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

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DEAN OF FACULTY DENTISTRY HANG TUAH UNIVERSITY WELCOME NOTE

Welcome to Surabaya!
Is a great honor for us to welcome you all at the International Seminar “Dentisphere 2016”. This international seminar is the third time we have held at the Shangri La Hotel Surabaya. This Seminar which held on 26-27 August 2016 is one of my pride as the Dean of Dentistry Faculty of Hang Tuah University. This is also proofing one of Hang Tuah University’s contribution both nationally and internationally in the field of dentistry.

The theme of International Seminar 3rd Dentisphere is "Current Concepts and Technology in Improving Dental and Oral Health Care", which aim is to provide a new generation of dentists who are experts and professionals with the knowledge that continues to grow for the Indonesian nation and the world. We hope that through this event we can raise the professionalism in the field of dentistry for all participants.

I would like to say a very big thanks to our speakers from home and abroad: Japan, Korea, Thailand, and Singapore. Thanks for all contributions and participation and your willingness to come and share your knowledge and experience in dentistry. It is an honor for us that the events will also have an important role in the quality control mechanisms to ensure stability and increased periodically in the field of dentistry.

Also for all the participants, thank you very much for joining the International Seminar 3rd Dentisphere, I hope you can all enjoy the entire summary of the seminar. Hopefully this seminar that we held useful for the advancement of knowledge of dentistry you all peers. I apologize if there are less pleasing for the organization of this seminar.

Enjoy the 3rd international seminar Dentisphere!

CHAIRMAN 3RD DENTISPHERE WELCOME NOTE

Hello Dentists!
Welcome to the International Seminar 3rd Dentisphere. It's an honor for us, Dentistry Faculty of Hang Tuah University to host the International Seminar 3rd Dentisphere. We are welcoming all of our sponsors, speakers and participants from both inside and outside Indonesia who contribute to this International event. Welcome to Surabaya!

The theme of this time seminar is "Current Concepts and Technology in Improving Dental and Oral Health Care", as the committee we offers a place to learn and exchange dental knowledge with national and international facilitators. International Seminar 3rd Dentisphere will also provide a unique opportunity for participants to develop the knowledge, skills and professionalism with the interaction with other participants. Do not miss the opportunity to interact directly and do hands on with the speakers and experts which are amazingly competent in the field of dentistry from different countries (Indonesia, Japan, Korea, Singapore, and Thailand).

After all, we apologize if if there are less pleasing for the organization of this seminar . Enjoy the beauty of the city of Surabaya while you also explore the dental sciences!

God bless us always.

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RESEARCH ARTICLE

Integrin A2β1 And Bmp-2 Regulated In Bone Remodelling To Accelerate Orthodontic Tooth Movement By Giving Stichopus Hermanii

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ABSTRACT

Background: *Orthodontic tooth movement is a continual and balanced process between bone deposition and bone resorption on pressure and tension sites. Integrin α2β1 is the major collagen type 1 receptor and BMP-2 is the parameter of osteoblast proliferation that have role in bone remodeling. Stichopus hermanii is one of the best fishery commodities in Indonesia, its contain various active ingredients such as hyaluronic acid,chondroitin sulphate,cell growth factor, EPA DHA, flavonoid that might have role in orthodontic tooth movement.* ***Objectives:*** *The aim of this study is to investigate Integrin α2β1 and BMP-2 regulated in bone remodelling to accelerate orthodontic tooth movement by giving Stichopus hermanii.* ***Material and Method:*** *Thirty two male Cavia Cobaya were divided into four groups. K(-) group as negative control group (without treatment), K(+) group as positive control group which were applied separator rubber for orthodontic tooth movement, and P1, P2 groups, were applied for orthodontic tooth movement and Stichopus hermanii 3 % and 3,5 %. After treatment the cavia cobaya were sacrificed. Integrin α2β1 and BMP2 expression were examined with immunohistochemistry.* ***Results:*** *This study showed Integrin α2β1 means and SD in K(-), K(+), P1,and P2 are 7,5±1,77; 3±1,07; 11,1±3,3 and 14,13±4,55. BMP-2 have means and SD : 5,38±2,72; 2,62±1,77; 10,88±3,64 and 18,63±1,5. Integrin was significantly increased in P2 and P1 compare to K(+), K(-), while BMP2 increased too.* ***Conclusion :*** *Stichopus hermanii active component could increase integrin α2β1 and BMP2 that regulate bone remodelling,while 3,5 % Stichopus hermanii had the best to accelerate orthodontic tooth movement.*

