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Editor :
Dhona Afriza
Rifani
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Okmes Fadriyanti

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Analysis of IL-1 β levels in male wistar rats induced by Porphyromonas gingivalis with intervention of Dadiah isolate bacteria Pediococcus pentosaceus

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ABSTRACT

Periodontal disease is dominated by gram-negative bacteria *Porphyromonas gingivalis* which produce endotoxin lipopolysaccharide (LPS), which would induce an inflammatory response that will induce the release of mediators such as pro-inflammatory Cytokine IL-1B. In controlling the inflammatory process, evolving thinking use of probiotics to reduce the use of antibiotics. Dadiah is a traditional probiotic of West Sumatra that are rich lactic acid bacteria, including bacteria *Pediococcus pentosaceus*. The aim of this study was to analyze the levels of interleukin-1B (IL-1B) bacterial isolates Dadiah *Pediococcus pentosaceus* on male Wistar rats induced by *Porphyromonas gingivalis*. This research is an experimental laboratory with a sample of 30 rats Wistar male were divided into 3 groups firstly was negative control without any treatment, secondly was positive control which was induced by *Porphyromonas gingivalis* for 15 days, thirdly was the group which was induced by *Porphyromonas gingivalis* for 15 days and the intervention of bacteria isolates Dadiah *Pediococcus pentosaceus* for 5 days. Levels of IL-1B each group were analyzed using ELISA method. The levels of IL-1B average of each group are; the negative control group was 10.44 ± 4.34 pg /mL; Positive control was 65.19 ± 34.07 pg /mL; the treatment group was 21.42 ± 7.55 pg /mL. Kruskal-Wallis analysis showed $p < 0.05$, followed post Hoc Mann Whitney showed significant differences in all groups with $p < 0.05$. From this study it can be concluded that the bacterial isolates Dadiah *Pediococcus pentosaceus* shown to lower levels of IL-1B on male Wistar rats were induced by *Porphyromonas gingivalis*.

Keywords: Periodontal inflammation, IL-1B, *Porphyromonas gingivalis*, *Pediococcus pentosaceus*

INTRODUCTION

Periodontal disease is one of the most common diseases of man and is responsible for most of the tooth loss in adults. Number of the adult population world wide suffer from periodontal disease was 50%¹. Based on the data profile of health Indonesia in 2011, issued by the Ministry of Health of the Republic of Indonesia in 2012, as many as 72 223

residents of Indonesia underwent periodontal treatment at the Provincial General Hospital and Regional Hospital. The highest morbidity rate was in East Java province with 35,326 patients followed by Central Java as many as 5,205 patients, while the West Sumatra is ranked sixth by the number of patients as many as 2,317 people. The primary etiology of periodontal disease is bacterial irritation specific pathogens (Charles, 2008). Secondary etiology is poor oral conditions, smoking, educational level, socioeconomic status, age, pregnancy, genetic factors and systemic disease².

Specific pathogenic bacteria that have the ability to penetrate and damage the periodontal tissue is *Porphyromonas gingivalis* and *Actinobacillus actinomyces comitans*³. *Porphyromonas gingivalis* is a bacteria that has the fastest growth at the time of periodontitis. In normal conditions the number of bacteria in periodontitis just 10.6 while the number increased to 59.5⁴. *Porphyromonas gingivalis* lipopolysaccharide will remove toxins (LPS), which in turn these toxins can induce cellular events in periodontal tissues, especially in the alveolar bone (Amin, 2010). Cellular incident that occurred the initial protection mechanisms of the innate immune system. Phagocytic cells such as polymorphonuclear neutrophils, monocytes and macrophages trigger the release of chemical mediators such as cytokines. Cell cytokine that plays a role in periodontal disease are: Interleukin -1 (IL - 1), Interleukin - 6 (IL-6), tumor necrosis factor- α (TNF- α) and Interferon - gamma (IFN- γ)⁵.

