



HANG TUAH UNIVERSITY  
FACULTY OF DENTISTRY  
PRESENT

INTERNATIONAL SCIENTIFIC MEETING

PROCEEDING BOOK

# Dentisphere 3

26TH-27TH, AUGUST 2018  
SHANGRI-LA HOTEL, SURABAYA



*Current Concepts and Technology  
in Improving Dental and Oral Health Care*

ISBN 978-602-145-90-1-0

**PROCEEDING BOOK INTERNATIONAL SCIENTIFIC MEETING**

**3rd DENTISPHERE (DENTISTRY UPDATE & SCIENTIFIC ATMOSPHERE)  
CURRENT CONCEPTS AND TECHNOLOGY IN IMPROVING DENTAL AND ORAL  
HEALTH CARE**

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**COVER DESIGN :**

MONICA VITA, SKG

**PRINTED AND PUBLISHED BY:**

FKG HANG TUAH SURABAYA.PRESS  
JL. ARIF RAHMAN HAKIM NO.150 SURABAYA 60111  
TELP. 031-5945864, FAX. 031-5946261  
WEBSITE: [www.hangtuah.ac.id](http://www.hangtuah.ac.id)  
Cetakan : SURABAYA, 2016-06-29

ISBN 978-602-14590-1-0

SL 2.26

RESEARCH ARTICLE

## ***Sticophus Hermanii* Extract Affected The Eepression Of TLR 4 And TNF-A In Periodontitis Induced By *Porphyromonas Gingivalis***

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### **ABSTRACT**

**Background :** *Porphyromonas gingivalis* is one of major pathogens associated with chronic periodontitis. Toll-like receptor (TLR) 4 recognize bacterial lipopolysaccharides and tumor necrosis factor (TNF)- $\alpha$  the inflammatory cytokine, both play important role in pathogenesis of periodontitis. Sea cucumber (*Sticophus hermanii*) possess antibacterial and antiinflammatory properties which potentially to be explored as strategic therapy. **Purpose :** This study was aimed to examine the effect of *Sticophus hermanii* extract to the expression of TLR-4 and TNF- $\alpha$  in periodontitis. **Methods :** The study was an experimental laboratories research with post test only control group design. Twenty four male wistar rats aged 8-10 weeks were divided equally into 4 groups. Group-1 was normal group, while group 2-4 were periodontitis group induced by  $10^9$  *Porphyromonas gingivalis* ATCC 33277 three times in 4 days. Treatment groups were given *Sticophus hermanii* extract by the route of : per oral 0,025mg/gBW (group-3) and applied topically 0.01ml on gingival sulcus with 3% extract gel (group-4). Control groups (group-1 and 2) were given 0.2% CMC Na only, all were done once daily for 14 consecutive days. The expression of TLR 4 and TNF- $\alpha$  on mandibular periodontal ligament were examined by immunohistochemistry. **Result :** The expression of TLR-4 and TNF- $\alpha$  were raised on group-2 compare to group-1 ( $p < 0.05$ ). Treatment with *Sticophus hermanii* extract not significantly decreased the expression of TLR 4 and TNF- $\alpha$  ( $p > 0.05$ ) on group-3 but decreased those on group-4 ( $p < 0.05$ ). **Conclusion :** *Sticophus hermanii* extract decreased the expression of TLR 4 and TNF- $\alpha$  in periodontitis induced by *P. gingivalis*.

**Keywords :** *Sticophus hermanii*, TLR-4, TNF- $\alpha$ , periodontitis, *Porphyromonas gingivalis*

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## BACKGROUND

Periodontitis has been defined as "an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, and bone loss<sup>1,2</sup>. The bacterial complex named "red complex" and composed of *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* has been strongly associated with advanced periodontal lesions. Impairment of homeostatic balance leads the destructive inflammation in periodontitis.

*Porphyromonas gingivalis* -the Gram-negative oral anaerobe- has been recognized statistically involved and strongly correlated with chronic periodontitis.<sup>3,4</sup> It implicated as a major periodontal pathogen by orchestrates inflammation through manipulation of host immunity and periodontal microbiota and that periodontal disease is initiated by polymicrobial synergy and dysbiosis.<sup>5</sup> Studies stated that *P. gingivalis* have been could act as a keystone pathogen, which reshapes an otherwise harmless periodontal microbiota into a disease-provoking microbiota in dysbiosis and because it has an ability to exploit complement and Toll-like receptor (TLRs).<sup>4,5</sup>

Toll-like receptors (TLRs) are type I transmembrane proteins found on the surface of mammalian cells. A broad variety of pathogen-associated molecular patterns (PAMPs) could interact with high specificity with TLRs, which leads to the secretion of proinflammatory cytokines, anti-inflammatory cytokines and chemokines, and initiates inflammation processes.<sup>1,6</sup> Upon interaction with these PAMPs, TLRs

activate the innate immunecells through intracellular signaling pathways.

