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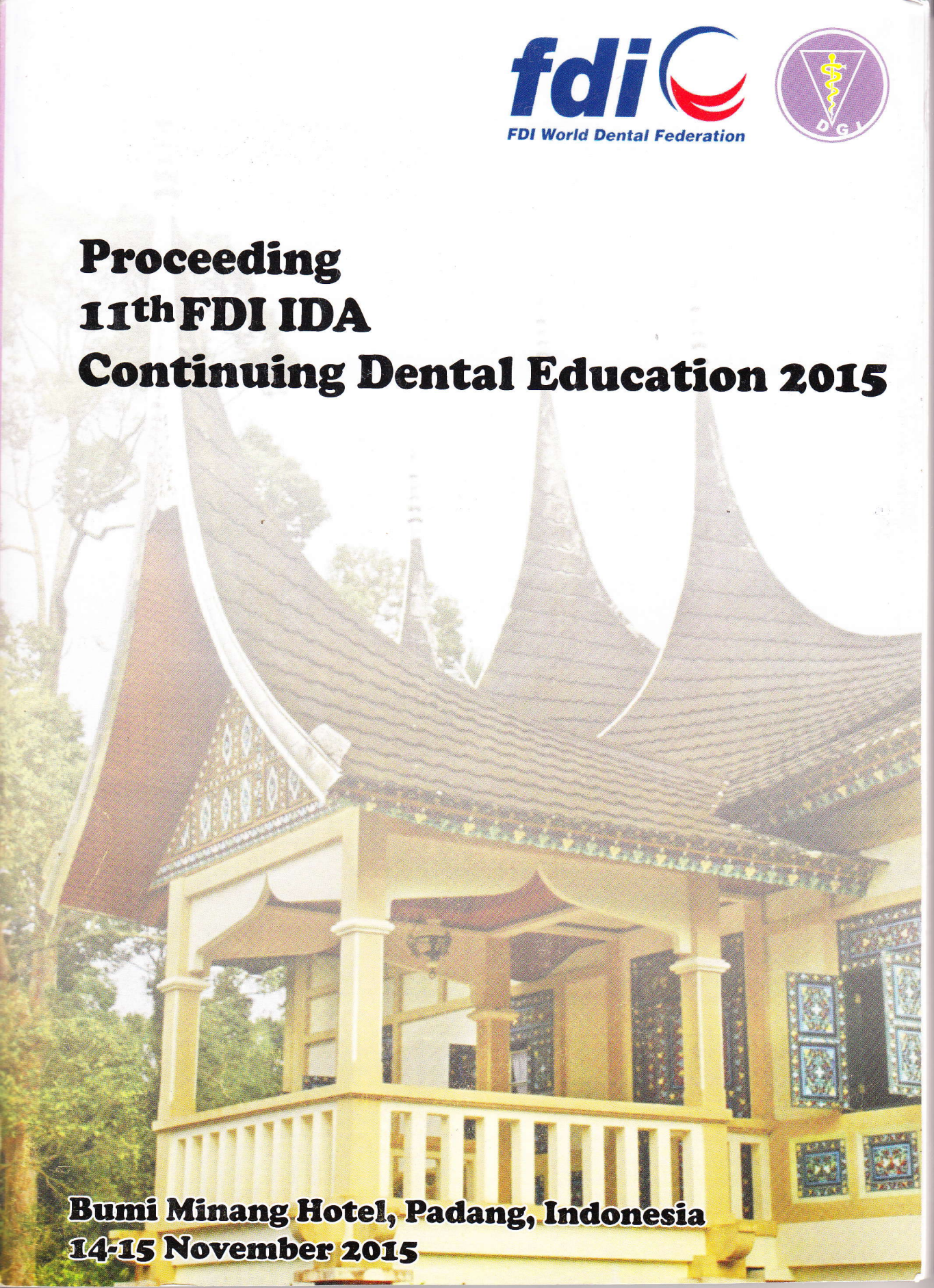

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Analysis Of Il-1β Levels In Male Wistar Rats Induced By PorphyromonasGingivalis With Intervention OfDadiah Isolate Bacteria PediococcusPentosaceus

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Abstract

Periodontal disease is dominated by gram-negative bacteria Porphyromonasgingivalis which produce endotoxin lipopolysaccharide (LPS), which would induce an inflammatory responsethat 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.Dadiahis a traditional probiotic of West Sumatra that are rich lactic acid bacteria, including bacteria Pediococcuspentosaceus. The aim of this study was to analyze the levels of interleukin-1B (IL-1B) bacterial isolates DadiahPediococcuspentosaceus on male Wistar rats induced by Porphyromonasgingivalis.

This research is an experimental laboratory with a sample of 30 rats Wistar male were divided into 3 groups firstlywas negative control without any treatment, secondlywas positive control which wasinduce byPorphyromonasgingivalis for 15 days, thirdly was the group which was induced by Porphyromonasgingivalis for 15 days and the intervention of bacteria isolates DadiahPediococcuspentosaceus 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 DadiahPediococcuspentosaceus shown to lower levels of IL-1B on male Wistar rats were induced by Porphyromonasgingivalis.

***Keywords**: Periodontal inflamation,* IL-1B, *Porphyromonasgingivalis.Pediococcuspentosaceus*

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BACKGROUND

Periodontaldiseaseisone ofthe-mostcommondiseasesofmanandis-responsibleformostofthetoothlossinadults. Number of the adult population worldwide 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 disease[2]. Specific pathogenic bacteria that have the ability to penetrate and damage the periodontal tissue isPorphyromonasgingivalis and Actinobacillusactinomycetemcomitans[3]. Porphyromonasgingivalis 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 59.5[4]. Porphyromonasgingivalis 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 periodontitis[4] 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-1B[6]. 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 periodontium[7.] 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 body[8].

