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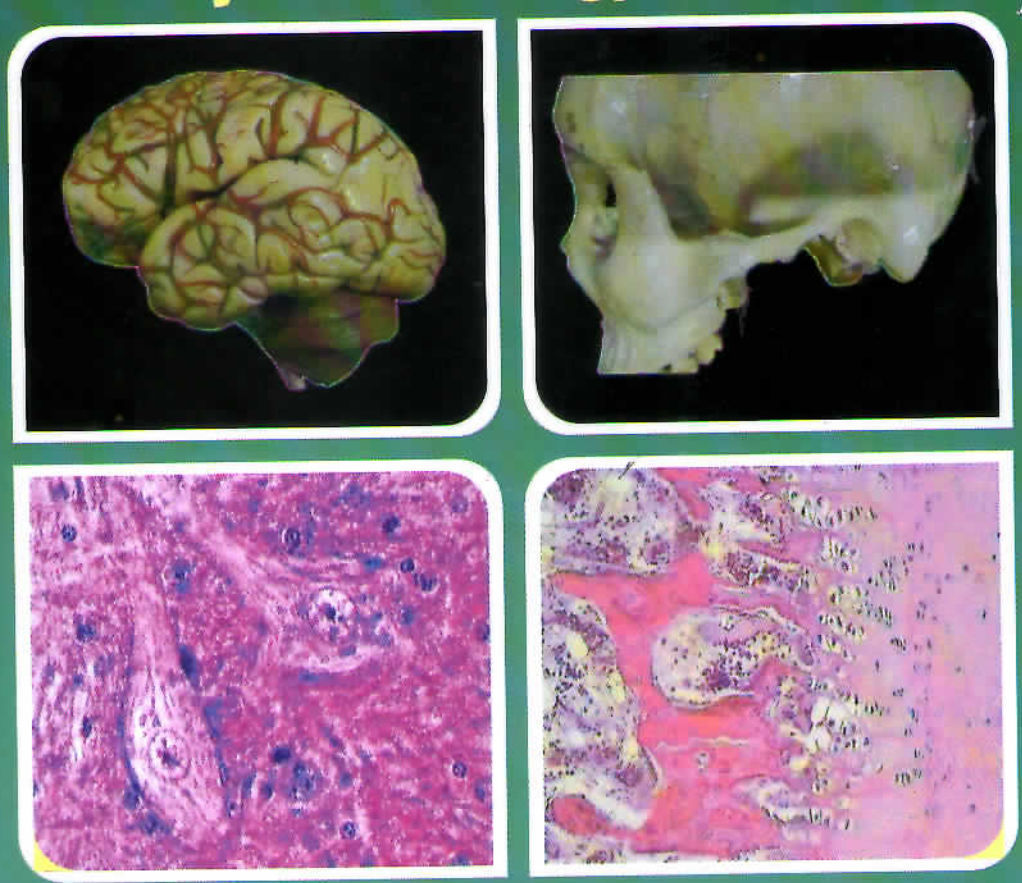

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ANTIOXIDATIVE CAPABILITY OF SYNBIOTIC YOGHURT IN LIVER AND KIDNEY TISSUES OF RATS: AN IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT

Introduction: Tractus digestivus, especially intestine, is the place that easily gets contact with patogenic microorganisms. To increase the balance of intestinal flora, it is very important for improving human health. It was reported that probiotic to have a favorable influence on physiological processes of the host by their effect on intestinal flora. Probiotic was also reported to have effect on immune status, especially IgA in diarrhea. However, only a few reports in the effect of probiotic and prebiotic (synbiotic) on the antioxidant status exist, that can support in the immunomodulation effect. **Objectives:** The present study was conducted to observe the immnohistochemical profile of antioxidant superoxide dismutase (C,Zn-SOD) in the liver and kidney of synbiotic yoghurt treatment rats. **Methods and materials:** A total of 70 male white rats (*Sprague dawley*) were divided into 5 treatment groups; (1) negative control group, (2) treated with synbiotic yoghurt, (3) treated with synbiotic yoghurt and EPEC, (4) treated with EPEC only, and (5) treated with standard yoghurt. The treatments were carried out for 3 weeks. The liver and kidney were obtained every week and then processed using paraffin embedding standard method. The tissues slices were then stained with immunohistochemical technique using monoclonal antibody of Cu,Zn-SOD. **Results:** Antioxidant Cu,Zn-SOD was localized in the nuclear and cytoplasm of both hepatocytes and renal tubule cells. Synbiotic yoghurt treatment for 7, 14, and 21 days showed increased the content of the antioxidant in the liver tissues. It was the highest compared to other treatments. Synbiotic yoghurt treatment also showed increased the content of Cu,Zn-SOD in the kidney tissues. Following 14 days of treatment, the content of Cu,Zn-SOD in the kidney tissues of the symbiotic treatment group increased but it was not significantly different compared to the positive control group. After 21 days treatment, synbiotic yoghurt treatment also increased the content of Cu,Zn-SOD in the kidney tissues, and it was the highest compared to other treatments. **Conclusion:** The synbiotic yoghurt had antioxidative effect in both liver and kidney of rats. In the EPEC intervention rats, synbiotic yoghurt treatment could maintain the content of antioxidant Cu,Zn-SOD as high as the content in the negative control group.