Keywords*: Stichopus hermanii, Integrin α2β1, BMP-2, orthodontic tooth movement.*

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BACKGROUND

Orthodontic tooth movement occurs in the presence of a mechanical stimuli sequenced by remodeling of the alveolar bone and periodontal ligament (PDL). Bone remodeling is a process of both bone resorption on the pressure site and bone formation on the tension site. Orthodontic tooth movement can be controlled by the size of the applied force and the biological responses from the PDL. The force applied on the teeth will cause changes in the microenvironment around the PDL due to alterations of blood flow, leading to the secretion of different inflammatory mediators such as cytokines, growth factors, neurotransmitters, colony-stimulating factors, and arachidonic acid metabolites. As a result of these secretions, remodeling of the bone occurs. [1,2]

Today, it is challenging to reduce the duration of orthodontic treatments. Long orthodontic treatment have potensial risk such as caries, gingival recession and root resorption. There are many ways to accelerate orthodontic tooth movement based on phases of tooth movement. There are three phases of tooth movement: the initial phase, which is characterized by rapid movement after the application of force; followed by a lag period, where little or no movement, and the last phase, where gradual or sudden increase of movement occurs. The early phase of tooth movement involves acute inflammatory responses characterized by leucocytes migrating out of blood capillaries and producing cytokines, which stimulates the excretion of prostaglandins and growth factors. The acute phase is followed by the chronic phase that involves the proliferation of fibroblast, endothelial cells, osteoblasts, and alveolar bone marrow cells remodeling process. [1, 2, 3]

High concentration of cytokines such as interleukins IL-1, IL-2, IL-3 IL-6, IL-8, and tumor necrosis factor alpha (TNF) were found to play a major role in bone remodeling; moreover, interleukin-1 (IL-1) stimulates osteoclast function through its receptor on osteoclasts. [4,5] Other cytokines which are also involved in the acceleration of tooth movement are RANKL, which is a membrane-bound protein on the osteoblasts that bind to the RANK on the osteoclasts and causes osteoclastogenesis.[6] Prostaglandins (PGs) are inflammatory mediator and a paracrine hormone that acts on nearby cells; it stimulates bone resorption by increasing directly the number of osteoclasts. *In vivo* and *in vitro* experiments were conducted to show clearly the relation between PGs, applied forces, and the acceleration of tooth movement. [7] Another set of investigators has made an experiment where they have injected vitamin D metabolite on the PDL of cats for several weeks; it was found that vitamin D had accelerated tooth movement at 60% more than the control group due to the increasement of osteoclasts on the pressure site.[8] Integrin α2β1 and BMP-2 regulate bone remodelling in last phase / chronic phase.

Many but there is no natural has been used for accelerating orthodontic tooth movement. Stichopus hermanii is one of the best fishery commodities in Indonesia. It is natural and contain various active ingredient such as collagen, hyaluronic acid, chondroitin sulphate,cell growth factor, EPA DHA, flavonoid that has been proved as [9] that might reduce relapse

orthodontic. Previous research showed that stichopus hermanii stimulated the activation and proliferation of fibroblasts, and enhanced rapid production of collagen fiber network with shorter healing time. The level of proinflammatory cytokines; IL-1α, IL-1β, and IL-6, were significantly reduced in Stichopus hermanii treated wounds and stimulation tissue regeneration. [10] Stichopus hermanii at 5 mg/ml and 10 mg/ml can increased osteoblast cell function. The other study show that studies have shown that the extract of Stichopus species also affects viability or proliferation of human fibroblasts and osteoclast cells in a negative manner. [11] So, in this study, we investigate Integrin α2β1 and BMP-2 regulated in bone remodelling to accelerate orthodontic tooth movement by giving Stichopus hermanii

MATERIAL AND METHOD:

This study was performed on 32 male Cavia Cobaya 2,5 months old with 200-300 g weight. The Wistar rats was divided into 4 groups. K(-) group as negative control group (without treatment), K(+) group as positive control group which were applied with relaps orthodontic forces, and the other groups P1, P2, were applied with relaps orthodontic forces and Stichopus hermanii 2,5 % and 3 %.

