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EXPRESSION OF COX-2 ON DEFENSES OF THE GINGIVAL EPITHELIUM INJECTED WITH *PORPHYROMONAS GINGIVALIS* LIPOPOLYSACCHARIDE AFTER CURCUMIN ADMINISTRATION

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ABSTRACT : The lipopolysaccharide (LPS) of *Porphyromonas gingivalis* is a crucial virulence factor critically involved in the regulation of immune inflammatory responses. Curcumin is a medicinal plant that can be used as an anti-inflammatory therapy. The aim of the study is to determine the role of COX-2 in the defense of the gingival epithelium in intervention by LPS *P. gingivalis* by administering curcumin. Forty wistar rats, aged 2-3 months divided into control and treatment group. which got 5 heads for each group. Animal production of gingivitis, performed by induction of LPS *P. gingivalis* is injected into the gingival sulcus of the mandibular incisor proximal portion and since 48 hours from injection of LPS *P. gingivalis* done giving curcumin. Days 1, 7 and 14, wistar is sacrificed according to the group and gingival epithelial tissue is taken for immunohistochemical examination procedure to determine expressions of COX-2. The result is COX-2 expression decreased after administration of curcumin in wistar treated with LPS Pg. Administration of curcumin in wistar rats after induction with LPS *P. gingivalis* decreased COX-2 expression. Decreased expression of inflammatory mediators can keep the gingival epithelial defenses.

Key words : Gingiva epithelium, lipopolysaccharide, *Porphyromonas gingivalis*, herbal medicine, COX-2, curcumin.

INTRODUCTION

Periodontitis is an infection caused by microorganisms on the tooth surface, which form subgingival biofilms, are irreversible and can result in tooth loss (Chapple *et al*, 2015). Gingivitis in the final stages is reversible if adequate treatment is taken, but can also turn into destructive lesions. At this stage there can be continued damage to bone tissue known as periodontal breakdown (Reina *et al*, 2013). Proper treatment of gingivitis can prevent periodontitis. If someone has suffered from periodontitis, the periodontitis tissue will not be healthy again after treatment has been done, what can be done is to stop the progression (Nugraha *et al*, 2020). Therefore, effective prevention against the progression of periodontitis is more important than treating it because of the irreversible nature of periodontitis and has a broad impact. It is therefore necessary early prevention of the onset of periodontitis, especially in tissues that have been exposed to gingivitis by maintaining the primary defenses of the gingival epithelium. Various efforts have been made in the treatment of periodontitis, among others by the use of local or systemic antibiotics, periodontal surgery, but the results are still less satisfactory. One of the effective and efficient efforts by utilizing the plant nutritious plant extracts. One of the plant extract is efficacious as a drug that is turmeric (*Curcuma longa*) (Akram *et al*, 2010). The main active ingredient of turmeric is curcuminoid. The main content of curcuminoid from turmeric is curcumin, which is the most active ingredient (Gottumukkala *et al*, 2013). Various studies have shown that curcumin has antioxidant, anti-inflammatory, and anti-cancer properties (Akram *et al*, 2010; Guimarães *et al*, 2011; Rezkita *et al*, 2020; Kharisma *et al,* 2020). The role of 1% curcumin as subgingival irrigation can lead to a decrease in gingival inflammastion with bleeding on probing indicators (Suhag *et al*, 2007).

The bacterial product, along with the inflammatory factor released from inflammatory cells, induces damage to connective and bone tissue, altering the structure of the supporting tissue of the teeth. Therefore, epithelial barrier damage in the development of gingivitis has a significant important role for the health of periodontal tissue (Damek-Poprawa *et al*, 2013). Damage to the gingival epithelial defenses occurs due to bacterial invasion, causing inflammation, so one of the efforts to prevent it is by giving anti-inflammatory, one alternative is medicinal plant extracts. Curcumin is a medicinal plant ingredient that has anti-inflammatory properties, as an inhibitor of NFκB transcription factors. Obstacles to NF-

κB are expected to reduce the expression of pro-inflammatory mediators that contribute to the destruction of the gingival epithelial defenses in gingivitis, among them is COX-2. With efforts to prevent the occurrence of periodontitis in gingivitis cases, it is expected that the periodontitis cases will decrease more, and gingivitis cases can become a healthy tissue (reversible). The aim of the study is to determine the role of COX-2 in the defense of the gingival epithelium in intervention by *P. gingivalis* LPS by administering curcumin.