Periodontal disease, with characteristic bone loss around the tooth supporting tissue inflammation caused by the expansion of the network to advocate marginal gingiva. Invasion of gingival inflammation to the bone surface and the onset of bone loss is a major feature of the transition from gingivitis to periodontitis⁴ Inflammation that occurs can be increased osteoclastic processes that lead to bone resorption. This resorption much mediated by increased local production of pro-inflammatory cytokines such as IL-1B⁶. Bone resorption on these conditions (1) induces the proliferation and differentiation of progenitor-progenitor osteoclasts and enable the formation of osteoclasts indirectly, (2) stimulates collagenase and degradation of type I collagen by fibroblasts that trigger tissue destruction periodontium⁷.

Today the treatment of periodontitis continue to be developed either by using probiotics. Research conducted by Shah, et al (2013) regarding the comparison of the effects of probiotics pure, probiotic mixed with antibiotics and antibiotic pure show the results that the probiotic potential as drug peridodontitis future because the function of probiotics together with antibiotics, but probiotics do not cause resistennya bacteria and do not upset the balance micro-flora of the body⁸. One traditional probiotics are widely consumed by people is Dadiah. Dadiah is one of the traditional Indonesian fermented milk and is very well known in West Sumatra, Jambi and Riau⁹. Purwanti(2010) in his research found that in Dadiah buffalo milk containing lactic acid bacteria, dominated by the bacterium *Lactococcus*, *Pediococcus pentosaceus*, *Enterococcus faecalis* and *Weisella*¹⁰.

One product of this lactic acid bacterium is nisin. Nisin is a natural antibiotic that contains bacteriocins and has the most broad-spectrum activity¹¹. Bacteriocin is a protein compound that is excreted by the bacteria that are inhibiting the growth of other bacteria that are pathogenic especially¹². Given this nisin make bacterial isolates Dadiah

can prevent further inflammation caused by pathogenic bacteria in periodontal disease. *Pediococcus pentosaceus* is typical of bacterial isolates Dadiah West Sumatra which is now known to have the ability to inhibit the growth of gram-positive bacteria and gram-negative. Research conducted by Yuliawati, et al ¹¹(2012) about the influence of *Pediococcus pentosaceus* on mice diarrhea, *Pediococcus pentosaceus* able to reduce the frequency of bowel movements rat diarrhea, inhibits TNF- α levels and improving the balance of intestinal microflora. Based on the above researchers are interested to test the effect of bacterial isolates *Pediococcus pentosaceus* Dadiah to the amount of IL-1 β -induced rat bacterium *Porphyromonas gingivalis* (laboratory experimental study in rats Wistar species) which may be a reference to the development of information-based medicine of traditional food such as Dadiah.

OBJECTIVES

The purpose of this research is: To determine the effect of bacterial isolates *Pediococcus pentosaceus* Dadiah on levels of IL-1 β in rat periodontal tissue inflammation induced bacterium *Porphyromonas gingivalis*. This study is expected to provide the following benefits: 1. Provide information on the effect of bacterial isolates *Pediococcus pentosaceus* Dadiah to the amount of IL-1 β -induced rat bacterium *Porphyromonas gingivalis*. 2. Provide information on the potential of Lactic Acid Bacteria Isolates Dadiah which Dadiah a traditional West Sumatran feed on the health of the oral cavity especially in periodontal disease. 3. As reference and additional information for further research so that the results that have been obtained in this study can be completed.

METHODS

Type of research is an experimental laboratory. The study design is the posttest with control group. The sample size in this study as many as 10 per group of samples. A total of 30 samples. Animals that have been adapted to be grouped into 3 groups: Group 1 is a negative control that is a control group that was not given treatment. Group 2 was the positive control group treated by induction of the bacteria *Porphyromonas gingivalis* for 15 days. Group 3 is an induction treatment groups were given the bacterium *Porphyromonas gingivalis* for 15 days and given Bacteria *Pediococcus pentosaceus* isolates Dadiah for 5 days.