Keywords: Synbiotic, yoghurt, antioxidative, liver, kidney, SOD, immunohistochemistry.

INTRODUCTION

Human gastrointestinal tract is a tube that is rolled over approximately 9 m through the center of the body. The human gastrointestinal tract has a surface area about 300 m^2 (compared with skin that has a surface area of 2 m^2 and lungs that have a surface area of 100 m^2)[1]. This large surface is easily get contact with certain agents including pathogenic microorganisms during the digestive process[2]. These pathogenic microorganisms caused several diseases, such as diarrhea[3].

Leomil *et al.*[4] reported enteropathogenic *Escherichia coli* (EPEC) as a major bacteria that caused diarrhea in human and animals. EPEC was also reported as one major cause of children diarrhea in Indonesia which the prevalence reached 55% of children with diarrhea[5]. In addition, EPEC infection can also cause oxidative stress and affect the other organs. It is because blood from the intestine flows to the other organs including liver and kidney. Liver detoxification function is processing of hazardous substances into harmless which then it will be removed by the kidney[6]. *E. coli* strain can cause extraintestinal infection such as urinary tract infection, include kidney infection.

When foreign microorganisms, such as EPEC, come into the body, there are two main defenses that act. They are the effect of destruction by dissolved chemicals (such as bactericidal enzymes) and the mechanism of phagocytosis[7]. Macrophages do the phagocytosis in the body tissues. In phagocytosis process, there will be destruction of the foreign materials (EPEC). Then, the expenditure of free radicals will be occured in the destruction of the foreign materials (EPEC) by macrophages and cause inflammation to the surrounding body cells. Symptoms appeared in the systemic inflammatory syndrome are allegedly caused by PMN (polymorphonuclear) phagocyte dysregulation, which produces excessive superoxide radicals and

peroxide[8]. If the number of free radical remarkable increased and cellular antioxidant cannot handle it, the e stress will occur, which in turn can cause tissue damage.

Arief *et al.*[9], isolated 10 indigenous lactic acid bacteria from beef meat at some traditional markets in Then, our previous study showed that only two of them to have probiotic characteristic. They are *cillus fermentum 2B4* and *Lactobacillus plantarum 2C12*[10]. Wresdiyati *et al.*[11], reported that *cillus fermentum 2B4* showed increased antioxidant Cu,Zn-SOD, immunohistochemically, in rats ine.

It was reported that probiotic has a favorable influence on physiological processes of the host by their on intestinal flora[1]. Probiotic was also reported has effect on immune status, especially IgA in the on of diarrhea. However, only a few report in the effect of probiotic and prebiotic (synbiotic) on the dant status related to immunomodulation effect.

ECTIVES

The present study was conducted to observe the immnohistochemical profile of antioxidant superoxide se (C,Zn-SOD) in the liver and kidney of indigenous *Lactobacillus fermentum 2B4*–contained synbiotic urt treatment rats, that were intervented by EPEC.

THODS AND MATERIALS

erials

The materials used for making yoghurt were: *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, local probiotic lactic acid bacterias or LAB (*Lactobacillus fermentum* 2B4) cultures, *enteropathogenic Escherichia coli* K1.1 (EPEC K1.1) culture, *de Man Rogosa Sharpe Broth* (MRSB), *de Man Rogosa Sharpe* (MRSA), *Nutrient Broth* (NB), *Eosin Methylene Blue Agar* (EMBA), KH_2PO_4, aquadest (distilled water), NaOH 1N, glucose, bacto agar (Difco), $CaCO_3$, skim milk, sugar, fructooligosaccharide (FOS), alcohol 70%, spirit.

