Expression of IL-1β in Periodontitis Post Oral Administration of Papaya Seed Extract

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Abstract

Periodontitis is a chronic inflammation induced by bacteria that can increase the expression and secretion of IL-1β. Papaya (Carica papaya linn) seed extract is rich in alkaloids, flavonoids, and phenolic acids that act as antiinflammatory.

The aim of this study is to evaluate the role of papaya seed ethanol extract in periodontitis trough the expression of IL-1β.

Rattus novergicus were randomly divided into five group and subject to induced LPS of P.gingivalis + ligatures on the mandibular incisive region except group C as the control group. The first group of Periodontitis (P1) was given standard diet. Meanwhile P2, P3, P4 were given extract of papaya seeds respectively 200 mg/kgBW, 300 mg/kgBW, and 400 mg/kgBW. Papaya seed extract was administered orally for 15 days. On day 16th, the rats were sacrificed and prepared for immunohistochemical evaluation with the data being analyzed using One Way ANOVA.

There was a significant difference in IL-1β expression between P2,P3, P4 and the control group. Papaya (Carica papaya linn) seed extract can decrease IL-1β expression in periodontitis.

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Introduction

Considered as the most prevalent inflammatory disease in the world, chronic periodontitis affects nearly 50% of adult population and 60% of aged population globally.[1,2] Periodontitis is commonly not recognized by the patient because there is no symptom but leads to a systemic disease that can potentially threaten life such as atherosclerosis, myocardial infarction, stroke, rheumatoid arthritis, diabetes mellitus and premature birth with severe low body weight. This is caused by toxic components that easily spread to the systemic blood circulation and other organs of the body.[3,4,5]

Periodontitis is a chronic inflammatory disease in the oral cavity that affects tooth-supporting tissues and alveolar bone, leading progressively to tooth loss. Bacterial infection is the primary cause in triggering periodontitis. Lipopolysaccharide (LPS) P. *gingivalis* is a key factor in the development of periodontitis. Gingival fibroblasts which are the main forming cells of the gingival connective tissue can directly interact with the bacteria P. *gingivalis* and its products including LPS in periodontitis lesions.[6]

In several studies, it has been found that among the inflammatory mediators that are potentially as biomarkers of periodontitis, IL-1β has shown a more consistent relationship compared to other cytokines. Its response to treatments has also shown a significant expression decrease so that IL-1β can be used as a potential therapeutic target for periodontitis[7,8,9,10]

The potential of papaya ethnobotanics has been known for a long time, which is traditionally used to treat a toothache by inhaling the steam resulted from the stewing process of papaya leaves [11] According to Amazu[12], papaya seeds have the potential as an antioxidant and anti-inflammatory compound by reducing the production and expression of cytokines, and modulation of transcription factors. This is presumably because papaya seed extract is rich in polyphenol content (alkaloids, flavonoids, and phenolic acids). However, the active polyphenol compounds in papaya seed extract have not still

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been widely exploited and utilized for therapeutic treatment in dentistry, particularly on periodontitis. Meanwhile, according to Cai[14], flavonoids can decrease the degree of alveolar bone in rats with periodontitis induced using ligatures. This study was aimed to evaluate the role of papaya seed ethanol extract in periodontitis trough the expression of IL-1β.

Materials and methods

Research Design

Thirty five Rattus *novergicus* were randomly divided into five group and subject to induced *LPS* of *P.gingivalis* + *ligatures* on the mandibular incisive region except group C as the control group. The first group of Periodontitis (P1) was given standard diet. Meanwhile P2, P3, P4 were given extract of papaya seeds respectively 200 mg/kgBW, 300 mg/kgBW, and 400 mg/kgBW. Papaya seed extract was administered orally for 15 days. The dose referred to a study of Amazu[12] which used a maximum dose of 200 mg/kgBW, modified by adding two larger doses.