MATERIALS AND METHODS

This study was an experimental study in vivo using a 20-week-old white rats (*Rattus norvegicus*) male, weighing 325-350 grams, was obtained from the Biochemistry Laboratory of the Faculty of Medicine, Universitas Airlangga, Surabaya. The research design used is randomized control design. This study received ethical approval from The Ethical Committee of Dental Research Airlangga University 2017 (Number 012/HRECC.FODM/1/2017). Animals were divided into 4 groups: group K (n = 5) control group without treatment, sacrificed on the first day. Animal model gingivitis without treatment group K1 (n = 5) was sacrificed on the first day, group K2 (n = 5) was sacrificed on day 7, group K3 (n = 5) was sacrificed on day 14 and group K4 (n = 5) with the provision of corn oil as a curcumin carrier sacrificed on day 14. Animal model gingivitis with treatment of curcumin, group P1 (n = 5) sacrificed on the first day, group P2 (n = 5) sacrificed on day 7, group P3 (n = 5) was sacrificed on the 14th day. Curcumin is obtained from Tokyo Chemical Industry, purity is 95%. Preparation of 1% curcumin solution using curcumin in powder form which has been standardized and certified, using solvent corn oil as a carrier, curcumin powder weight 1 gram then dissolved in corn oil until 100 ml. Gingival epithelial tissue was taken for immunohistochemical procedures to determine the expression of COX-2.

Animal production of gingivitis, performed by induction of *P. gingivalis* LPS, injected into the gingival sulcus of the mandibular incisor proximal portion. Each experimental animal injected by *P. gingivalis* LPS with volume 10 µl and concentration 1 mg/ml with syringe tuberculin 1 cc/ml (terumo) and needle 30 G (BD) (Mysak *et al*, 2014; Damek-Poprawa *et al*, 2013). Consecutive days and since 48 hours of injection of *P. gingivalis* LPS, curcumin is given (Mysak *et al*, 2014). Curcumin active ingredients derived from turmeric rhizome (*Curcuma longa*) in the form of certified powder, from Tokyo Chemical Industry. Curcumin is made in concentrations of 1% in corn oil, administered 0.03 ml (30 µl), twice daily for 2 weeks, topically on the gingival sulcus of the mandibular incisor proximal portion.

Immunohistochemical staining used in this study was for the examination of COX-2 expression from gingival tissues of the mandibular incisor. The primary antibodies COX-2 from Santa Cruz Biotechnology, inc (Cox-2 (29): sc-19999). Histologic samples of the gingival epithelium performed immunohistochemical tests for COX-2. Statistical analyses were performed using SPSS software version 21. All data were expressed as mean±standard deviation (SD). Differences between experimental groups were analyzed with ANOVA. A p value <0.05 was considered statistically significant.

RESULTS

COX-2 expression in response to *P. gingivalis* LPS stimulation and with the curcumin administration,

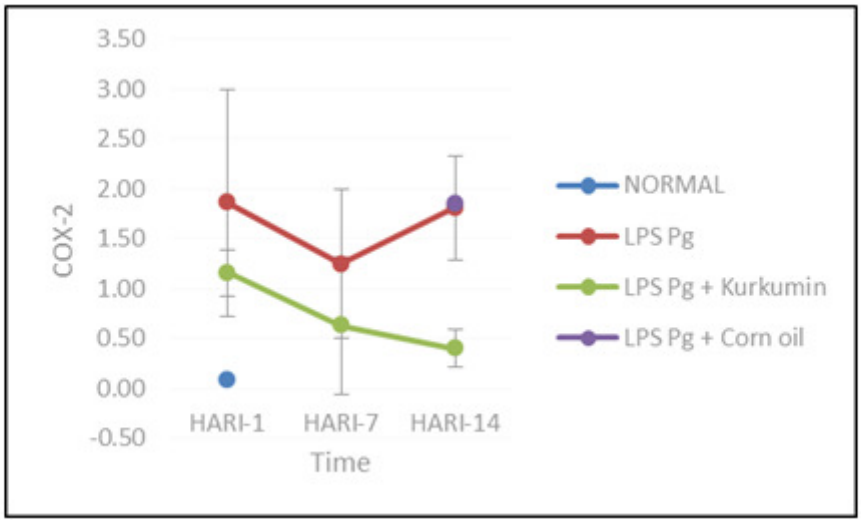

Fig. 1 : An increased in COX-2 expression in the LPS Pg treatment group (red line) on day 1, decreased on day 7 and increased on day 14, compared with control group (blue dot). Compared with green line that showed increased COX-2 expression day 1, but decreased on day 7 and day 14. The blue dot, showed COX-2 expression in the LPS Pg on control group.