The materials used for analysing antioxidative capability of the synbiotic yoghurt were: rats feed sein, corn oil, carboxymethylcellulose, mineral mix, vitamin mix, corn starch, and water), liver and kidneys rats, Bouin solution (saturated picrate acid, 37-40% formalin, and glacial acetic acid with a ratio of 15: 5: 1), 80, 90, 95, and 100% (absolute) alcohol, xylol, paraffin, aquadest (distilled water), NaCl crystal, siological solution of 0.9% NaCl, toluene, neophrene, tap water, $Na_2HPO_4.12H_2O$, $NaH_2PO_4.2H_2O$, sphate Buffered Saline (PBS), aquabidest, methanol, H_2O_2, normal serum, Cu,Zn-SOD primary antibody, Envision System Peroxidase as a secondary antibody, diamino benzidine solution (DAB) as a chromogen, matoxylin, and entellan.

The 70 rats used in this research were male-sex white rats, Sprague *Dawley* strain with a weight range een 80-100 g. The rats were from *Pusat Studi Biofarmaka Lembaga Penelitian dan Pengabdian arakat Institut Pertanian Bogor (LPPM IPB).*

thods

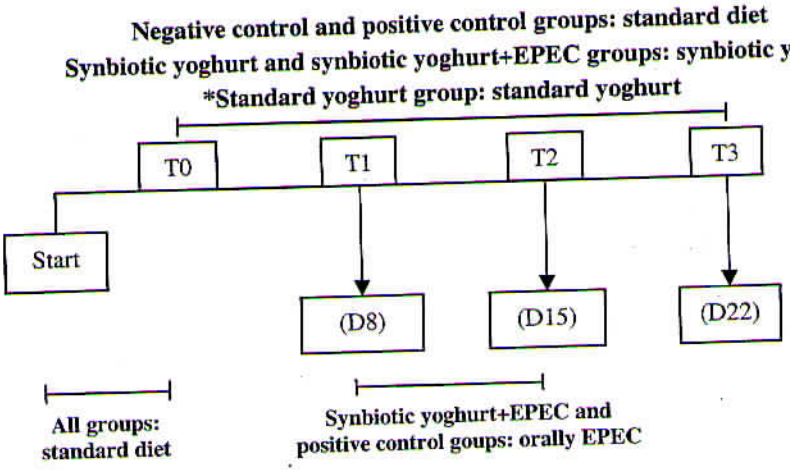
eatments to The Experimental Rats

The rats (70 rats) were divided into five treatment groups: (1) negative control group, (2) treated with biotic yoghurt, (3) treated with synbiotic yoghurt and EPEC, (4) treated with EPEC only, and (5) treated with dard yoghurt (Table 1). Each group consisted of 15 rats as repetitions, except for the standard yoghurt up, which only consisted of 10 rats. Before the treatments began, the standard diet was given to the all rats as adaptation period to the environment for three days. The treatments were carried out for 3 weeks. Then, after treatments were done, the liver and kidney were obtained every week and then processed using paraffin bedding standard methods. The termination process was carried out as shown in Figure 1.

Table 1. The groups of rats according to the given treatments

No.	Rats Groups	Treatments
1.	Negative control	Normal rats that were fed with standard diet
2.	Synbiotic yoghurt	The rats that were fed with standard diet and synbiotic yoghurt
3.	Synbiotic yoghurt + EPEC	The rats that were fed with standard diet and synbiotic yoghurt, but interspersed with EPEC intervention
4.	Positive control	The rats that were fed with standard diet and EPEC intervention
5.	Standard yoghurt	The rats that were fed with standard diet and standard yoghurt

Notes:
- Synbiotic yoghurt contained *L. bulgaricus, S. thermophilus,* prebiotic FOS, and indigenous probiotic lactic acid bacterias or LAB (*Lactobacillus fermentum* 2B4)
- Standard yoghurt was the yoghurt containing *L. bulgaricus* and *S. thermophilus* and the prebiotic FOS
- Yoghurt was administered orally as much as 1 ml/day (LAB population of 10^9 cfu/ml) using the sonde from day 1 (the beginning of the treatment) until day 21 (the end of the treatment).
- EPEC intervention (caused diarrhea) was given orally using the sonde as much as 1 ml/day with a population of 10^7 cfu/ml for 7 days (day 8 to day 14).



Notes:

D8 = termination day 8 → negative control, synbiotic yoghurt, synbiotic yoghurt + EPEC, and positive control groups.