Preparation of orthodontic tooth movement

Orthodontic forces was applied with giving applied separator by separating plier in mesial left insisivus maxilla cavia cobaya 14 days to produce orthodontic tooth movement. Separator forces was 0,0474 kN, measured by autograph

Preparation of Powder Stichopus Hermanii

Stichopus hermanii were used in this study from coastal regions around Sumenep, East Java Indonesia. Stichopus hermanii was cleaned by making a longitudinal incision 3-5 cm on the ventral side of stichopus hermanii without damaging the internal organs using scalpel. Sticopus hermanii was dried but not be in direct sunlight for 7 days. After this, Stichopus hermanii was blender until get the powder.

Preparation and Applied Stichopus Hermanii gel

Stichopus hermanii gel 2,5% was made from 0,25 gr Stichopus hermanii powder was diluted with NaCMC 2% in DMSO 5 % until 10 ml. Stichopus hermanii gel 3% was made from 0,3 gr Stichopus hermanii powder was diluted with NaCMC 2% in DMSO 5 % until 10 ml. Stichopus hermanii gel was applied in gingival sulcus with insulin syringe 0,025 ml once per day

The research was conducted in Biochemistry Laboratory Medical Faculty of Airlangga University. After 14 days of treatment. the cavia cobaya were sacrificed. The jaw was sectioned. Integrin α2β1 and BMP-2 (Bone Morphogenetic Protein-2) expression were examined with immunohistochemistry method in tension side.

The research data result tabulated and planned to analyze by descriptive statistic test, normality distribution test to know if the data that obtained come from population with normal distribution, ANOVA test

(analysis of varians) to analyze the difference of each variable compared with control. Then the data were tested with LSD Test

RESULTS

The aim of this study is to investigate Integrin α2β1 and BMP-2 regulated in bone remodelling to accelerate orthodontic tooth movement by giving Stichopus hermanii. The result in this experiment show the the expression of integrin α2β1 in accelerating orthodontic tooth movement as shown as table 1

Table 1 : Integrin α2β1 in accelerating orthodontic tooth movement applied with Stichopus hermanii

Group	Mean± Standart Deviation
K(-)	7,5±1,77
K(+)	3±1,07
P1	11,1±3,3
P2	14,13±4,55

Table 1 show means and SD in K(-), K(+), P1,and P2 are 7,5±1,77; 3±1,07; 11,1±3,3 and 14,13±4,55.Then the data were tested with normality test, homogenity test and show the data was homogen and have a normal distribution. ANOVA test (p=0.05) for the expression of integrin α2β1 in accelerating orthodontic tooth movement applied with Stichopus hermanii showed significantly differences. With the LSD test, showed that integrin α2β1 expression : P1 and P2 showed increased integrin α2β1 expression whether P2 has the best expression as seen as table 2

Table 2 : LSD Test expression in relaps orthodontics Cavia Cobaya applied with Stichopus hermanii

Group	**K(-)**	**K(+)**	**P1**	**P2**
K(-)		0,006*	0,022*	0,000*
K(+)	0,006*		0,000*	0,000*
P1	0,022*	0,000*		0,055
P2	0,000*	0,000*	0,055	

*Significantly different

So, the expression of Integrin α2β1 was significantly increased in P2 and P1 compare to K(+) and K(-).

The result showed BMP-2 expresion were increased in accelerating orthodontic tooth movement by giving Stichopus hermanii as sees as table 3

Table 3 : The Expression BMP-2 as osteoblast activity in accelerating orthodontics tooth movement applied with Stichopus hermanii

Group	Mean± Standart Deviation
K(-)	5,38±2,72
K(+)	2,62±1,77
P1	10,88±3,64
P2	18,63±1,5

Table 3 show means and SD in K(-), K(+), P1,and P2 are 5,38±2,72; 2,62±1,77; 10,88±3,64 and 18,63±1,5 Then the data were tested with normality test, homogenity test and show the data was homogen and have a normal distribution. ANOVA test (p=0.05) for the expression of BMP-2 as osteoblast activity in accelerating orthodontic tooth movement applied with Stichopus hermanii showed significantly differences. With the LSD test, showed that BMP-2 expression : P1 and P2 showed increased BMP-2 expression whether P2 has the best expression as seen as table 4

Table 4 : LSD Test expression BMP-2 as osteoblast activity in accelerating orthodontics tooth movement applied with Stichopus hermanii

Group	K(-)	K(+)	P1	P2
K(-)		0,004*	0,000*	0,000*
K(+)	0,004*		0,000*	0,000*
P1	0,000*	0,000*		0,000*
P2	0,000*	0,000*	0,000*	

*Significantly different

So, the expression of BMP-2 was significantly increased in P2 compare to K(+), K(-) and P1.