Research has established that TLRs are also expressed in periodontal tissues and plays an important role in the innate immune response and provide first line of defense in maintaining periodontal health.<sup>7</sup> Surface components of *P. gingivalis*, such as LPS, lipoproteins, and fimbriae, interact with TLR2 and TLR4 expressed by host cells and stimulate production of proinflammatory cytokine.<sup>1,4,5</sup> However, over-production of proinflammatory cytokines due to chronic stimulation of TLRs may lead to tissue destruction. It can be stated that TLRs act as a double-edged sword, not only maintaining periodontal health but also contributing to periodontal tissue destruction.<sup>7</sup>

Destructive periodontal diseases, when left untreated could become a chronic inflammatory condition. Periodontally associated inflammatory processes contribute to an increase in the levels of local and systemic inflammatory mediators, including tumor necrosis factor alpha (TNF- $\alpha$ ).<sup>2</sup> Bacterial lipopolysaccharides (LPSs) of *P. gingivalis* can induce TNF- $\alpha$  production, this cytokine has a fundamental rôle in the innate response. TNF- $\alpha$  increases the activity of phagocytes, such as neutrophils, mediates cell and tissue turnover by inducing matrix metalloproteinase secretion and by stimulating the development of cells of the myeloid lineage (e.g. osteoclasts). It also limits tissue repair by the induction of apoptosis in fibroblasts. Periodontal pathogens can stimulate excessive production of TNF- $\alpha$  leading to



periodontal tissue destruction.<sup>2,8</sup> Regarding to the immune response, pointing at primary receptor of periodontal pathogens and inhibition of inflammatory cytokine may provide an efficacious therapeutic strategy.

Sea cucumber have many variety species and it has been known to have beneficial medical properties.<sup>9,10</sup> The result of our previous study stated that extract of sea cucumber *Sticohus hermanii* possessed antibacterial activity against *P. gingivalis* in vitro.<sup>11</sup> Golden sea cucumber (*Sticophus hermanii*) extract have been known to have antibacterial,<sup>9,10,11</sup> antioxidant,<sup>12,13</sup> and anti-inflammatory<sup>9,10,13</sup> Sea cucumber (*Sticophus hermanii*) possess antibacterial and antiinflammatory properties which potentially to be explored as strategic therapy. This study was aimed to examine the effect of *Sticophus hermanii* extract to the expression of TLR-4 and TNF- $\alpha$  in periodontiti

## MATERIAL AND METHODS

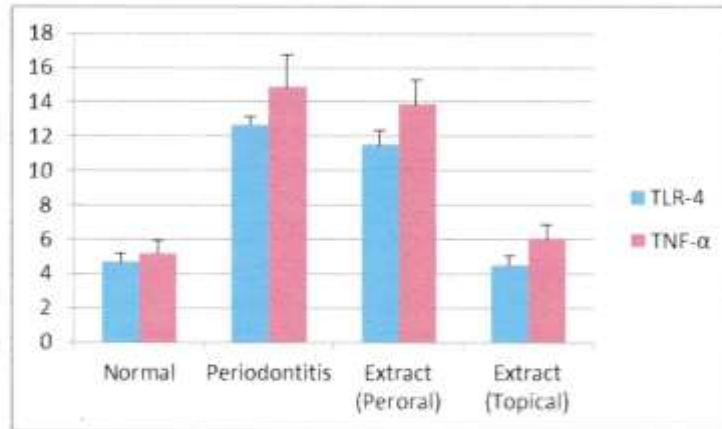
The study was an experimental laboratories research with post test only control group design. Sample of sea cucumber *Sticophus hermanii* were collected from Sumenep coastal. *Sticophus hermanii* extract was prepared from sea cucumber bodywall that have been freeze dried and powdered, formulated with 135mg/ kg BW in 0.2% CMC Na for peroral

route and 3% extract gel for topical use.

Twenty four male wistar rats aged 8-10 weeks were divided into 4 groups. Group 1 was normal group, while group 2-4 were periodontitis groups. Oral preconditioning were set up by the administration of 0,1% chlorhexidine topically and 20 mg Kanamycin and Ampicilin/ rat in the drinking water as once daily for 4 consecutive days.<sup>14</sup> Periodontitis induction were performed by inoculation of *P. gingivalis* ATCC 33277 bacterial suspension containing  $10^9$  CFU/ml in 2 ml PBS by peroral administration with nasogastric tube, swabbed in buccal/labial-palatal gingiva along molar to molar regio and anal regio. The administration of *P. gingivalis* were done 3 times in 4 days. After 3 weeks periodontitis condition was achieved<sup>15</sup> and were started to get the treatment.