On day 16th, the rats were sacrificed and prepared for immunohistochemical evaluation with the data being analyzed using One Way ANOVA. All treatments in the experimental rats have been approved by the Ethics Commission of Faculty of Dentistry, Airlangga University, number: 004/HRECC.FDOM/I/2017.

Sample

In the experimental unit, it was selected healthy male *Wistar* rats aged 2-3 months with 180-200 grams of weight. The experimental ones used in this research amounted to 35 rats that were divided into 5 groups, each of which included 7 rats.

Periodontitis Induction

The *LPS P.gingivalis* (LPS PG standard from InvivoGen Hongkong) was induced in the rats by dripping it on the gingival sulcus in the area of mandibular-central incisive buccal as much as 5 mg in 0.05 ml of *PBS* once a day for 7 days. Prior to the induction, the rats were anesthetized with ketamine (80 mg/kg) injected in the left side area of hind foot in quadricep/tricep musculus.[16] Then, it was followed by an induction with 0.01" ligature wire attached to the lower mandibular incisors forming an eight-figure for 7 days.[14] At the end of the induction, the periodontitis symptoms/signs in the rats were observed clinically, *i.e.* the pocket depth and the resorption of alveolar bone.

Administration of Papaya Seed Ethanol Extract

The ethanol extract of papaya seeds used derived from the papaya seeds aerated for several days until being dry in a place protected from direct sunlight, which then was processed into an extract in *Materia Medika Batu, Malang.* The extract was given to P2, P3, and P4 groups for 15 days by oral administration respectively 200 mg/kgBW, 300 mg/kgBW, and 400 mg/kgBW starting on the 15th day after the induction of periodontitis is completed, in accordance with a research conducted by Kose *et al.*[17] The papaya seed extract was sucked using a sonde with a tip made of rubber. The rats were held on the skin of the head until the mouth was facing upwards, then . the sonde was inserted through the mouth slowly until it reached the stomach and the papaya seed extract was sprayed. The administration of the extract was done once a day at 08.00 – 09.00 AM.[18]

Experimental Animal Sacrifice

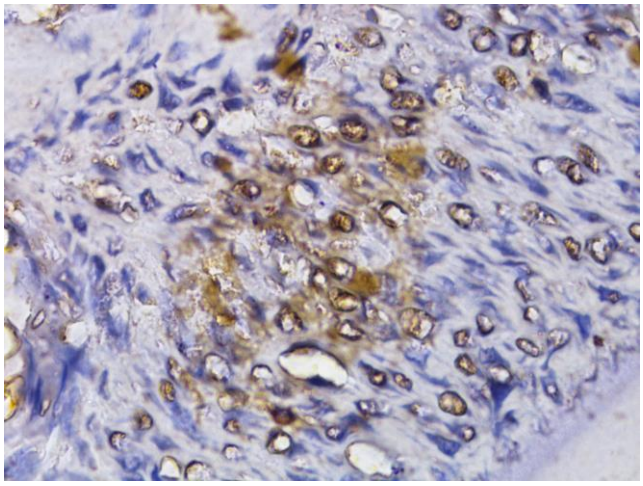
In the 15th day after the administration of papaya seed extract, the rats were sacrificed with inhalational chloroform. The rats were fixed on the worktable, and then a mandibular bone removal was performed. The rat mandibular bones were inserted in sealed platinum tubes containing a 10% formalin buffer solution for fixation, labeled according to the groups and then sent to the laboratory for paraffin block preparations.

Preparation of Specimen (Preparat)

The tissue was softened in a 10% EDTA liquid for the decalcification process for 30 days at room temperature and the liquid was replaced / renewed in daily. The tissue softness was observed by piercing the tissue using a needle. After that, the tissue was cut as thick as approximately 4 microns, affixed to the object glass for immunohistochemistry examination, use antibody IL-1β from InvivoGen Hongkong.