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 Riau[9]. Purwanti(2010) in his research found that in Dadiah buffalo milk containing lactic acid bacteria, dominated by the bacterium Lactococcus, Pediococcuspentosaceus, 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 activity[11]. Bacteriocin is a protein compound that is excreted by the bacteria that are inhibiting the growth of other bacteria that are pathogenic especially[12]. Given this nisin make bacterial isolates Dadiah can prevent further inflammation caused by pathogenic bacteria in periodontal disease. Pediococcuspentosaceus 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 Pediococcuspentosaceus on mice diarrhea, Pediococcuspentosaceus 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 PediococcuspentosaceusDadiah to the amount of IL-1β-induced rat bacterium Porphyromonasgingivalis (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 PediococcuspentosaceusDadiah on levels of IL-1β in rat periodontal tissue inflammation induced bacterium Porphyromonasgingivalis. This study is expected to provide the following benefits:

1. Provide information on the effect of bacterial isolates PediococcuspentosaceusDadiah to the amount of IL-1β-induced rat bacterium Porphyromonasgingivalis.
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 Porphyromonasgingivalis for 15 days. Group 3 is an induction treatment groups were given the bacterium Porphyromonasgingivalis for 15 days and given Bacteria Pediococcuspentosaceus isolates Dadiah for 5 days.

Treatment procedures

Application materials treated with the bacterium Porphyromonasgingivalis Injection Each treatment will be given in the form of an injection of bacterium Porphyromonasgingivalis 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 Porphyromonasgingivalis in experimental animals is to inject the bacteria on the Junctional epithelium in the gingival sulcus in the maxillary first molar teeth left. Porphyromonasgingivalis dose based on research Permana, et al (2013) which is the concentration of 5μg / 0,05mL with each application 0,02mL treatment as well as the needles used size 30G[13]. 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 high[14].

Applications Bacteria Pediococcuspentosaceus isolates Dadiah

Preparations Bacteria Pediococcuspentosaceus 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)[11]ie 2 x 108 cfu / g.

b. Intake of Blood Serum

1. 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 Porphyromonasgingivalis were conducted over 15 days. Group III blood serum is taken on day 21 after the induction of the bacteria Porphyromonasgingivalis 15 days followed by induction of bacteria Pediococcuspentosaceus 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-1β 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 Kruskall Wallis test to see the difference in all three treatment groups. Kruskal-Wallis analysis showedp<0.05, Mann Whitney followed post hoc showed significant differences in all groups with p <0.05.

Tabel1.Mean levels of IL-1B in each group

Group	n	Mean ± s.d	p
Negative control	10	*10.44 ± 4.34*	*<0,05*
Positive control	10	*65.19 ± 34.07*	
Treatment	10	*21.42 ± 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 aboveshows 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 PediococcuspentosaceusDadiah can reduce levels of IL-1B in Wistar rats induced periodontitis with Porphyromonasgingivalis. 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. Porphyromonasgingivalis is a major putative periodontophatic bacteria[15]. One of the virulence factors common to periodontopathic bacteria is lipopolysacharide (LPS), the which is known to penetrate the periodontal tissues and subsequently interacts with the host immune and non-immune cells cells.This interaction leads to cell activation and release of inflammatory mediators such as cytokines ,chemokines and prostaglandin[16]. 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 ith group of non-periodontitis.

This research currently using rats as a model research. This study uses induction of bacteria Porphyromonasgingivalis 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.

Porphyromonasgingivalis

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 groupbacteriumPorphyromonasgingivalis ATCC 33 277 increased Expression of cytokines pro inflammation of the examination days to 15 days[17],

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 Porphyromonasgingivalis, which in its activitywill produce endotoxins form lipopolysaccharide. Lipopolysaccharide from Porphyromonasgingivalis is a major factor the progression of periodontal disease. Gingival fibroblasts are connective tissue The main gingiva can directly interact with Porphyromonasgingivalis and bacteria productssuch as lipopolysaccharide[18]. lipopolysaccharideactivate 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 anesthesichloroform 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 startson day 2 post-induction of bacteria Porphyromonasgingivalis

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 eyes[19]

After induced periodontal inflammation for 15 days, the treatment group was given probiotic bacteria derived from bacteria Pedicoccuspentosaceus whey isolates for 5 daysonce that is done and the blood draw examined the levels of IL-1B in this group. Resultexamination showed the treatment grouphas 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 declineTNF-α levels after administration of bacterial isolates curd Pediococcuspentosaceus in inflammatory rats diarrhea[12],

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 mechanismsthe work is not yet known with certainty, but

There are several mechanisms that seem play a role in the treatment of periodontal diseasenamely:

1. Probiotic bacteria secrete two types bacteriocinsyakninyareuterin 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 bulgaricu and Lactobacilli strains capable of inhibiting the growth of Porphyromonasgingivalis up to 82% and is able to inhibit the effects produced by bacterial pathogens mentioned[20].
2. Probiotic bacteria have the ability to competing with pathogenic bacteria[21]
3. Probiotic bacteria have anti-inflammatoryeffectswhich can inhibit the secretion of cytokines pro inflammatory.

These three mechanisms have the effect of directly and indirectly to periodontal disease treatment[20.]

CONCLUSION

From this study it can be concluded that the bacterial isolates DadiahPediococcuspentosaceussuppress the levels of IL-1B on male Wistar rats were induced by Porphyromonasgingivalis.

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