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EFFECTS OF *Pediococcus pentosaceus* BACTERIA ISOLATED FROM *DADIH* TO THE LEVEL OF TNF-α ON THE INFLAMMATORY PERIODONTAL TISSUE INDUCED BY *Porphyromonas gingivalis* BACTERIA

EXPERIMENTAL RESEARCH LABORATORY AT *Sprague - dawley* RAT

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ABSTRACT

Introduction : The primary aetiology of periodontal disease, one of which is irritation of specific pathogens which will produce lipopolysaccharide toxins. These toxins will trigger the production of tumour necroting factor alpha (TNF-α) whom the high productivity can lead to bone resorbtion. The purpose of this study was to determine the effects of the *Pediococcus pentosaceus* bacteria which were isolated from *dadih* to the amount of TNF-α in rat periodontal tissue inflammation induced by bacteria *Porphyromonas gingivalis*. **Method :**The study was conducted on 27 Sprague Dawley Rats consists of 3 treatment groups. Examination of TNF-α was conducted by using Enzyme-Linked Immune Sorbent Assay (ELISA). **Results :**The result of ANOVA statistical test was p = 0.01 where p <0.05 means that all treatment groups had significant differences. **Conclusion**: *Dadih* can reduce the amount of TNF-α in periodontal tissue inflammation.

Keywords : Periodontal tissue inflammation, *Porphyromonas gingivalis*, *Pediococcus pentosaceus*

I. INTRODUCTION

Periodontal disease is a disease with a high rate of complaints. The number of the worldwide adult population who suffer from periodontal disease is about 50% (Wahyukundari, 2009). The primary aetiology of periodontal disease is irritation of specific pathogens (Charles, 2008). The secondary aetiology is poor condition of mouth cavity, smoking, education level, socioeconomic status, age, pregnancy, genetic factors and systemic disease (Widyastuti, 2009). Specific pathogens that have the ability to penetrate and damage the periodontal tissue are *Porphyromonas gingivalis* which have the most rapid growth at the time of periodontitis. In normal conditions, the amount of bacteria was only 10.6, while at the time of periodontitis, the number had risen into 59.5 (Fauziah and Herawati 2008, Carranza and Camargo 2006). *Porphyromonas gingivalis* will remove lipopolysaccharide toxins (LPS), after that this toxin will induce cellular events which are the beginning of the protection mechanisms of the innate immune system. Next, phagocytic cells such as polymorphonuclear neutrophils, monocytes and macrophages trigger the release of chemical mediators such as cytokines. Cytokine Cells that play a role in periodontal disease are: Interleukin -1

(IL - 1), Interleukin - 6 (IL-6), tumour necrosis factor-α (TNF-α) and Interferon - gamma (IFN-γ) (Amin *et al. 2010*, Eley et al. 2010).

The inflammation can increase osteoplastic processes that result in bone resorption. The TNF-α activity can improve the function of osteoclast genesis and reduce the function of many osteoblast genesis (Boyce *et al. 2005*). Many resorptions are mediated by the increased local production of pro-inflammatory cytokines such as TNF-α. (TNF-α can stimulate bone resorption by: (1) inducing the proliferation and differentiation of osteoclast progenitors and activate the osteoclast formation indirectly, (2) stimulating collagenase and degradation of type I collagen by fibroblasts that trigger tissue destruction periodonsium (Erica *et al. 2000*). By looking at the effects of the high level activity of TNF-α, we need a medication that can inhibit or reduce the activity of TNF-α in inflammatory conditions.