The observation of IL-1β expression were performed on the preparation of specimen using *Nikon H600L* light microscope equipped with 300-megapixel camera digital of *DS Fi2* and an image processing software of *Nikon Image System.* The IL-1β expression score data were obtained using modified *Remmele* method[17] in which the scale index of *Remmele* (Immuno Reactive Score / IRS) was the result of

multiplication between immunoreactive cell percentage and color intensity scores on immunoreactive cells. The data of each sample were the average IRS value observed at 5 (five) Fields of View with 1000x magnification. The views of the five fields were different at 1000x magnification.



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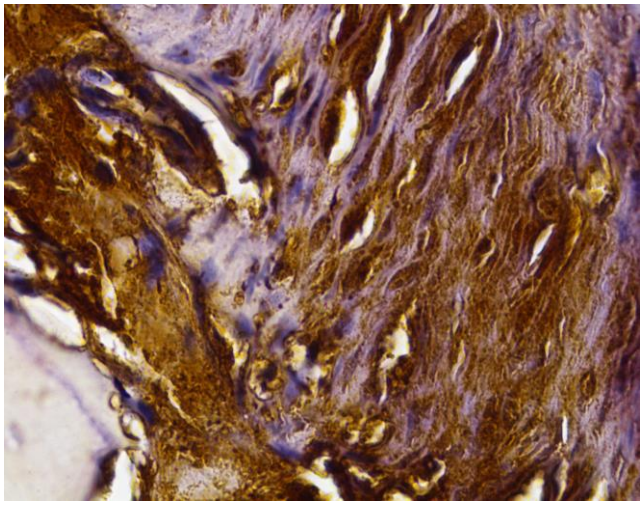
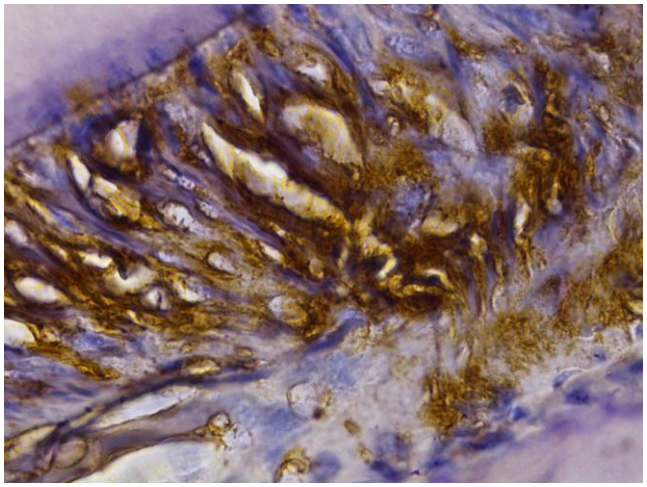


P1



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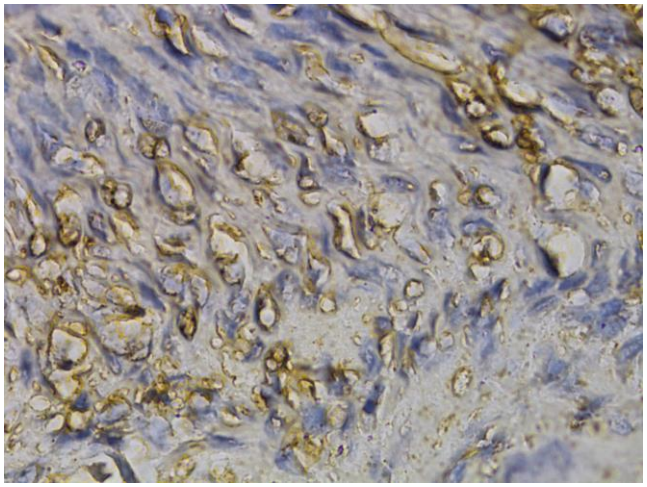