Treatment groups were given *Sticophus hermanii* extract by the route of : per oral 0,025mg/gBW (group-3) and applied topically 0.01ml on gingival sulcus with 3% extract gel (group-4). Control groups (group-1 and 2) were given 0.2% CMC Na only, all were done once daily for 14 consecutive days. Mandibular section were performed and the expression of TLR-4 and TNF- $\alpha$  on mandibular periodontal ligament were examined by immunohistochemistry. Data were analyzed by Manova and LSD test.

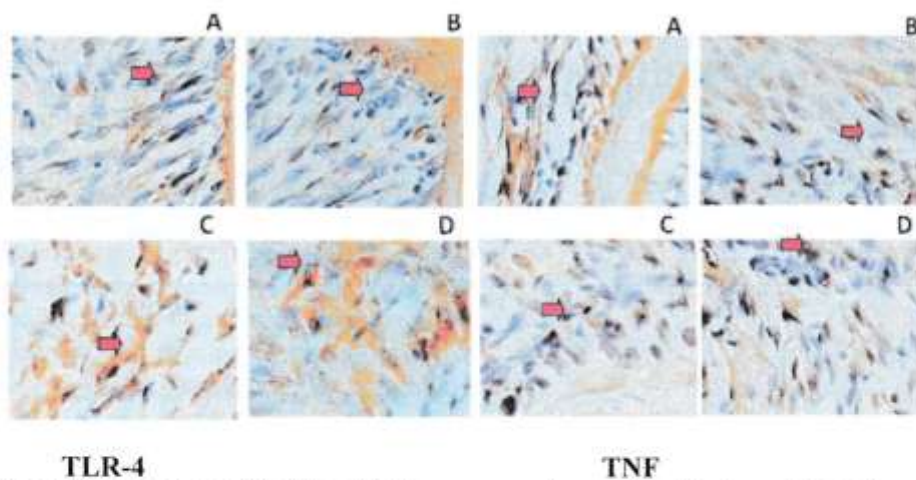
## RESULT



**Fig 1.** Mean of TLR-4 and TNF expression on macrophage of Wistar rat mandibular periodontal ligament given extract of *Sticophus hermanii* peroral and topical on sulcus gingiva

The expression of TLR-4 and TNF- $\alpha$  were raised on periodontitis untreated group compare to normal group ( $p < 0.05$ ). Treatment with *Sticophus hermanii* extract perorally on group 3 were not significantly

decreased the expression of TLR-4 and TNF- $\alpha$  ( $p > 0.05$ ) while treatment with *Sticophus hermanii* extract perorally on group 4 were decreased the expression of TLR-4 and TNF- $\alpha$  ( $p < 0.05$ ).



**Fig 2.** The expression of TLR-4 and TNF- $\alpha$  on macrophage of mandibular periodontal ligament

- A. The expression of TLR-4 / TNF- $\alpha$  in normal group (group 1)
- B. The expression of TLR-4 / TNF- $\alpha$  in periodontitis untreated group (group 2)
- C. The expression of TLR-4 / TNF- $\alpha$  in periodontitis treated by extract of *Sticophus hermanii* peroral ly(group 3)
- D. The expression of TLR-4 / TNF- $\alpha$  in periodontitis treated by extract of *Sticophus hermanii* topically (group 4)

## DISCUSSION

The Toll-like receptor plays an instructive role in innate immune responses against microbial pathogens as well as the subsequent induction of adaptive immune.<sup>16</sup> In periodontitis, bacteria and pathogen-associated molecular patterns are sensed by Toll-like receptors (TLRs), which initiate intracellular signaling cascades that may lead to host inflammation.<sup>6,17</sup> Both healthy and periodontitis gingival tissues expressed all TLRs except TLR-10. In patients with periodontitis, epithelial cells showed increased TLR expression towards the basal layer. In the connective tissue, consistently higher TLR expression was found within the periodontitis group compared to the healthy group.<sup>17</sup>

Fig 1. showed the increasing of TLR 4 and TNF- $\alpha$  expression on periodontitis untreated group compare to normal group ( $p < 0.05$ ). Induction of *P. gingivalis* ATCC 33277 bacterial suspension proved to lead to periodontitis condition and raised the recognition of host receptors as periodontitis immune response. Lipopolysaccharide is an essential macromolecule that comprises the outer surface of gram-negative bacteria. Numerous studies have shown that *P. gingivalis* lipopolysaccharide is less potent than that of *E. coli* and also elicits a different repertoire of inflammatory mediators in innate immune cells. Lipid A from *P. gingivalis* is unique in that it exhibits remarkable structural heterogeneity. Four lipid A structural subgroups have been identified in *P. gingivalis* which, remarkably, can be stimulatory, inert or antagonistic with