Today, the treatment of periodontitis is continuously being developed by one of them is using probiotics. Probiotic is defined as living microorganism, specifically bacteria that is safe to be consumed by human and will give benefit for human if it is consumed in adequate amount. This definition has been approved by UN through FAO and WHO (Bonifait *et al.* 2009). One of the traditional probiotics that many people are consumed is *dadih*. *Dadih* is a homemade probiotic done by the people of West Sumatera which originated from fermented buffalo milk in a bamboo tube by lactic acid-producing microorganism which are naturally found in the buffalo milk. *Dadih* has Lactic Acid Bacteria (LAB) which are different in each region and can be identified by using 16S rRNA. LAB are a big cluster of beneficial bacteria which have relatively common characteristics (Purwanti *et al.* 2011). The study of Purwanti (2010) found that in buffalo milk *dadih* dominated by the *Lactococcus* bacteria, *Pediococcus pentosaceus*, *Enterococcus faecalis* and *Weisella*. One product of lactic acid bacteria is nisin. Nisin is a natural antibiotic that has bacteriocins and the widest activity of spectrum (Yuliawati *et al.* 2012). The nisin can make the *dadih* -isolated bacteria prevent further inflammation caused by pathogens in periodontal disease. *Pediococcus pentosaceus* is typical of *dadih* -isolated pathogen in West Sumatra which is now known to have the ability to inhibit the growth of gram-positive bacteria and gram-negative.
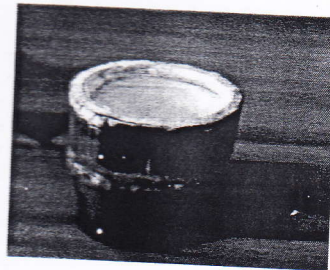

Figure 1 *Dadih* (Source : Purwanti *et al.* 2011)

II. RESEARCH METHOD

This study has received ethical permission which was from the Research Ethics Committee of the Faculty of Medicine, University of Andalas No: 006/KEP/FK/2015. The purpose of this study is to determine the effect of *Pediococcus pentosaceus* bacteria which were isolated from *dadih* to the level of TNF-α in rat periodontal tissue inflammation induced by bacteria *Porphyromonas gingivalis*. This study is an experimental laboratory study conducted at the Laboratory of Livestock Production Technology Faculty of Animal Science, Biomedical Laboratory and animal house of Faculty of Medicine, University of Andalas from January to April 2015. The samples in this study were 27 male Wistar divided into three groups. Group 1 was the negative control group which was not given any

treatment, group 2 was the positive control group which was given *Porphyromonas gingivalis* bacteria for 15 days, group 3 was given treatment groups by *Porphyromonas gingivalis* bacteria for 15 days and *Pediococcus pentosaceus* bacteria for 5 days.

Materials used for this study are: 27 male Wistar rats, *Porphyromonas gingivalis* bacteria, *Pediococcus pentosaceus* bacteria isolated from *dadih* , the liquid of de Monitol ROGOSA Salt Broth (MRS-B), the liquid of de Monitol Media ROGOSA Salt Agar (MRS-A), Brain Heart Infusion Agar (BHI - A), Brain Heart Infusion Broth (BHI - B), Yeast Extract, Hemin, Vitamin K, chloroform, Phosphate Buffer Saline, Alcohol, distilled sterile, liquid spritus, sterile cotton, filter paper, standard drinks and food for wistar rats, Preenrichment, aluminum foil, Washing Buffer.

The tools used for this study are: Anaerobic jar, Eppendrof, serum separator tube, Magnetic striter, Hockey sticks, lamp spritus, Lamina flow, Quebec Colony Counter. Cage + a place to eat and drink for Wistar rats, Syringe size 30G, test tube, petridish, ohaus balance, Incubator, Autoclave, measuring cup, flask, glass beaker, Ose, Lamp spritus, Vibrator, Stopwatch, Stove, Pots, Gloves, masks, Refrigerators, eBioscience ELISA Kit with catalogue number 88-7340, Micro plate reader set, Spectrophotometer.