Treatment procedures

Application materials treated with the bacterium *Porphyromonas gingivalis* Injection Each treatment will be given in the form of an injection of bacterium *Porphyromonas gingivalis* previous experimental animals sedated with chloroform. Rat inserted into the appropriate jars then chloroform cotton drops at a dose of 0.5 ml jar is closed and waited until the mice did not react. Application of the bacteria *Porphyromonas gingivalis* in experimental animals is to inject the bacteria on the Junctional epithelium in the gingival sulcus in the maxillary

first molar teeth left. *Porphyromonas gingivalis* dose based on research Permana, et al (2013) which is the concentration of $5\mu\text{g} / 0,05\text{mL}$ with each application $0,02\text{mL}$ treatment as well as the needles used size 30G¹³. Positive control group and the treatment group induced 3x a week for 15 days. The timing of induction didadasarkan on research Molon, et al (2014) in which the intervention group was observed at day 15 was found the expression of pro-inflammatory cytokines are high¹⁴.

Applications Bacteria *Pediococcus pentosaceus* isolates Dadiah

Preparations Bacteria *Pediococcus pentosaceus* isolates Dadiah applied via sublingual animal for 5 days. Method of application: animals that had previously been anaesthetized, his mouth opened by the operator and the tongue of mice raised, sublingual dosage of bacteria was applied to the animal and then the animal is released back. Dose study is based on research Yuliawati, et al (2012)¹¹ie $2 \times 10^8 \text{ cfu} / \text{g.b}$. Intake of Blood Serum¹. Taking blood serum at the time the study was conducted based on the intervention each group. Group I and II, blood serum is taken on day 16 after the induction of the bacteria *Porphyromonas gingivalis* were conducted over 15 days. Group III blood serum is taken on day 21 after the induction of the bacteria *Porphyromonas gingivalis* 15 days followed by induction of bacteria *Pediococcus pentosaceus* for 20 days.² Blood samples each taken through a vein sub-lingual much as 0.5 cc using microhematocrit.³ Blood serum and centrifuged and taken in groups I and II, while the serum was stored in the freezer.⁴ After all complete serum, ELISA preparations were immediately made use of the mouse IL-1 β Elisa Test Kit (metaphor, 2014).⁵ Serum is stored in the cupboard of ice with temperature - 100C.

RESULTS

The statistical test used in this study using Shapiro-Wilk normality test. Results showed the data were not normally distributed, then proceed with the Kruskal Wallis test to see the difference in all three treatment groups. Kruskal-Wallis analysis showed $p < 0.05$, Mann Whitney followed post hoc showed significant differences in all groups with $p < 0.05$. Tabel 1. Mean levels of IL-1B in each group.

Group	n	Mean \pm s.d	p
Negative control	10	10.44 \pm 4.34	<0,05
Positive control	10	65.19 \pm 34.07	
Treatment	10	21.42 \pm 7.55	

post Hoc Mann-Whitney test: Negative control vs. positive control, $p = 0.000$; negative control vs treatment $p = 0.003$; positive control vs treatment $p = 0.001$.

The table above shows that there are significant differences in each group with a significance value < 0.05 . The results of post hoc Mann Whitney likewise showed a significant difference between each group demonstrated the value of $p < 0.05$.

DISCUSSION

In this study, it was found that the bacterial isolates that *Pediococcus pentosaceus* Dadiah can reduce levels of IL-1B in Wistar rats induced periodontitis with *Porphyromonas gingivalis*. In periodontitis caused by bacteria, the body will respond with an inflammatory response characterized by active mediators in the form of pro-inflammatory cytokines such as IL-1B. *Porphyromonas gingivalis* is a major putative periodontopathic bacteria¹⁵. One of the virulence factors common to periodontopathic bacteria is lipopolysaccharide (LPS), the which is known to penetrate the periodontal tissues and subsequently interacts with the host immune and non-immune cells. This interaction leads to cell activation and release of inflammatory mediators such as cytokines, chemokines and prostaglandin¹⁶. IL-1B has been implicated in the pathogenesis of periodontal disease, evidenced by increased levels of IL-1B were higher in the group with periodontitis compared with group of non-periodontitis. This research currently using rats as a model research. This study uses induction of bacteria *Porphyromonas gingivalis* to make the network into a rat periodontal inflammation. Induction conducted in the positive control group and the group treated for 15 days at the maxillary left first molar of rats. After 15 days of induction, the mice and the negative control group

Positive control blood drawn and checked the levels of IL-1B each group.