respect to Toll-like receptor 4 activation.<sup>18</sup>

TLR 4 functions as the principal innate sensor for lipopolysaccharide of Gram-negative bacteria in mammals. The expression of TLR 4 has been confirmed in some cell types in periodontal tissues, such as gingival fibroblasts and gingival epithelial cells and might be very important in the progress of periodontitis. Sun *et al*<sup>6</sup>, stated that expression of TLR 4 was detected on human periodontal ligament cells (HPDLCs). Lipopolysaccharide treatment may change the gene expression pattern of HPDLCs via TLR4 signaling and may control homeostasis in periodontal tissues. It also indicates that the NF $\kappa$ B and MAPK pathways may be involved in the regulation of periodontitis development.

One of the main consequences of signaling via pattern-recognition receptors is the modified synthesis, processing and secretion of cytokines that serve to transmit, modulate and amplify the host immune responses. Cytokines are central to the initiation, organization and maintenance of immune responses in periodontal disease and represent key diagnostic and therapeutic targets. TNF- $\alpha$  is one of major inflammatory cytokine<sup>8</sup>. The increasing expression of TNF- $\alpha$  in group-2 ( $p < 0.05$ ) showed the periodontitis condition as it has been known that the level of TNF- $\alpha$  are raised in periodontal diseases compare to the healthy ones. 1,2,8 periodontal diseases are infectious inflammatory conditions, and recent studies have demonstrated that cytokines (TNF- $\alpha$  and IFN- $\gamma$ ) considered harmful in the context of tissue destruction play

important roles in the control of periodontal infection.<sup>20</sup>

Manipulating TLR4 signaling may potentially become one of the recognized therapies for periodontitis. Recently, host modulation therapies are being proposed and developed to bring down excessive levels of enzymes, cytokines, prostanoids, as well modulate osteoclast functions.<sup>21</sup>

Administration of *Sticophus hermanii* extract in both treatment groups resulted in decreasing TLR 4 and TNF- $\alpha$  expression, significant in topical use as gel in sulcus gingival (group 4) ( $p < 0.05$ ) but not significant in per oral mode (group 3) ( $p > 0.05$ ). In the previous study, *Sticophus hermanii* extract have been known to have antibacterial activity to periodontopathogen bacteria<sup>18</sup> and *P.gingivalis*.<sup>11</sup> Some sea cucumber extract has been known to have antibacterial properties regarding its content of saponin and triterpene glycoside.<sup>18</sup> Sea cucumbers are rich in glycosides, particularly triterpene glycosides which are proven to have antibacterial and antitumor activities. Mechanism of terpenoid or triterpene as antibacterial agent is to interact with porin in the bacterial outer membrane to form the strong polymer bond that make destruction to the porin that will reduce the cell wall permeability then caused the lost of nutrition which lead to the inhibition in bacterial growth.<sup>9,10,11,18</sup> The antibacterial activity of the extract may reduce the number of *P. gingivalis* thus reduce the PAMPs that will be recognized by TLR 4. The decreasing expression of TLR 4 may implicated in less production of abundant inflammatory cytokines that will lead to periodontal tissue destruction.

One of the main consequences of signaling via pattern-recognition receptors is the modified synthesis, processing and secretion of cytokines that serve to transmit, modulate and amplify the host immune responses.<sup>8</sup> The decreasing expression of TLR 4 may affect to the TNF- $\alpha$  production. Administration with antagonist TNF- $\alpha$  have been known to be strategic therapy in host modulation and decrease periodontal tissue damage.<sup>21</sup> The bioactive content of *Sticophus hermanii* extract triterpene glycoside may have the role to enhance the macrophage phagocyte activity and flavonoid had its role as anti inflammatory component.<sup>9,10</sup> *Sticophus hermanii* bodywall contain the component of glysin, arginine and collagen. Glysin could stimulate the release of IL-12 and B cell that enhance phagocytosis and arginin enhance T cell activity and proliferation.<sup>9,10</sup>

Administration of *Sticophus hermanii* extract as topical use in group 4 yield the better significant result than per oral mode in group 3. It has been observed that the local route of drug delivery can attain 100-fold higher concentrations of an antimicrobial agent in subgingival sites compared with a systemic drug regimen. This reduces the total patient dose by over 400 fold thereby reducing the potential problems with the use of systemic drug regimen.<sup>22</sup> In conjunction with scaling and root planing, the adjunctive use of local drug delivery may enhance the results of periodontitis therapy.



## CONCLUSION

*Sticophus hermanii* extract decreased the expression of TLR 4 and TNF- $\alpha$  in periodontitis induced by *P. gingivalis*.

## ACKNOWLEDGEMENT :

This research was supported by a grant from Doctoral Research Program, funded by Ministry of Education and Culture Indonesia

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