Treatment procedure was done by the injection of *Porphyromonas gingivalis* bacteria in the positive control and treatment group. The injection was performed at the junctional epithelium of the upper molar teeth left at a dose of 0.5 ml. Before the treatment, rats were anaesthetized beforehand by using chloroform. Procedures of injecting *Pediococcus pentosaceus* bacteria isolated from *dadih* were applied via sublingual administration for 5 days. The dose was about 1 cc/treatment with a total minimal of bacteria colonies was about 2×10^8 cfu/ml. Blood sampling procedure for inspection of TNF-α is taken from the rat aorta and the amount of the blood sample was 1 cc. After that, the serum was separated and had TNF-α tested by using ELISA.
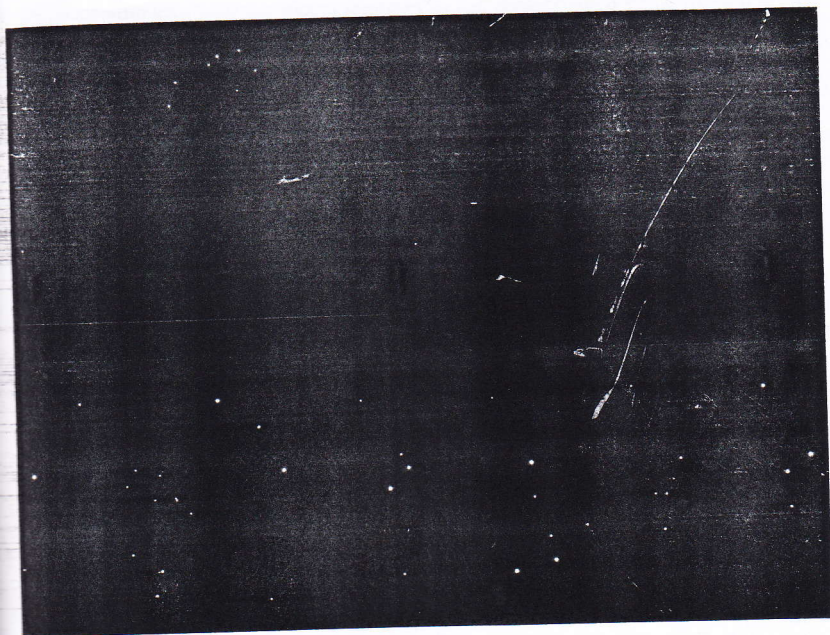

Figure 2 Procedure of research

III. RESULT

The data analysis was done by using ANOVA test. The data normality test was done previously by using Shapiro Wilk, while the homogeneity test was performed by using Lavene test. The data results had data normality test performed by using test of normality Shapiro Wilk. Shapiro Wilk test results showed abnormal data, hence data transformation must be done first. The data transformation showed that all data normally distributed. Levene homogeneity test showed that all of the data have the same variance.

Table 1 The Results of TNF-α level in the 3 groups

	TNF-α Level ng/ml	
	n	x ± SD
Negative control group	7	0,22 ± 0,11
Positive control group	7	0,32 ± 0,29
Treatment group	7	0,06 ± 0,01

Homogeneity test and Levene test were qualified for the Anova test. One way ANOVA test showed p = 0.01 where p <0.05 means that all treatment groups had significant differences. After the ANOVA results obtained, then the Least Significant Difference (LSD) test was performed to determine the differences of average level of TNF-α in each group.

Table 2 LSD Test

Treatment group	Comparison	P
Negative Control	Positive Control Treatment	0,555
		0,002*
Positive Control	Negative Control	0,555
	Treatment	0,001*
Treatment	Positive Control	0,001*
	Negative Control	0,002*

LSD test results showed that there were significant differences between the treatment, positive control and negative control (P <0.005) and there was no significant difference between the positive control and negative control (P> 0.005).

IV. DISCUSSION

Periodontal disease is a common infection that requires serious mouth cavity health care. Periodontal disease is associated with infection of gram-negative bacteria. Gram-negative may release endotoxin to perform repeat infection in the host, which usually starts from the edge of the gingival sulcus, and gradually to the deeper parts, including the destruction of the periodontal ligament and bone alveolar. Therefore, it is necessary to do a research on changes of periodontal tissue inflammatory (Liao *et al*. 2013). The study nowadays is using rats as a research model.

This study used the induction method of *Porphyromonas gingivalis* bacteria to make periodontal tissues of rat become inflammatory. The induction was performed in the positive control group and the treatment group during the first 15 days in the first molar of left upper jaw of rats. After

...ys of induction, the rat negative control and positive control group had their blood drawn and ...ked the level of TNF-α from each group. Blood sampling were originally done in the lingual ...s, yet it was cancelled due to the amount minimum of blood from the lingual vein, based on this ...rence, the blood sampling is done from a vein aorta.

Scans showed that the negative control group had an average value of 0.22 ± 0.11 ng/ml ...le the positive control group had an average value of 0.32 ± 0.29 ng/ml. There was increasing ...el in the TNF-α level of negative control group which is associated with increased levels of stress ...s at the time of blood sampling where negative controls had never given any treatment before. This ...n line with a research conducted by Madrigal et al. (2002) regarding increased levels of TNF-α ...er given a stress exposure which results in an increase of the level of TNF-α in cortex after 1 hour ...ess induced. This incident was preceded by an increase in TNF converter (TACE) in the cortex of ...e brain after 30 minutes of exposure to stress. One of the weaknesses of TNF-α test is there is no ...ecific inflammatory source of an increase in TNF-α, so further research is needed for more specific ...formation. No significant difference between the negative control and positive control could be due ...e the increased time of proinflammatory cytokines in acute condition occurred not long after ...llowed by increasing anti-inflammatory cytokines. Hence, these conditions need to be considered if ...e researchers want to assess the level of cytokines that appear during inflammation.