Porphyromonas gingivalis Elevated levels of proinflammatory cytokines in periodontal tissues according to research conducted by Molon, et al (2014), regarding the variation of the response of mice induced periodontal disease. Wherein the induced group bacterium *Porphyromonas gingivalis* ATCC 33 277 increased Expression of cytokines proinflammation of the examination days to 15 days¹⁷, Increased pro-inflammatory cytokine according to studied by Mysak et al (2013). In the periodontal disease where one a gram-negative bacteria that act is *Porphyromonas gingivalis*, which in its activity will produce endotoxins form lipopolysaccharide. Lipopolysaccharide from *Porphyromonas gingivalis* is a major factor the progression of periodontal disease. Gingival fibroblasts are connective tissue. The main gingiva can directly interact with *Porphyromonas gingivalis* and bacteria products such as lipopolysaccharide¹⁸. Lipopolysaccharide activate inflammatory reaction in host may be a sign to the host that the infection is going on. One form inflammatory reaction is spending cytokines. Clinical signs appear in the cavity mouth rats occurred in the positive control and treatment at day 7 is the area around the gums induced colored red and swollen when compared with the other side, color redness clearly visible when the effect of anesthetic chloroform already started to disappear in mice. Redness and swelling in the area induced together with the study observed by Nitawati, et al (2014)¹⁹. In that study redness, swelling accompanied by bleeding start on day 2 post-induction of bacteria *Porphyromonas gingivalis*. One other inflammatory clinical signs is the discovery Chromodacryorrhea on Day 9 - 15. As disclosed American College of Laboratory Animal Medicine Series In 2006, a Chromodacryorrhea conditions often found in mice laboratory and widely reported. Fueled by stress conditions and an early sign of infections in the body

of mice. Clinical signs The most common is the red color appear around the eyes¹⁹ After induced periodontal inflammation for 15 days, the treatment group was given probiotic bacteria derived from bacteria *Pediococcus pentosaceus* whey isolates for 5 days once that is done and the blood draw examined the levels of IL-1B in this group. Result examination showed the treatment group has an average value of 0.06 ± 0.05 ng / ml. Results obtained from group treatment showed that the occurrence of a decrease in the amount of TNF- α in group treatment compared with the control group The positive thing according to research performed by Yuliawati (2012)¹¹ on the decline TNF- α levels after administration of bacterial isolates curd *Pediococcus pentosaceus* in inflammatory rats diarrhea¹², Changes in clinical signs most look at giving the bacteria *Pediococcus pentosaceus* day 5 was started disappearance Chromodacryorrhea on rats around the eyes, where previously evident in the group treatment. Decreased levels of IL-1B after administration of probiotic bacteria have mechanisms the work is not yet known with certainty, but There are several mechanisms that seem play a role in the treatment of periodontal disease namely: 1. Probiotic bacteria secrete two types bacteriocins yakninyareuterin and reutericyclin which may hamper 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 bulgaricus* and *Lactobacilli* strains capable of inhibiting the growth of *Porphyromonas gingivalis* up to 82% and is able to inhibit the effects produced by bacterial pathogens mentioned²⁰. 2. Probiotic bacteria have the ability to competing with pathogenic bacteria²¹ 3. Probiotic bacteria have anti-inflammatory effects which can inhibit the secretion of cytokines pro inflammatory.

These three mechanisms have the effect of directly and indirectly to periodontal disease treatment²⁰

CONCLUSION

From this study it can be concluded that the bacterial isolates Dadiah *Pediococcus pentosaceus* suppress the levels of IL-1B on male Wistar rats were induced by *Porphyromonas gingivalis*.

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