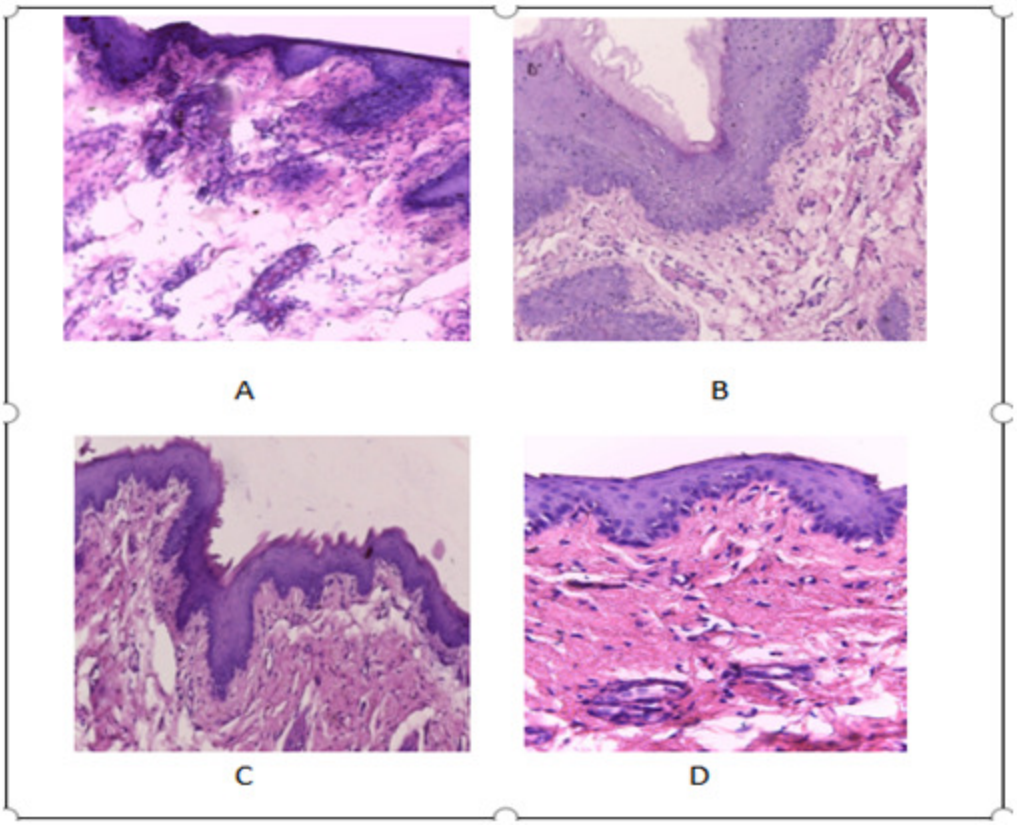

Fig. 2 : Histological compared to **a:** normal appearance in the control group (Original magnification, ×400), epithelial cells and few inflammatory cells **b:** Moderate inflammation histological appearance in the LPS Pg group. **c:** Severe inflammation histological appearance in the LPS Pg group. **d:** Mild inflammation histological appearance in the LPS Pg group administered curcumin.