D15 = termination day 15 → negative control, synbiotic yoghurt, synbiotic yoghurt + EPEC, and positive control groups.

D22 = termination day 22 → negative control, synbiotic yoghurt, synbiotic yoghurt + EPEC, positive control and standard yoghurt groups.

* = the standard yoghurt group experienced only a one-time termination on day 22 (D22).

Figure 1. The treatments and termination schema of the experimental rats groups

Analysis of Superoxide Dismutase (SOD) Enzyme Content Immunohistochemically in Liver and Kidney[12,13]

The livers and kidneys of the rats were washed with 0.9% physiological NaCl and fixed in Bouin solution for 24 hours. Afterwards, these tissues were processed by standard method using paraffin to obtain tissue blocks. Then, the tissue blocks were sliced with a thickness of 4 µm using a rotary microtome. Furthermore, the tissues were placed on the object glass coated with neophrene in toluene. Afterwards, these sliced tissues were stained with immunohistochemical staining technique[12,13].

The observations on cells producing Cu,Zn-SOD was held by comparing the distribution and intensity of brown color that appeared in tissue preparations observed. The differences in intensity of color formed were divided into positive and negative reactions. Brown color indicated a positive reaction to the Cu,Zn-SOD enzyme. It meant these cells contained the Cu,Zn-SOD enzyme. The older brown color in the tissues showed the higher content of Cu,Zn-SOD enzyme. The differences of Cu,Zn-SOD enzyme content in the hepatocytes and the renal tubules nucleus were divided into :

- Strong positive (+++) indicated by the dark brown color.
- Moderate positive (++) indicated by the medium brown color.
- Weak positive (+) indicated by the brown mixed with blue color.
- Negative (-) indicated by the blue color.

Furthermore, quantitative observations were held by counting the number of nucleus at various intensity of brown color in five areas of view of each slide. The results were statistically analyzed by analysis of variance (Anova) and Duncan test.

RESULTS

Superoxide Dismutase (Cu,Zn-SOD) Enzyme Content in Liver Tissue

The immunohistochemical staining results of Cu,Zn-SOD enzyme in liver tissue could be shown in the nucleus and cytoplasm of hepatocytes. The quantitative observation on the hepatocytes at the terminations on day 8, 15, and 22 are presented in Table 2. Termination on day 8 (before EPEC intervention) shown that the negative control group, the group treated by the synbiotic yoghurt, the group treated by the synbiotic yoghurt and EPEC intervention, and the positive control group had the similar Cu,Zn-SOD content statistically ($p > 0.05$).

Termination on day 15 (after EPEC intervention for 7 days) shown that the group treated by the synbiotic yoghurt had the highest Cu,Zn-SOD content. It was proved by the number of hepatocytes with strongly positive reaction was the highest very significantly ($p < 0.01$) in the rats group treated by the synbiotic yoghurt compared with other groups. Thus, the synbiotic yoghurt could increase the content of Cu,Zn-SOD in the liver tissue.

The Cu,Zn-SOD content of the positive control group was high due to the EPEC that infected for a week could trigger the production of the Cu, Zn-SOD enzyme. In addition, the termination on day 15 also shown that the positive control group, which received the EPEC intervention treatment, contained a high Cu,Zn-SOD content too. It was presented by the number of hepatocytes with strongly positive reaction in positive control group (59.67) was not significantly different ($p > 0.05$) compared with the group treated by the synbiotic yoghurt (63.00).

As the termination on day 15, the termination on day 22 also shown that the group treated by the synbiotic yoghurt had the highest Cu,Zn-SOD content. It was indicated by the number of hepatocytes with strongly positive reaction which was the significantly highest number ($p < 0.01$). This report shown that the synbiotic yoghurt still could provide the positive benefit during three weeks consumption.

At the termination on day 22, it was found that the synbiotic yoghurt had a better effect in liver tissue compared with the standard yoghurt. This was shown by the number of hepatocytes with strongly positive reaction in the group treated by the synbiotic yoghurt was significantly higher ($p < 0.01$) compared with the group treated by standard yogurt.