DISCUSSION

The aim to this study was to investigate integrin α2β1 and BMP-2 regulated in bone remodelling to accelerate orthodontic tooth movement by giving Stichopus hermanii. This study showed the result Integrin α2β1expression means and SD in K(-), K(+), P1,and P2 were 7,5±1,77; 3±1,07; 11,1±3,3 and 14,13±4,55. BMP-2 had means and SD : 5,38±2,72; 2,62±1,77; 10,88±3,64 and 18,63±1,5. Integrin was significantly increased in P2 and P1 compare to K(+), K(-), while BMP2 increased too.

Orthodontic tooth movement in cavia cobaya models occurs when separator rubber applying in the left first insisivus compressed towards the distal side during 14 days orthodontic tooth movement. Increasing integrin and BMP-2 expresion by applying Stichopus hermanii during orthodontic tooth movement means there are processes for bone remodeling because integrin and BMP-2 plays a central role for alveolar bone osteogenesis.

Integrins are cell surface receptors composed of α- and β-subunits. Integrins enable cell adhesion (cell-matrix, cell-cell) and transduce both chemical and mechanical signals. Certain integrins have function to mediate mechanical stress-induced proliferation, shear stress activated extracellular regulated-protein kinases (ERKs) and c-Jun kinases (JNKs) and integrin may function as mechanotranduction.[12] A2β1 integrin is the major collagen type 1 receptor expressed on Th 17 cells that mediates attachment of collagen type 1.[13] α2β1 integrin increases collagen type 1 synthesis and turnover.[14]

The bone morphogenetic proteins (BMPs) included BMP-2, is the second family of growth factors, unique: these are the growth factors involved in the process of osteoblast differentiation that drive the process of bone formation and mineralization. Since the late 1980s, BMPs have been known to stimulate new bone formation. BMPs represent molecular targets used to identify and develop new agents to simulate the bone-forming process. Much is understood about the signal transduction pathway for the BMPs. BMP-2 stimulates the differentiation of mesenchymal cells into osteoblasts and chondrocytes. BMP-2 binds to its receptor, a Ser/Thr kinase, which phosphorylates and activates the intracellular signaling molecules Smad 1 and Smad 5. This in turn leads to the expression of the transcription factor Cbfa1 (Runx2), which results in the expression of several proteins critical for bone formation. Wnt/LRP5 pathway is also linked to the BMP pathway by a cascade of anabolic transcriptional events. The signal starts at the Hedgehog signaling pathway, moving through the BMPs and Wnt/LRP5, and ultimately leads to expression of the critical genes involved in osteoblast differentiation. This pathway provides multiple potential molecular targets that may be manipulated in the process

of bone formation. [15] The process that been needed to accelerated orthodontic tooth movement.

Stichopus hermanii contain various active ingredient such as collagen, hyaluronic acid, chondroitin sulphate,cell growth factor, EPA DHA, flavonoid.[9] In a previous in-vitro study showed that there was a positive promoting effect of stichopus hermanii water extract on osteoblast functional activity when 1.6mg/ml, 3.1mg/ml, 6.3mg/ml, 12.5mg/ml, and 25mg/ml of stichopus hermanii concentrations were used. Microscopic examination showed adequate cell confluency in the wells with stichopus hermanii concentration from 1.6 mg/ml up to 25mg/ml. Previous studies showed that the water extract of Stichopus contains high amino acid concentrations (37%)34 as well as calcium, magnesium, iron and zinc that may play an important role in osteoblast molecular activities. [11]

Previous study showed that increasing integrin α2β1 mediates cell adhesion to and spreading on fibrillary collagen. Integrin α2β1 also can mediate collagen gel contraction and promote the integrin-mediated formation of long cellular projections typically that has role in mechanical tension. Condroitin Sulphate on the surface of bone matrix binds to cell adhesion molecule such as integrin. Ascorbic acid is also can promote collagen integrin.[16] Collagen type 1 is a major type for matrix composition in alveolar bone formation of orthodontic tooth movement.