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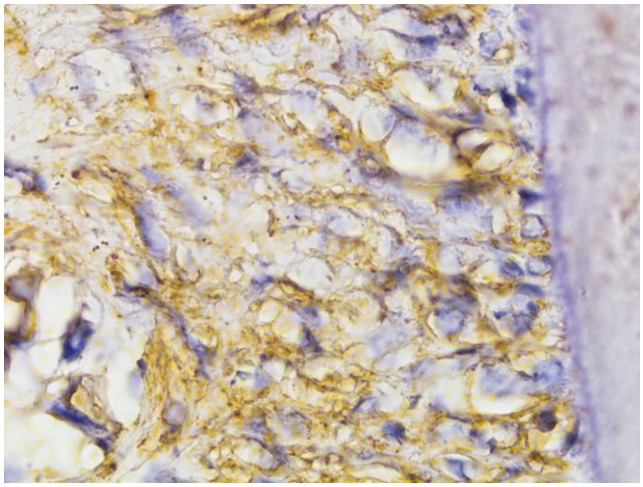

P4

Figure1-4. IL-1β expression comparison on periodontal tissue composing cells in rat tooth (yellow arrows) between treatment groups (histochemical immuno-staining, 400x magnification; *Nikon H600L* microscope; *DS Fi2* 300-megapixel camera). (picture on files named:IHC C, P1, P2, P3, P4).

Results

On the average IL-1β expression (table 1) it was found a sharp difference between the P1 and P4 group, that P1 group obtained twice higher average score than the P4 group. There was also a decreased IL-1β expression in the group P1, compared to the group P2. However, there was no statistically significant difference between the two. The results of this research indicate that the dose of papaya seed ethanol extract effectively decreases the expression of IL-1β at a minimum of 300 mg/kgBW dose. Different results are shown in a study conducted by Amazu[12], stating that at a dose of 200 mg/kgBW of rats given the extract of papaya seeds can reduce the diameter of the edema in rat legs, but the observation conducted in the study is clinical, *i.e.* measuring the diameter of edema, so the actual degree of IL-1β expression is unknown.

Variable observation was carried out on the 16th day after being given ethanol extract of papaya seeds in the treatment group for 15 days. The difference in the control and periodontitis groups was analyzed with one-way ANOVA. A series of statistical tests to meet the requirements of one-way ANOVA testing were carried out and showed that all mean of IL-1β expression data in all control and periodontitis groups had significant differences, P <0.05, as seen on Table 2.

Parameters	Treatment					Sign.
	C	P1	P2	P3	P4	
IL-1β	0.8 ± 0.25	8.08 ± 1.31	6.43 ± 2.05	5.86 ± 1.86	3.83 ± 1.26	0.000

Table 1. Average IL-1β expression number of periodontitis in rats given different dose of papaya seed ethanol extract (mg/kgBW).

ANOVA

IL-1β expression

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	145.863	4	36.466	9.667	.000
Within Groups	109.394	29	3.772		
Total	255.258	33			

Table 2. Analysis ANOVA of IL-1β expression.

Discussion

Among different pro-inflammatory cytokines, interleukin-1 (IL-1) has been attributed as a key marker of periodontal inflammation and disease progression, including bone loss. Many studies have been conducted on reducing inflammation and stopping periodontal bone resorption by inhibiting IL-1β. Treatment Short-term low-level laser therapy attenuates inflammation and production of interleukin-1β.[19]

IL-1β belongs to the super family of cytokines and plays an important role in the immune system, inflammation, replacement of connective tissue, and homeostasis, produced by various cells including lymphocytes, fibroblasts, epithelial cells, macrophages and monocytes. Some studies has shown an increase in IL-1β levels in gingival crevicular fluid in periodontitis patients compared to healthy controls.[13] Increased levels of IL-1 have been associated with increased production of enzymes for tissue degradation such as prostaglandin E2 and MMP which can cause tissue damage and attachment loss. IL-1β has also been known to play a role in triggering bone resorption and inhibiting bone formation. [15]