Table 2. The average number of hepatocytes at a variety levels of Cu,Zn-SOD content in rat liver tissue at the termination on day 8, 15, and 22 per area of view with a magnification of 200×

Termination on Day 8	**The Number of Rat Hepatocytes at different level of Cu,Zn-SOD**			
	+++	++	+	-
Negative control	48.67 ± 2.08^{d}	$43.00 \pm 5.00^{a,b}$	22.33 ± 3.21^{a}	$21.33 \pm 2.08^{b,c}$
Synbiotic yoghurt	$46.33 \pm 2.89^{c,d}$	48.67 ± 4.04^{b}	18.67 ± 2.52^{a}	$24.67 \pm 3.06^{b,c}$
Synbiotic yoghurt + EPEC	$40.67 \pm 3.51^{b,c}$	41.00 ± 4.58^{a}	27.00 ± 1.00^{a}	14.00 ± 3.60^{a}
Positive control	32.67 ± 2.08^{a}	37.67 ± 3.06^{a}	28.33 ± 6.81^{a}	$18.00 \pm 3.60^{a,b}$
Termination on Day 15				
Negative control	43.33 ± 5.13^{a}	36.67 ± 1.53^{a}	22.67 ± 1.53^{b}	33.00 ± 5.29^{b}
Synbiotic yoghurt	63.00 ± 3.60^{b}	46.33 ± 3.51^{b}	15.00 ± 1.73^{a}	13.33 ± 5.13^{a}
Synbiotic yoghurt + EPEC	32.67 ± 1.53^{a}	35.33 ± 1.15^{a}	14.00 ± 2.00^{a}	48.33 ± 2.52^{c}
Positive control	59.67 ± 7.23^{b}	36.67 ± 1.53^{a}	12.33 ± 3.78^{a}	$23.33 \pm 6.81^{a,b}$
Termination on Day 22				
Negative control	$43.00 \pm 4.36^{a,b}$	45.67 ± 5.86^{b}	34.67 ± 2.52^{c}	17.67 ± 2.08^{a}
Synbiotic yoghurt	66.00 ± 5.20^{c}	43.00 ± 1.00^{b}	$15.33 \pm 5.13^{a,b}$	18.67 ± 5.69^{a}
Synbiotic yoghurt + EPEC	$43.67 \pm 1.53^{a,b}$	36.00 ± 2.00^{b}	18.67 ± 1.53^{b}	21.33 ± 5.03^{a}
Positive control	36.67 ± 1.53^{a}	26.33 ± 8.14^{a}	11.33 ± 3.78^{a}	41.00 ± 2.00^{c}
Standard yoghurt	48.00 ± 5.29^{b}	37.33 ± 1.53^{b}	$12.67 \pm 3.06^{a,b}$	33.67 ± 3.06^{b}

Notes:

- The data were analyzed statistically (by Anova dan Duncan tests) in every same column and termination time of the table
- The values followed by the same letters indicated those were not significantly different ($p > 0.05$)

Superoxide Dismutase (Cu,Zn-SOD) Enzyme Content in Kidney Tissue

The immunohistochemical staining results of Cu,Zn-SOD enzyme in kidney tissue could also be s in the nucleus and cytoplasm of renal tubule cells. The number of renal tubule cells in different intensity c of Cu,Zn-SOD at the terminations on day 8, 15, and 22 are presented in Table 3.

Termination on day 8 (before EPEC intervention) shown that the synbiotic yoghurt group and sy yoghurt + EPEC group, which was just treated by the synbiotic yoghurt on day 1 to day 7, contained the h Cu,Zn-SOD than the negative control and positive control groups, which did not receive the synbiotic yo treatment. This was presented by the number of renal tubules cells with strongly positive reaction i synbiotic yoghurt and synbiotic yoghurt + EPEC groups which were significantly higher ($p < 0.01$) th negative control and positive control groups. Thus, the synbiotic yoghurt could improve the content of Cu, Z SOD in kidney tissue.

Termination on day 15 also shown that the group treated by synbiotic yoghurt and EPEC interv contained the higher Cu,Zn-SOD than that of the positive control group. It was proved by the number of r tubules cells with strongly positive reaction in the group treated by synbiotic yoghurt and EPEC interv were significantly higher ($p < 0.01$) compared to that of the positive control group.

Synbiotic yoghurt treatment until day 21 still shown better rat kidney condition. This was indicate the number of renal tubules cells with negative reaction which was the lowest very significantly ($p <$ compared with other groups. It was proved that the synbiotic yoghurt still had the benefits until the day 21.

At termination on day 22, the group treated by the synbiotic yoghurt and EPEC intervention show its Cu,Zn-SOD content was higher than the positive control group, which only got EPEC intervention treatm This was indicated by the number of renal tubules cells with strongly positive reaction in the group treate the synbiotic yoghurt EPEC intervention which was significantly higher ($p < 0.01$) compared to that o positive control group. It suggested that the administration of synbiotic yoghurt provided better conditio body in preventing the free radical formation by infectious pathogens.