Flavonoid, inhibits osteoclast differentiation and bone resorption in vitro but also stimulates human osteoblast differentiation. In vivo, flavonoid increases bone mass in immobilized rats and also the biomechanical properties of rat bone.[15,17] Flavonoid treatment resulted in a significant elevation of alkaline phosphatase (ALP) activity, collagen contents and osteoblast differentiation genes [ALP, collagen, osteopontin (OPN), osteoprotegerin (OPG) and osteocalcin (OC)] and bone morphogenetic protein (BMP) genes (BMP2, BMP4 and BMP7). [18] Flavonoid activated BMP signaling by inducing Smad1, 5 phosphorylation, as well as Id1 and Id2 protein expression in a dose-dependent manner. [19]

The effect of glycosaminoglycan (GAG) such as chondroitin sulphate, oral administration had been shown to increase the total calcium pool and intestinal absorption of calcium, which may lead to an increased capacity for injured bone to regenerate during osteogenesis.[11] Condroitin Sulphate on the surface of osteoblasts or bone matrix binds to cell adhesion molecule such as integrin on the pre-osteoclastic cells and inhibits the differentiation into osteoclasts so bone formation can occurred. [16]

Stichopus hermanii accelerate tooth movement through integrin α2β1 and BMP-2 in bone remodelling cycle. Stichopus hermanii Bone remodeling process is a last phase in orthodontic tooth movement that occur after rapid movement stops. When tooth movement occurs, bone resorption have role in bone remodeling. Bone formation is a phase after bone resorption. Increasing integrin α2β1 and BMP2 regulate bone formation process. Bone formation process increasing so that bone remodelling cycle.

CONCLUSION

Stichopus hermanii active component could increase integrin α2β1 and BMP2 that regulate bone remodelling,while 3,5 % Stichopus hermanii had the best to accelerate bone remodelling in orthodontic tooth movement.

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SL 2.28

RESEARCH ARTICLE

The Expression Of Macrophage Cell On Wound Healing Process In Rattus Norvegicus Using Chitosan Gel With Different Molecular Weight

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ABSTRACT

Objectives: *The infiltration of macrophage cell on wound healing process has important role to release a number of cytokines and synthesize extracellular matrix. The aim of this study was to account the the expression of macrophage cell on wound healing process of dental extraction in Rattus norvegicus for 3 and 4 days using chitosan gel with different molecular weight.* ***Methods:*** *Rattus nornegicus strain wistar male, aged 8-16 weeks, divided into 3 groups, namely group 1 which given chitosan gel 1% with high molecular weight, group II which given chitosan gel 1% with low molecular weight and group III as control which were not given chitosan gel. Chitosan gel 1% were applied into the socket of dental extraction. Rat was decaputated 3 and 4 days after chitosan gel application and the jaw in the treated regions and control group were cut for immunohistochemical examination using macrophage cell monoclonal antibody to observethe expression of macrophage cell. Data were analyzed using ANOVA test.* ***Results:*** *The expression of macrophage cells were found higher in the group which given chitosan gel 1% with high molecular weight. The result showed significant differences in expression of macrophage cell for 3 and 4 days observation compared to control group ($p<0,05$).* ***Conclusion:*** *The application chitosan gel 1 % with high molecular weight stimulates macrophages cells on wound healing process of dental extraction.*

Keywords: *Chtosan gel 1 %, molecular weight, macrophage cell*

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BACKGROUND

The macrophage is important inflammatory cell in wounds healing process. The macrophage cells have role to many functions in wound healing, including host defense, promotion of inflammation and support of cell proliferation on wound healing process.[1] It include the following growth factors that promote cellular proliferation and protein synthesis, proteases and extra-cellular matrix molecules. It produce a large number of mediators and cytokines including interleukin-1, interleukin-6, interleukin-12, TNFα, and inducible nitric oxide synthase (iNOS). The macrophage cell stimulate the production of growth factors such as TGF-beta1, vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF)-1. These growth factor promote the proliferation cells in wound healing process.[1,2,3]

Chitosan is naturally derived polysaccharide that have many application in tissue engineering due to its antimicrobial activity ,biocompatibility and having some properties to accelerate wound healing process.[4] In recent study the application of chitosan gel on wound healing process of dental extraction can increase the number of type 1 collagen on remodeling process of dental extraction.[5] Kojima *et al*. reported that chitosan is able to stimulates Platelets derived growth factor (PDGF). It can stimulates the migration and proliferation of macrophages and fibroblast cell on wound healing. Futhermore, PDGF activates the synthesis of Tranforming growth factor beta (TGF β) in macrofag, which also activates the synthesis of collagen in fibroblast.[5,6,7] The application of chitosan depends on the characteristic of chitosan include the molecular weight and deacetylation degree.[8,9]