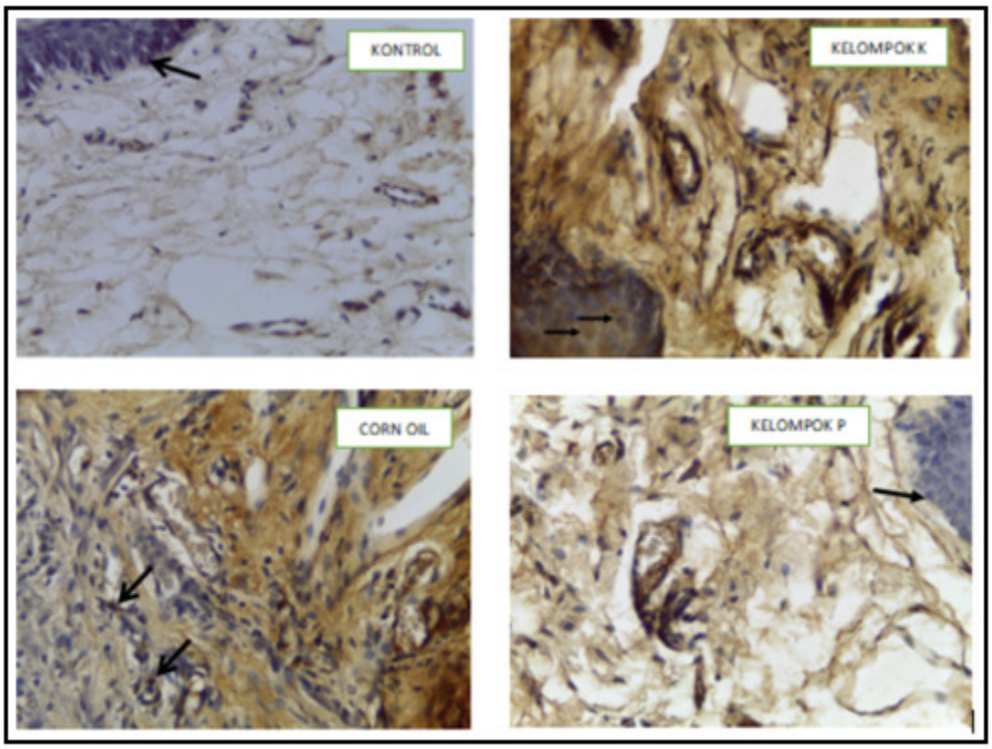

Fig. 3 : Immunohistochemical staining COX-2 in the gingiva epihelial after injection LPS *P. gingivalis* administered curcumin. COX-2 expression in control group (A), in LPS Pg group (B), in LPS Pg and administered curcumin (C) and in corn oil LPS Pg group (D).

determination by immunohistochemical examination procedured in gingival epithelial. Based on immunohistochemical examination, increased expression of COX-2 after treatment of *P. gingivalis* LPS.

Immunohistochemical measurement of COX-2 showed the following results:

The normality test with Shapiro Wilk was carried out on COX-2. Data for COX-2 is not normally distributed. Mann Whitney Post Hoc Testing for COX-2 showed significant differences ($p < 0.05$). This shows that the observations on day 1 have increased expression of COX-2.

Immunohistochemical staining for COX-2 in the gingiva epihelial after injection LPS P gingivalis and administered curcumin (Fig. 3).

DISCUSSION

The environment in the gingival epithelium is constantly surrounded by bacteria, therefore the gingival epithelium plays an important role in maintaining tissue homeostasis from bacterial attack (Hayashi *et al*, 2010). Porphyromonas gingivalis lipopolysaccharide (LPS Pg) can interfere with epithelial integrity and contribute to the destruction of junctional epithelium. In the present study, we analysed COX-2 expression in the gingiva epihelial of the anterior lower incisor after injection LPS P gingivalis. Porphyromonas gingivalis is an anaerobic gram-negative bacteria that is involved in the pathogenesis of periodontitis (Mysak *et al*, 2014). Lipopolysaccharide from Porphyromonas gingivalis is the key leading cause of periodontitis (Chen *et al*, 2008). LPS is a bioactive component and can induce IL-1 and mononuclear cell immunoregulation in multiple doses of pg / ml (Dixon and Darveau, 2005).

The experimental animals used in this study were Wistar rats exposed to Lipopolisacaharide Porphyromonas gingivalis (LPS Pg) for two consecutive days as a model of gingivitis. Wistar rats were used in this study, because they are animal models for gingivitis. All of the animals in the positive (LPS Pg) group showed macroscopic signs of inflammation, which was confirmed by histological examination. These findings are consistent with those of the study by Dumitrescu *et al* (2004). Several studies have used LPS to induce periodontal inflammation in rats. Topical application of LPS results in typical inflammatory changes such as junctional epithelial disruption, infiltration of leukocytes and oedema of the subepithelial connective tissue (Mysak *et al*, 2014). Similarly, Fujita *et al* (2018) in his study, found that LPS from *P. gingivalis* was injected into the palatine gingiva of BD and HD rats, epithelial hyperplasia, rete ridge elongation, collagen destruction and infifiltration of inflammatory cells were distinctly observed in the interdental gingiva.