Besides, the termination on day 22 also shown that the synbiotic yoghurt provided better benefits the standard yoghurt. It was presented by the number of renal tubules cells with strongly positive reaction i group treated by synbiotic yoghurt which was significantly higher ($p < 0.01$) compared with the group treate standard yoghurt. This was also indicated by the number of renal tubules cells with negative reaction i group treated by synbiotic yoghurt which was significantly lower ($p < 0.1$) compared with the group treate standard yoghurt. Better effect of the synbiotic yoghurt was related to the presence of the probiotic *L. ferme* 2B4 which can modulate the host immune system, thus it can provide better health benefits.

Table 3. The average number of renal tubules cells at a variety levels of Cu,Zn-SOD content in rat kidney t at the termination on day 8, 15, and 22 per area of view with a magnification of 200×

Termination on Day 8	**The Number of Rat Renal Tubules Cells**			
	+++	++	+	-
Negative control	73.67 ± 3.06^{a}	47.00 ± 5.00^{a}	18.00 ± 2.00^{a}	81.00 ± 10.00^{c}
Synbiotic yoghurt	100.00 ± 3.60^{b}	51.00 ± 7.55^{a}	22.33 ± 1.53^{a}	$51.33 \pm 7.02^{a,b}$
Synbiotic yoghurt + EPEC	114.00 ± 11.79^{b}	48.33 ± 8.74^{a}	21.33 ± 2.08^{a}	48.67 ± 6.51^{a}
Positive control	77.00 ± 7.55^{a}	49.33 ± 8.39^{a}	21.00 ± 2.64^{a}	63.67 ± 1.53^{b}
Termination on Day 15				
Negative control	59.00 ± 4.00^{b}	45.67 ± 8.14^{c}	25.67 ± 4.93^{c}	96.33 ± 5.51^{b}
Synbiotic yoghurt	76.00 ± 6.08^{c}	63.67 ± 4.72^{d}	34.33 ± 1.53^{d}	54.67 ± 3.51^{a}
Synbiotic yoghurt + EPEC	52.00 ± 4.58^{b}	33.67 ± 1.53^{b}	17.33 ± 3.06^{b}	128.33 ± 3.06^{c}
Positive control	5.33 ± 4.16^{a}	19.67 ± 3.06^{a}	9.33 ± 1.53^{a}	203.33 ± 8.14^{d}
Termination on Day 22				
Negative control	$63.33 \pm 4.04^{b,c}$	$47.00 \pm 1.73^{b,c}$	34.00 ± 2.64^{b}	94.00 ± 7.00^{c}
Synbiotic yoghurt	68.00 ± 6.56^{c}	55.67 ± 5.86^{d}	32.67 ± 3.78^{b}	51.33 ± 4.16^{a}
Synbiotic yoghurt + EPEC	53.33 ± 7.37^{b}	41.00 ± 3.60^{b}	19.67 ± 2.31^{a}	98.33 ± 5.03^{c}
Positive control	22.33 ± 2.08^{a}	22.33 ± 1.53^{a}	17.00 ± 4.58^{a}	153.00 ± 5.29^{d}
Standard yoghurt	56.33 ± 4.62^{b}	$52.67 \pm 4.16^{c,d}$	34.67 ± 3.51^{b}	75.00 ± 4.58^{b}

Notes:

- The data were analyzed statistically (by Anova dan Duncan tests) in every same column and termination time of the table
- The values followed by the same letters indicated those were not significantly different ($p > 0.05$)

DISCUSSION

Songisepp *et al.*[14] mentioned *in vitro* that *L. fermentum* ME-3 has a high antioxidative potential. *L. fermentum* ME-3 as a probiotic with antimicrobial and antioxidative activity was beneficial to improve the oxidative stress status of organisms that consume and could reduce the risk of infection[15]. The synbiotic yoghurt, which contained *L. fermentum* 2B4, was also has antioxidant capability. Therefore, it able to increase the content of the Cu,Zn-SOD enzyme in the rat liver and kidney tissue.

The Cu,Zn-SOD content in liver tissues of the positive control group was high due to the EPEC that infected for a week could trigger the production of the Cu, Zn-SOD enzyme. This result might occur as the explanation of Halliwell and Gutteridge[16]. They explained that exposure to the organism by a mild oxidative stress could cause the increased synthesis of antioxidant defense system quickly. This response helps to protect the cells against stronger oxidative stress and radicals attack in the following time so that the cells become resistant to the presence of the stronger free radicals. This mechanism of adaptation generally involves the gene expression changes that lead to the increased antioxidant defenses[16].

