at seven-day interval (days 0, 7, 14, and 21). The onset, frequency, and duration of diarrhea were evaluated on the 7th to 18th day).

2.6 Data Analysis

All data were presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS software with normality test and analysis of variance for normally distributed data and Kruskal Wallis test for data that were not normally distributed.

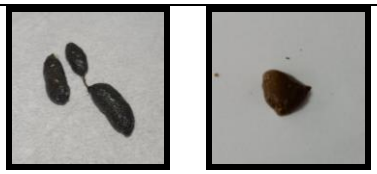
3. Results and Discussion

3.1 Weight of the Experimental Rats

Weight of the experimental rats is presented in Figure 2(a). Weight of the experimental rats in all groups was observed in day 0, day 7th, day 14th, and day 21st. Figure 2(a) shows that the weight of rats in group 1 (negative control) decreased on the day 14th of the experiment because the group was only induced with *E.coli*. However, groups 3, 4, and 5 given *Lactobacillus fermentum* showed an increase in their weight. The results of the homogeneity test indicated that the data were homogeneous (p=0.378). ANOVA with post hoc Tuckey and LSD proved that there was a significant difference between group 1 and other groups in term of weight gain (group 2, p=0.000; group 3, p=0.005; group 4, p= 0.000; group 5, p=0.000; group 6, p=0.000; group 7, p=0.000). Meanwhile, there was a significant difference in term of weight gain of rats between the 0th and the 14th day of observations (p=0.001). There was also a significant difference in term of weight gain of rats between the 0th and the 21st day of observations (p=0.000). The decrease in weight of the rats in the control group was caused by *E.coli* that induced digestive tract infections which consequently disrupted the absorption of nutrients in the feed in the rat's intestines. In contrast, increase in weight was observed in the groups treated with *Lactobacillus fermentum.* This fact indicated that the intestinal mucosas of the rats were protected by the presence of these bacteria. Diarrhea can also result in fever, abdominal pain, decreased appetite, fatigue, and weight loss [3]. Diarrhea is usually associated with weight loss which ultimately leads to malnutrition. This clinical condition and requires nutrition intake to improve nutritional status [12].

3.2 The Consistency of Feces

Consistency of the feces is shown in Figure 1. The consistency of feces was visually observed on 0-day, 7th day, 14th day, and 21st day. Group 1 (negative control) was observed to have soft stool consistency on the 8th day (1 day after inducing with *E.coli*). Meanwhile, in the other group, there was no evidence of soft or massless stools. This means that *Lactobacillus fermentum* bacteria were able to balance the excessive number of *E.coli* in the intestine. *Lactobacillus fermentum* secretes antimicrobial proteinaceous compound(s) that was active to exclude the enteropathogenic *E.coli* in the digestive tract [13]. Rats that experienced diarrhea were characterized by the discharge of feces in a soft, watery to the shapeless condition with a frequency of more than 4 times daily.



(a) (b)

Figure 1 The consistency of the rat feces. (a) normal. (b) soft (diarrhea).

3.3 Onset of Diarrhea

Onset of diarrhea in all groups is shown in Figure 2(b). Diarrhea in rats was observed to occur on the 8th day (1 day after inducing with *Escherichia coli* in group 1 (negative control). The onset of diarrhea occurred at 123.25 ± 3.30 minutes. As shown in Figure 2(b) diarrhea occurred more quickly on the 12th day with the onset of 76.00 ± 8.83 minutes. The results of the homogeneity test of the data showed that the data were not normally distributed (p=0.000). Based on the Kruskal Wallis test, there was a significant difference between groups with the control group (p=0.000), but between days there was no significant difference with the control group (p=0.600). *Escherichia coli* can cause diarrhea by producing enterotoxins by forming colonization in the intestine. *Escherichia coli* will damage the epithelial cells in the small intestine, which will then be replaced by immature epithelial cells that in-optimal function. This condition causes fluids and food not to be absorbed properly and increases the osmotic pressure in the intestines. Fluids and food that are not absorbed will be pushed out through the anus and diarrhea [3]. Diarrhea did not occur in the group given *Lactobacillus fermentum* because the probiotic mechanism in overcoming the diarrhea prevention effect was by causing changes in the intestinal lumen microenvironment (pH, oxygen); production of antimicrobial agents against several pathogens; nutrient competition; prevents adhesion of pathogens to enterocytes; modification of the toxin or toxin receptor; trophic effects on the intestinal mucosa through the provision of nutrients; and immunomodulation [12].

3.4 Frequency of Diarrhea

The frequency of diarrhea is demonstrated in Figure 2 (c) The frequency of diarrhea on the 8th day showed that the rats had diarrhea 5.75 ± 0.96 times in 1 day on group 1 (negative control). In Figure 1(c) the frequency of diarrhea, increased on the 14th day, which was 9.25 ± 0.50 times per day. The results of the homogeneity test of the data showed that the data were not normally distributed (sig. 0.000). Based on the Kruskal Wallis test, there was a significant difference between the groups with the control group (Asimp. sig. 0.000) and between days there was no significant difference with the control group (Asimp. sig. 0.602). With the increase in the number of *E.coli* bacterial colonies in the intestines of rats, which causes the intestines to be unable to absorb fluids and food, it also causes an increase in osmotic pressure in the intestines of rats, thereby increasing the frequency of diarrhea [3]. While the other group did not experience diarrhea because the intestine was protected from the activity of *Lactobacillus fermentum* in the intestine. The frequency of diarrhea, decreased after 3 days without the induction of *E.coli* bacteria on the 17th day (4.25 ± 0.50 times), and on the 18th day the rat

feces began to return to their normal shape (solid stool). Determination of the frequency of diarrhea shows that the more diarrhea occurs, the more severe the diarrheal infection that occurs and vice versa [14].