We found that the expression of COX-2 in the positive (LPS Pg) group were higher than those in the negative group. We also found COX-2 levels in the LPS Pg group were higher than the treatment group of LPS Pg given curcumin. Similarly, Park *et al* (2017) in his study, found that enhanced expression of TNF-α and IL-6, after buccal injection of one microgram pg-LPS in 1 µl sterile PBS in mice. Pg bacterial lipopolysacharide can activate epithelial cell membrane receptors namely Toll-like receptor-4 (TLR-4) (Yarmolyuk, 2012; Køllgaard *et al*, 2017). Signals from the Toll Like receptor, via the adapter protein myeloid differentiation factor 88 (MyD88), will cause degradation of the inhibitor of nuclear factor-κB, release NFκB and translocate to the nucleus. Activation of NFêB will trigger the process of transcription and translation resulting in proinflammatory cytokines Pro-inflammatory cytokines produced include: IL-1β, TNF-α and IL-6 (Hans, Mayank, 2011). Activation of the NF-κB pathway occurs in the presence of many pro inflammatory mediators present in large quantities in tissues with periodontal diseases such as bacterial LPS, TNF-α, IL-1, MMPs, COX-2 and inducible nitric oxide synthase (iNOS) (Gharib *et al*, 2013).

From this study, a significant increase in expression of TLR-4 after exposure to LPS Pg. Increased expression of TLR-4 occurs because LPS Pg exposure causes cells to respond with an immune response and produce proinflammatory cytokines. TLR-4 is responsible for exposure to pathogens that enter the tissue by recognizing and binding to pathogen associated molecular patterns (PAMP), which are specific molecules of pathogens that attack tissues. When TLR-4 expression increases, there will be an increase in excessive pro-inflammatory cytokine production. Excessive pro-inflammatory production will cause excessive inflammatory reactions and causing tissue damage (Song *et al*, 2017). Increased expression of TLR-4 after LPS Pg exposure also causes an increase in NF-κB expression. TLR activates the same signal component as used in IL-1 receptor (IL-1R) signals, which produce the appropriate immune response needed for host defenses. TLR recruits a set of adapter proteins with Toll / IL-1 Receptor (TIR) domains. This interaction results in the activation of nuclear Kappa β (NF-κB) transcription factors, which control the induction of proinflammatory and chemokine cytokines and the increase in the induction of co-stimulation molecules. Increased expression of NF-κB causes an increase in other inflammatory mediators that play a role in damage

to periodontal tissue, namely COX-2. Prostaglandin 2 (PGE-2) production, which is an important factor in periodontal tissue damage is catalyzed by cyclooxygenase-2 (COX-2), which plays an important role in the host response of periodontal tissue. COX-2 expression and prostaglandin formation are due to the presence of inflammatory bacterial products and cytokines (Pesevska *et al*, 2017).

In this study, COX-2 expression showed increased in LPS group. This suggests that there is a role of COX-2 in the gingival epithelium that is inflamed by invading pathogenic bacteria. The expression of COX-2 on gingival wistar rats exposed to LPS of Pg bacteria in group K without treatment was significantly different from LPS *P. gingivalis* group; K1, K2, K3, K4 and LPS *P. gingivalis* group administered curcumin; P1, P2 and P3. When viewed from the average in Figs. 1 and 2, control group has average COX-2 expression lowered than LPS *P. gingivalis*. In the LPS, *P. gingivalis* group showed higher COX-2 expression than LPS *P. gingivalis* group with curcumin administration.

Curcumin is a polyphenol compound that is widely used as traditional medicine with various benefits such as anti-inflammatory, anti-microbial, anti-oxidant and anticancer (Anand *et al*, 2007). In this study, curcumin was used as an anti-inflammatory therapy thereby increasing the gingival epithelial barrier defense. In this study, the results showed that topical administration of curcumin 1% to gingiva exposed to Pg bacterial LPS could reduce expression of and COX-2.

In this study, the LPS Pg treatment group by giving curcumin, can reduce COX-2 expression. Decreased COX-2 expression after administration of curcumin can occur due to curcumin activity in histone acetyltransferase (HAT) p300 CBP. As it is known that curcumin is a p300 / CBP HAT inhibitor, while COX-2 activity is regulated by p300 protein for transcription process, so that the curcumin activity on p300 / CBP HAT, thought to reduce COX-2 expression which increased after the application of LPS Pg (Wang *et al*, 2015).

CONCLUSSION

The present investigation showed that expression of COX-2 on the wistar rat gingival epithelium increased at induction with *P. gingivalis* LPS. This suggests that COX-2 play an important role in the inflammatory response of inflammatory processes in the gingiva due to invasion of pathogenic bacteria. Administration of curcumin decreased the expression of COX-2 in the wistar gingival epithelium.

Conflict of interest

The authors declare no conflicts of interest.

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