The infiltration of macrophage cell on wound healing process has important role to release cytokines, some mediator and synthesize extracellular matrix. Chitosan gel is property to accelerate wound healing process of dental extraction. The aim of this study was to account the the expression of macrophage cell on wound healing process of dental extraction in Rattus norvegicus for 3 and 4 days using chitosan gel with different molecular weight**.**

MATERIALS AND METHODS

The material in this experiment were Chitosan powder purchased from Sigma chemical, St. Louis, USA. The degree of deacetylation was more than 75 %. Chitosan with high molecular weight (Product number: 419419, Lot number: MKBH5816V) and chitosan with low molecular weight (Product number= 448869, Lot number= MKBH7256V), asetat acid 2 % p.a (Merck, Germany), buffer formalin 4% and 10% , ketamin (Ketalar,Pfzer), xylazine, alkohol 80%, alkohol 95 %, alkohol 100 % (absolute), xylene, buffer Parafin, EDTA 10 % (JT Baker, USA), NaSO4 2 % (Merck, Germany), PBS,Tripsin 0,125 %, H_20_2 0,5 %, methanol (Merck, Germany), NaOH 1,25 % and Macrophage monoclonal antibody. The tools used in this experiment were Becker glass, Stirer, pipette pasteur, Autoclave (Foundry), 5 cc syringe injection (Terumo), 1 cc syringe tuberculin (Terumo), pinset, elevator, Needle holder, non resorbable silk sutures,

Bekker glass, Incubator memmert W Germany, Rotary microtome, Label, slide, cover glass, petri disk Poly-L-lysine, deck glass and mikroskop trinokuler Olympus CX 31 Japan).

Chitosan gel 1 % (w/v) was made with diluted one gram of chitosan powder in acetic acid 2 %. It added with NaOH 1,25 % solution to get neutral pH. The mixture was stirred until the gel was completely formed. After homogenization, the gels were stored in closed containers at ambient temperature until use. The characteristic of chitosan gel was evaluated includes solubility, pH, viscosity, physical characteristic, homogeneity, consistency, and duration of storage time. The homogeneity test of gel carried out using glass plates after the powder diluted in acetic acid 2 %. It should be observed on optimized homogeneous. Consistency test could be done by using a penetrometer or mechanically sentrifugator. Gel without precipitation will produce a good consistency. Physical characteristic test or Organoleptic analysis during the storage time includes change of colour, form of formulation gel and odorless.[10,11]

The research was an experimental laboratory study. Rattus nornegicus strain wistar male, aged 8-16 weeks, divided into 3 treatment groups namely group 1 which given chitosan gel 1 % with high molecular weight dan high viscosity. Group 2 given chitosan gel 1 % with low molecular weight and low viscosity, and group III as control which were not given chitosan gel. Chitosan gel were applied into the socket of dental extraction. Rat was decaputated 3 and 4 days after chitosan gel application and the jaw in the treated regions and control group were cut for immunohistochemical examination to analyze expression of Macrophage cell. Fixation was performed using 10 % buffer formalin and decalcification applying EDTA. Further process was dehydration and continued by clearance. The tissue could be cut using microtome in 4-6 µm thickness. Deparafin and rehydration were subsequently performed. Bone morphogenetic protein-2 monoclonal antibody was diluted by antibody diluents. Next, it was washes by PBS. Streptavidin-biotin was dropped and incubated for 30 minutes, washed by PBS. Counterstained using haematoxyline and washed by flowing water and dried. It was given entelan and covered by cover glass. Light microscope was applied and the evaluation was done. The measuring result were analyzed using ANOVA test. It analyzed the comparison between chitosan treated with high molecular weight group, lower molecular weight group and the control groups ($P<0,05$).

RESULTS

The mean and standard deviation of each group at 3 and 4 days after treatment. The expression of macrophage cell in 3 and 4 days after treatment using chitosan with high molecular weight and high viscosity more higher compared to group using chitosan with low molecular weight and low viscosity. The data was analysized using kolmogorov-smirnov statatistical test. It showed normal distribution ($p>0.05$) in which fulfiiling the requirement of parametric test. ANOVA test showed there were

significant difference ($p<0.05$) in all group.