The positive control group also experienced an increase in TNF-α caused by the induction of ...Porphyromonas gingivalis bacteria. The elevated levels of proinflammatory cytokines in periodontal ...issues is in line with a research conducted by Molon et al. (2014), regarding the variation of the ...esponse of rat which were induced by periodontal disease where the group of Porphyromonas ...gingivalis bacteria-induced ATCC 33 277 had their expression pro-inflammatory cytokines increasing ...n 15th examination day. An increase in pro-inflammatory cytokines is consistent with a research ...conducted by Mysak et al. (2013). One of the gram-negative bacteria in the periodontal disease is ...Porphyromonas gingivalis. One of its activities is to generate lipopolysaccharide endotoxin which is a ...ajor factor in the progression of periodontal disease. Moreover, gingival fibroblasts are the major ...onnective tissue gingival which can directly interact with Porphyromonas gingivalis and its products ...uch as lipopolysaccharide. Lipopolysaccharide can activate an inflammatory reaction in the host ...which may be a sign for the hosts that the infection was happening. One form of inflammatory ...esponse is cytokines generating. One of high cytokines at the time of the periodontal infection is ...TNF-α.

Clinical signs which appear in the mouth cavity of rat which occurs in the positive control and ...treatment group on day 7 is area-induced gums had red colour and swollen if it is compared with the ...ther side. Moreover, the reddish colour clearly visible when the effect of the anaesthetics chloroform ...already started to disappear in rat. Redness and swelling in the same area is in line with the research ...served by Nitawati et al. (2014). In the study, redness, swelling and bleeding started on day 2 post-...nduction of Porphyromonas gingivalis bacteria.

After periodontal inflammation was induced during 15 days, the treatment group was given ...the probiotic bacteria originating from dadih s-isolated Pediococcus pentosaceus bacteria for 5 days ...fter blood sampling was done and the level of TNF-α in this group has been checked. Test results ...howed the treatment group had an average value of 0.06 ± 0.05 ng/ml. The results from the treatment ...groups showed that the amount of TNF-α in the treatment group has decreased compared with the ...positive control group and it is consistent with the research conducted by Yuliawati (2012) about a ...ecrease in TNF-α levels after administration of Pediococcus pentosaceus bacteria in inflammatory ...darrhoea rats. This is due to Lactic Acid Bacteria found in dadih produces lactic acid which can ...amper the growth of harmful bacteria. Furthermore, nisin as a byproduct is natural antibiotic ...eneficial to neutralize pathogenic bacteria. This indicates that dadih may also be classified as ...robiotic food product due to the fact that it originates from fermented milk and contain lactic acid ...Purwanti et al. 2011).

Research on Pediococcus pentosaceus has not been popular and there are only few of them. It ...akes researches on probiotic that have been done in the past becoming a reference for the research ...on Pediococcus pentosaceus as all the probiotic bacteria have the same function and benefit. ...ncreased levels of TNF-α after administration of probiotic bacteria has a mechanism of action which

is not known with certainty, but there are several mechanisms that seem to involve in the treatment of periodontal disease (Bonifait *et al.* 2009):

1. Probiotic bacteria secrete two types of bacteriocins, two of them are reuterin and reutericyclin which can inhibit the growth of pathogenic bacteria; Research conducted by Stamatova *et al.* (2007) and Koll-Klais *et al.* (2005) in Barlow (2010) mentioned that Lactobacillus rhamnosus, Lactobacillus bulgaricu and Lactobacilli strains were able to inhibit the growth of *Porphyromonas gingivalis* up to 82% and is able to inhibit the pathogenic effects produced by those bacteria.
2. Probiotic bacteria have the ability to compete with the pathogen bacteria.
3. Probiotic bacteria have anti-inflammatory effects that can inhibit the secretion of pro-inflammatory cytokines. These three mechanisms have the directly and indirectly effect to cure periodontal disease
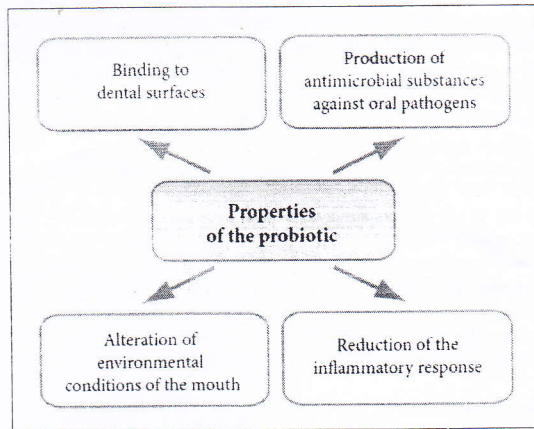