Hartanti[17] reported that probiotics could stimulate the immune system by enhancing the phagocytosis function of monocyte. According to Baratawidjaja[18], the monocytes not only attack the microbes, but also produce the cytokines (IL-6 and TNF-α) and mobilize the body defenses in response to infection. IL-6 (interleukin-6) and TNF-α (tumor necrosis factor-α) can modulate the production of copper (Cu) and zinc (Zn). The availability of Cu and Zn contributes to the formation or activation of Cu,Zn-SOD enzyme because the Cu,Zn-SOD requires Cu and Zn for its biological activity[19]. Thus, more Cu,Zn-SOD will be formed. This mechanism was probably carried out by probiotics to increase the content of Cu,Zn-SOD enzyme. Therefore, the synbiotic yoghurt provided better benefits against EPEC infection compared with the treatment without synbiotic yoghurt. In addition, the symbiotic yoghurt could also recover the body due to bacterial infection.

The synbiotic yoghurt had better benefits than the standard yoghurt because the synbiotic yoghurt contained probiotic. According to Langen and Madsen[20], probiotics had double benefits. They were modulate the intestinal microflora and reduce the oxidative stress and inflammation in hepatocytes. The decreasing in oxidative stress and inflammation caused the increased liver function and capacity to neutralize and reduce the absorption of toxins.

At the termination on day 8, 15, and 22, the group treated by synbiotic yoghurt shown the increased number of hepatocytes with strongly positive reaction. This was proved that administration of the synbiotic yoghurt could increase the content of Cu,Zn-SOD enzyme as the reported by Zubillaga *et al.*[21] that functional foods that contain probiotics can increase the expression of superoxide dismutase.

Besides, it was also found that the positive control group had Cu,Zn-SOD content which remained high at the termination on day 15 after the EPEC intervention, but then the Cu,Zn-SOD content decreased at the termination on day 22. This result may occur due to the infected EPEC only triggered the production of Cu,Zn-SOD enzyme in the beginning (as shown at the termination on day 15), but could not maintain the content of Cu,Zn-SOD continually. It was mentioned before that the increasing in Cu,Zn-SOD content at the termination on the day 15 was caused by the adaptation mechanism that involved the gene expression changes, then caused an increasing in antioxidant defenses. This adaptation process is a time-dependent process[22]. An extensive activation of phagocytic cells, which produces ROS, can exacerbate the tissue damage and inflammation[16]. Therefore, the more Cu,Zn-SOD enzyme exerted to scavenge ROS (reactive oxygen species), the less content in the liver tissue.

In addition, the synbiotic yoghurt + EPEC group shown high Cu,Zn-SOD content after it got the synbiotic yoghurt treatment (at termination on day 8). Then, after it had the EPEC intervention treatment when it was being treated by the synbiotic yoghurt (at termination on day 15), the group shown the decreasing in Cu,Zn-SOD content. Furthermore, the Cu,Zn-SOD content of the synbiotic yoghurt + EPEC group increased again after the EPEC intervention was stopped (at termination on day 22). It explained that the synbiotic yoghurt treatment was able to maintain the Cu,Zn-SOD content. Thus, it could maintain the antioxidants and free radicals in a balance composition and further keep the integrity of the body cells. The reason was the probiotic *L. fermentum* was able to stimulate the immune system.

The Cu,Zn-SOD content in the positive control group was low because of the EPEC infection which resulted the pathogenesis. Thus, the Cu,Zn-SOD content reduced. Cheng *et al.*[23] reported that the invasion of pathogenic bacteria and fungi into the host caused the decreasing in SOD activity. Thus, the synbiotic yoghurt could give a better effect to the host during the EPEC infection than without synbiotic yoghurt treatment because the synbiotic yoghurt could increase the content of Cu,Zn-SOD.

It suggested that the administration of synbiotic yoghurt provided better condition of body in preventing the free radical formation by infectious pathogens. This was probably due to the probiotic that had the ability to bind the free radicals. According to Mikelsaar and Zilmer[15], an independent laboratory confirmed that *L. fermentum* ME-3 had the ability in binding of superoxide anions (*in vitro*), 80-100 times more potential than the ability of ascorbic acid.