Table 1. The mean and standard deviation of each group at 3 and 4 days after treatment

Variable	Treatment	3 days	4 days
		Mean± SD	Mean± SD
The expression of macrophage cell	Chitosan high MW,visco	16.00±2.37	22.00±2.00
	Chitosan low MW,visco	12.40±2.30	13.33±2.25
	Control	2.83±0.98	3.40±1.14

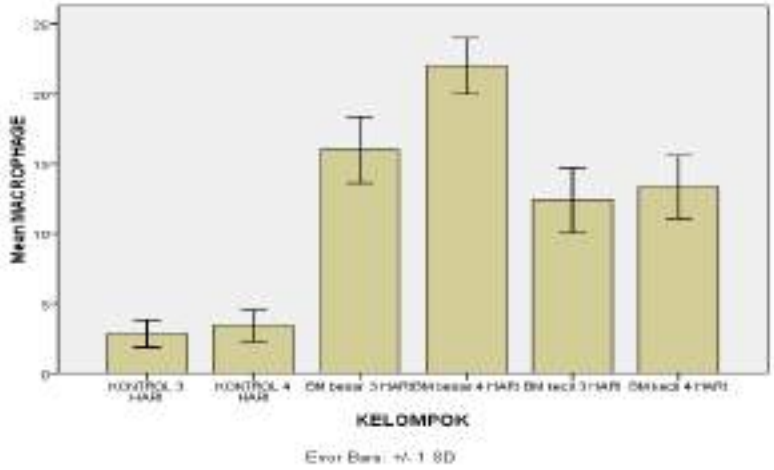

Figure 1. The graphic of expression macrophage cell on 3 and 4 days using chitosan with high molecular weight, lower molecular weight and control group

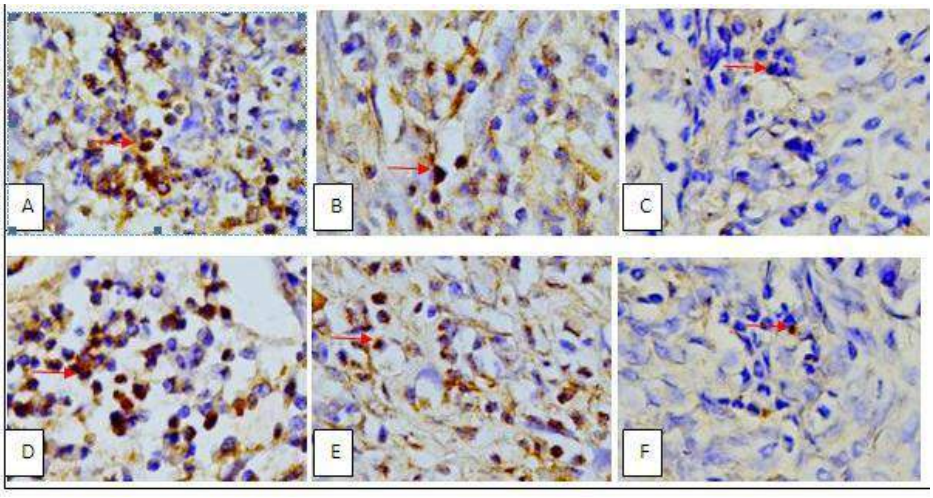

Figure 2. The expression of macrophage cell at 3 days observation: (A) Chitosan with high molecular weight and high viscosity, (B) Chitosan with low molecular weight and low viscosity, (C) Control group, without using chitosan; The expression of macrophage cell at 4days observation: (D) Chitosan with high molecular weight and high viscosity, (E) Chitosan with low molecular weight and low viscosity, (F) Control group, without using chitosan

The expression of macrophage cell on wound healing process of dental extraction using chitosan gel shown in figure 2. Figure 2 showing the expression of macrophage cell in 3 and 4 days after dental extraction. In our study, the expression of macrophage cell on wound healing process of dental extraction using chitosan was more higher compared to control group. The expression of macrophage cell in 3 and 4 days using chitosan gel with high molecular weight and high viscosity was more higher than using chitosan with low molecular weight and low viscosity.