3.5 Duration of Diarrhea

Duration of diarrhea experience by the experimental animals is shown in Figure (2d). The duration of diarrhea was determined by recording the time when the rats first experienced diarrhea water or soft stool consistency until it returned to form feces with a solid consistency (observed from the 7th day). Diarrhea occurred in an average of 250-350 minutes during the observation every day after being given *Escherichia coli* as inducer. The first time few rats experienced diarrhea was the 1st day after the induction of *Escherichia coli* bacteria was given on the 8th day for 266.60 ± 7.87 minutes. The longest duration of diarrhea experienced by the rats was on the 14th day, which was 358.25 ± 5.43 minutes. Based on the Kruskal Wallis test, there was a significant difference between the treatment groups and the control group (p=0.000) and between days there was no significant difference with the control group (p=0.599). The incidence with a long duration of diarrhea is caused by colonization of inducing bacteria in the digestive tract, especially the large intestine, thereby increasing the osmotic pressure in the intestine which further causes food not to be completely absorbed in the intestine and diarrhea occurs. [3]. Rats returned to normal heath condition for 4 days after the provision of *Escherichia coli* was stopped. Microvilli cells that are damaged due to pathological processes caused by diarrhea can carry out the regeneration process. The rate of regeneration of intestinal epithelial tissue generally takes place rapidly, ie every 2-5 days [14].

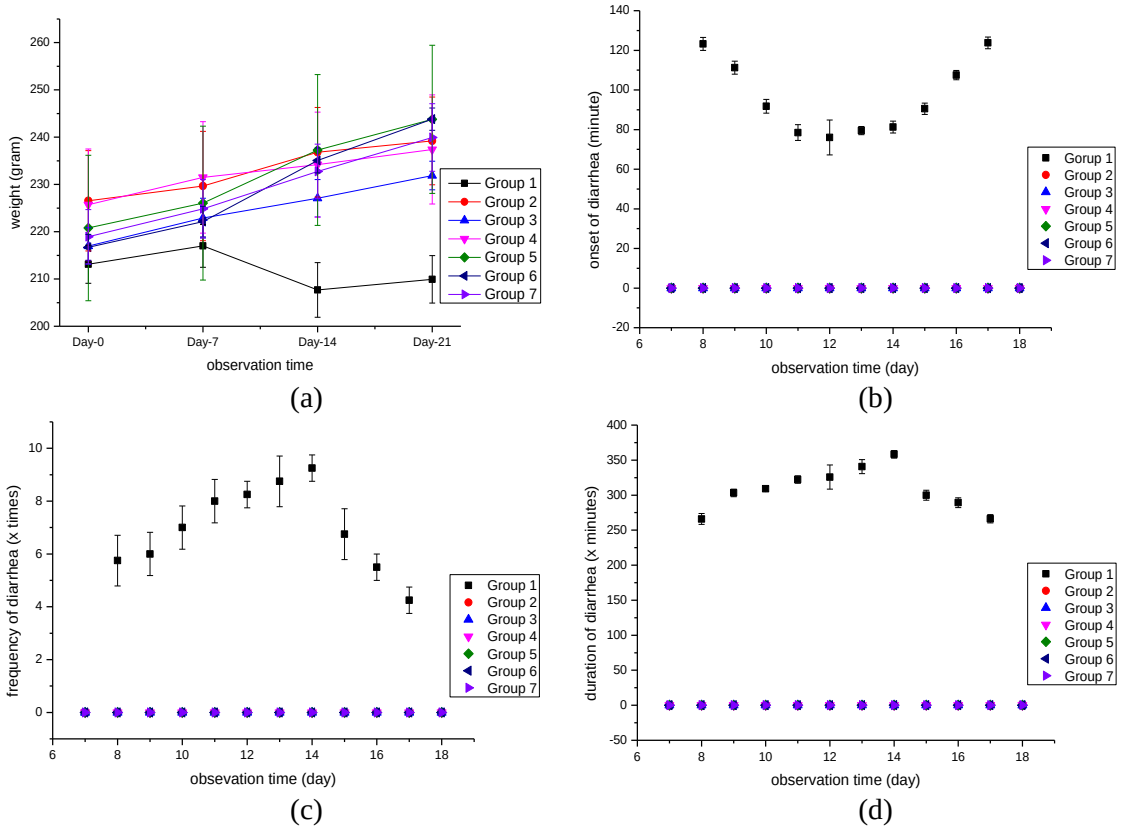


Figure 2 (a) Graph of the weight of the rats, (b) Graph of the onset of diarrhea, (c) Graph of the frequency of diarrhea, and (d) Graph of the duration of diarrhea.

4. Conclusion

Lactobacillus fermentum isolated from Dengke Naniura has a preventive effect on diarrhea in rats induced with *Escherichia coli.*

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