The potentials of papaya seed ethanol extract to be developed as an inflammatory inhibitor with working mechanism through IL-1β constraint are interesting to be observed. As it is known, the inflammatory process that triggers osteoclast genesis and bone resorption is also responsible for the failure of new bone formation in an adequate amount. Pro inflammatory cytokines such as IL-1β not only trigger and limit bone resorption but also can induce osteoblast apoptosis or osteoblast precursors directly or indirectly. The amount of bone loss occurring in periodontitis is due to the accumulative effects of inflammation that trigger bone resorption, which is unbalanced with new formation.[20]

Polyphenols have been shown to have anti-inflammatory and anti-oxidant properties.[21] In his study, Amazu[12] proved that there is anti-inflammatory activity in papaya seed extract in experimental animals. However, the active compounds of polyphenols found in papaya seed extract are still not widely exploited for therapy purpose in dentistry, especially in periodontitis.

According to Amazu[12] papaya seeds extract have potential as an antioxidant and anti-inflammatory compound by reducing the

production and expression of cytokines, and modulation of transcription factors. This is presumably because papaya seed extract is rich in the content of polyphenols (flavonoids and phenolic acids).The mechanism of action of polyphenols is suspected by inhibiting IκB phosphorylation, HAT activity, and activating HDAC, resulting in inhibition of IL-1 β expression.

When epithelial cells interact with inflammatory stimulus such as lipopolysaccharide (LPS), which is a product of bacteria identified as pathogen-associated molecular pattern (PAMP), it activates the adapter proteins such as *myeloid differentiation primary response protein 88* (MyD88) and then also activates specific protein kinase such as *mitogen-activated protein kinase* (MAPK). The protein kinase then activates *inhibitory κB kinase* (IKK), *i.e.* IKKα, IKKβ, IKKγ, which triggers phosphorylation, ubiquitination and a proteolysis degradation sequence of IκB proteins. The activated or free NF-κB translocates into the nucleus and binds to the specific DNA sequence that results in the expression of IL-1β.[15]

There were three doses of ethanol extract of papaya seeds used in this research, *i.e.* 200 mg/kgBW, 300 mg/kgBW and 400 mg/kgBW, referring to a study conducted by Amazu[12] stating that anti-inflammatory effects in experimental rats can be provided at a dose of 200 mg/kgBW. The administration of these three doses is provided to find out whether the doses have effects on the expression of IL-1β. The oral administration of papaya seed was performed for 15 days starting on the 16th day after the induction periodontitis was completed. This is also in line with the study conducted by Kose, *et al.*[17]

On the average IL-1β expression (table 1) it was found a sharp difference between P1 and P4, that P1 obtained twice higher average score than P4 group. There was also a decreased IL-1β expression in P1, compared to P2. However, there was no statistically significant difference between the two. The results of this research indicate that the dose of papaya seed ethanol extract effectively decreases the expression of IL-1β at a minimum of 300 mg/kgBW dose. Different results are shown in a study conducted by Amazu[12], stating that at a dose of 200 mg/kgBW of rats given the extract of papaya seeds can reduce the diameter of the edema in rat legs, but the observation conducted in the study is clinical, *i.e.* measuring the diameter of edema, so the actual degree of IL-1β expression is unknown.

Papaya seed ethanol extract in phytochemical screening conducted at *Materia Medika Batu* Malang qualitatively was mentioned to contain polyphenol compounds in the form of phenolic acid and flavonoids. The phenolic acid contained in the ethanol extract of papaya seeds presumably decrease the expression of IL-1β trough inhibition of inflammasome mediated inflammatory responses,[20] or by modulation of the signal transduction path through NF-κB path and *mitogen-activated protein kinase* (MAPK).[23]

Conclusions

Based on the results of the research, it is concluded that the ethanol extract of papaya seeds has the potential as anti-inflammation in periodontitis rats. This is indicated by decreased expression of IL-1β as pro-inflammatory cytokines.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

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