Overall, the synbiotic yoghurt + EPEC group shown the increasing in Cu,Zn-SOD content at the termination on day 8 because it just got the synbiotic yoghurt treatment. Then, at the termination on day 15, the Cu,Zn-SOD content decreased as a result of EPEC infection treatment. However, after no more EPEC intervention, the Cu,Zn-SOD increased again.

Mikelsaar and Zilmer[15] reported that *L. fermentum* could produce NO (nitric oxide). NO can induce the protection against inflammation, which can functionally activate the cellular antioxidant defense system. NO itself can act as powerful antioxidant that can quickly scavenge the peroxyl radical[24].

Probiotics can also inactivate the free radicals and degrade the superoxide anion and hydrogen peroxide by enzymatic mechanism, such as NADH oxidase/peroxidase, SOD, and catalase. Probiotics have defensive mechanisms, such as modulate the mucosal immune system by blocking the proinflammatory cytokines, have antagonistic activity against pathogens by producing antibacterial compounds or inhibit the attachment of pathogenic bacteria, and enhance the protective function of epithelial cells[25]. Therefore, the synbiotic yoghurt (formula 3), which contained the probiotic *L. fermentum* 2B4, could improve the profile of Cu,Zn-SOD in liver and kidney tissues so that the synbiotic yoghurt had potentially antioxidative activity. In other words, probiotics could induce the increased or decreased regulation of the immune response by maintaining the homeostatic of digestive tract[26].

The synbiotic yoghurt treatment for one to three weeks was able to raise the content of the Cu,Zn-SOD enzyme in the kidney. The effect of synbiotic yoghurt was also shown by the higher Cu, Zn-SOD content in kidney of the group treated by the synbiotic yoghurt and EPEC intervention compared with the positive control group. It explained that the administration of synbiotic yoghurt provided the benefits to the host during the EPEC infection better than without the synbiotic yoghurt treatment.

According to Mikelsaar *et al.*[27], *L. fermentum* has a unique carbohydrate profiles on its cell wall that allows it to attach to the receptors on mucosal epithelial cells of the upper urinary tract. It was the reason that caused *L. fermentum* was able to prevent the attachment of pathogenic *E. coli* on the epithelial cells of the upper urinary tract. As the result, EPEC did not get to express the pathogenicity effect because it was prevented by the presence of *L. fermentum*.

Another possible mechanism of probiotic is coaggregation with pathogenic bacteria. Rinkinen *et al.*[28] reported that some lactic acid bacterias made a coaggregation with *Escherichia coli* in the urogenital tract and the *Lactobacillus* in the gut were also known to make the coaggregation with *E. coli* K88. In these coaggregation, the lactic acid bacteria produced antimicrobial substances, thus it could inhibit the surrounding pathogens.

Adebayo-Tayo and Onilude[29] and Fukuda *et al.*[30] reported that *L. fermentum* was able to produce exopolysaccharide (EPS). The EPS secreted by probiotic bacteria might reduce the oxidative stress significantly[25]. The EPS was secreted by the probiotic to the cell surface and then it formed a capsule. Or it was secreted into the extracellular environment as slime. EPS can show antioxidative benefits to repair the oxidative damage of the mucosa.

Compared with Cu,Zn-SOD at the termination on day 8, the Cu, Zn-SOD content of liver tissues at the termination on day 15 was higher than in the kidney tissues. It was probably caused by the antioxidant defense system in liver was more effective than in the kidney[31]. It was also associated with the liver function as the main part of body that play a role in detoxification of toxic metabolites[32]. Therefore, the liver had abundant content of SOD and served as a major component of host defenses, produced acute phase proteins, and induced the tolerance action against antigen[33,34].

During the infection, bacterial products can activate the macrophages and other cells to release the various cytokines which can stimulate the liver to synthesize and release plasma proteins called acute phase proteins[18]. Baratawidjaja[18] mentioned that overall, acute phase protein response give the beneficial effects by improving the host resistance, reduce the tissue injury, and increase the resolution and recovery of inflammation. It allows the liver has a better defense when the infection was occuring.

CONCLUSION

Synbiotic yoghurt had antioxidative capability in the liver and kidney of EPEC treated-rats. The synbiotic yoghurt was able to maintain the high content of Cu, Zn-SOD in rat liver tissue similar to the negative control group and could increase the content of the Cu,Zn-SOD in rat kidney tissue. In rats that experienced the EPEC intervention, the synbiotic yoghurt could increase the content of Cu,Zn-SOD in both rat liver and kidney tissues.

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