Figure 3 : Ideal properties of a probiotic intended for use in disorders of the mouth (Bonifait *et al.* 2009)

Effects of probiotics in decreasing the secretion of proinflammatory cytokines are associated with the decreased activity of Nuclear factor kB (NFkB) (the exact mechanism is still unknown). This is consistent with a research conducted by Kusuma *et al.* 2008 regarding the effects of supplementation of Lactobacillus strain LIS10506 or LIS20506 that can inhibit the activity of NFkB and significantly inhibited the expression of TNFR 1 (which has a value equal to the TNF-α).

Research that supports this study is a research conducted by Shadnoush *et al.* 2013 about the effect of Lactobacillus paracasei B 20160 on the level of pro and anti-inflammatory cytokines. The results of multidimensional showed correlation between NFkB and pro-inflammatory cytokines. When the pro-inflammatory cytokines increase, it may also increase the activity of NFkB, and the NFkB brings positive effect on the expression of cytokines such as TNF-α which can aggravate inflammation. NFkB is a transcription factor that plays important role in inflammatory conditions especially the secretion of inflammatory cytokines.

Other research that supports this study about *lactobacillus* strain showed that probiotic can reduce gingiva inflammation and the number of black-pigmented bacteria such as *Porphyromonas gingivalis* which is saliva and subgingival plaque. The use of bacteria which has benefit as Lactic Acid Bacteria (LAB) supported with scaling and root planning (SRP) can block phatogenic bacteria growth in periodontal pocket and reducing probing bleeding in animal trial. The other clinical trial showed improvement in moderate to severe gingiva inflammation and probing of adult patient after

probiotic tablet regularly. Another research proved that consuming probiotic gum in two weeks decrease proinflammatory cytokine in gingivitis patient (Vivekananda *et al.* 2010).

The other research was done by Riccia *et al.* (2007) in Bonifait *et al.* (2009) about anti inflammation effect of *Lactobacillus brevis* in chronic periodontitis patient. This study used inhalation and the patient inhaled refreshing concentrate for throat that contain *Lactobacillus brevis* for four. After four days there was improvement in disease severity based on clinical parameters that can seen plaque index, gingival index and bleeding in probing of all patients. In addition to the there was also decreasing of prostaglandin E2 (PGE2) and matrix metalloproteinases (MMPs) of patient saliva. In this case Riccia *et al* concluded that *Lactobacillus brevis* had ability to nitrite oxide so that the activity of PGE2 and MMPs which are inducted by nitrite oxide can be Utilization of probiotic in periodontal disease very related to immune activity that is by probiotic bacteria (Joseph, 2014). In vitro study by Nara *et al* showed that *Lactobacillus* stimulated osteoblast cell formation. The osteoblast cell formation is very important in improving damage that is caused by periodontal disease (Chatterjee *et al.* 2010).

Based on the research author have suggestions :

1. Changing the design of research into pre and post is necessary because each mouse has different stress resistance, hence the increase in the levels of TNF-α can be definitely caused by the treatment given by the researchers.
2. The next study should provide training to rat blood sampling conditions particularly for the negative control group because that group were never given any treatment.
3. Research on the improvement of other pro-inflammatory cytokines such as interleukin should be more specific.
4. The next studies should compare the levels of TNF-α in inflammatory periodontal tissue of rat that were given and were not given the *dadih* .
5. The next research should compare the levels of pro and anti-inflammatory cytokines which are associated with *dadih* effectiveness in the inflammatory cytokines

CONCLUSION

Pediococcus pentosaceus bacteria which were isolated from *dadih* can affect the levels of TNF-α in periodontal tissue inflammation induced by *Porphyromonas gingivalis* bacteria.

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