DISCUSSION

Macrophage cell appear In inflammatory phase of wound healing process, 48 until 72 hours after injury and continue the process phagocytosis. These cells Attracted to the wound site by chemoattractive agents, including clotting factors, complement components, cytokines such as PDGF, TGF-β and platelet factor IV, as well as elastin and collagen. Macrophages cells have a longer lifespan than neutrophils. It has important role as regulatory cells and providing an abundant reservoir of potent tissue growth factors, TGF-β, as well as other mediators (TGF-α, heparin binding epidermal growth factor, fibroblast growth factor [FGF], collagenase), activating keratinocytes, fibroblasts and endothelial cells. If there no macrophage cell would cause delayed fibroblast proliferation, angiogenesis and maturation[2,12].

In our study the expression of macrophage cell in 3 and 4 days after treatment using chitosan gel have more higher than the treatment of group control. Chitosan exhibits several valuable properties such as antibacterial, antifungal, nontoxic, hemostatic, biodegradable as well as hydrogel formation properties. Which these properties, chitosan applications has important role in many fields for tissue engineering.[13] Chitosan gel also acts as an ideal wound dressing and more importantly chitosan gel accelerates wound healing.[14] Chitosan is metabolized by certain human enzymes, such as lysozyme. Thus, chitosan is biodegradable. It has structural similarities to glycosaminoglycans and is hydrophilic. Chitosan's monomeric unit, *N*-acetylglucosamine is an extracellular macromolecule that is important in wound healing.[14,15] When chitosan is applied to the wound, it biodegraded by lysozymes, Chitosan modulates macrophage function and the secretion of numerous enzymes collagenase and cytokines include interleukins and tumor necrosis factor during the wound healing process. Chitosan structurally glycosaminoglycans (GAG), which have long-chain, unbranched, repeating disaccharide units maintaining cell morphology, differentiation and function. Glycosaminoglycans and proteoglycans are widely distributed throughout modulate cytokines and growth factors, including heparin and heparan sulfate. Hence, the cell-binding and cell-activating properties of chitosan are important for wound healing. Moreover, *N*-acetylglucosamine is an anti-inflammatory drug and is synthesized in the human body from

glucose.[15] It is incorporated into glycosaminoglycans and glycoproteins. Chitosan exerts anti-inflammatory effects by inhibiting prostaglandin E2 (PGE2) and cyclooxygenase-2 (COX-2) protein expression. The application of chitosan increases the expression of the anti-inflammatory cytokine. The degradation of chitosan into monomers and oligomers at a wound site significantly accelerates the wound healing process. [13,15]

The characteristic of chitosan is related with its molecular weight. The expression of macrophage cell after treatment using chitosan gel with high molecular weight and high viscosity shown more higher than treatment using chitosan gel with low molecular weight and low viscosity. Chitosan gel has a strong tissue-adhesive property. When chitosan dissolved in acidic solution gives viscous solutions.The viscosity of chitosan is influenced by its molecular weight**.** The monomers of chitosan powder with high molecular weight and high viscosity were directly effective because it monomers more quickly absorbed and biodegraded by some enzymes. N-acetyl-D-glucosamine dimer active of chitosan cross-linked with glycosaminoglycan and glycoprotein that part of matrix macromolecules extracellular as well as stimulate increased.[16,17,18] The macrophage cell is key of inflmatory process in wound healing process. It produces some mediators, sitokin and growth factor which crucial role in wound healing process of dental extraction.[2] Chitosan gel were found to stimulate the expression of macrophage cell, significantly it could promote the the wound healing process of dental extraction.

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RESEARCH ARTICLE

Effects Of *Stichopus Hermanii* Ethanolic Extract On Tlr-2 And Il-17 Expression In Rats With Oral Candidiasis Immunosupressed Model

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ABSTRACT

Background: *TLR-2 and IL-17 play important role in signaling of C.albicans infection. Stichopus hermanii ethanolic extract have potency as inhibitor against C.albicans.* ***Purpose:*** *To find out the effect of stichopus hermanii ethanolic extract on the expression of TLR-2 and IL-17 in rats with oral candidiasis immunosupressed models.* ***Methods:*** *This study was true experimental with post test only control group design. Wistar rats were imunosupressed with dexamethasone and tetracycline peroral then induced by C.albicans ATCC 10231 $6x10^8$ on the tongue of rats for14 day. Groups were divided into : healthy (K1), candidiasis (K2), and two treatment groups P1: candidiasis+ 3% Stichopus hermanii ethanolic extract, P2: candidiasis+nystatin, P1 and P2 were treated for 14 days after induction of C.albicans. The expression of TLR-2 and IL-17in tongue specimen were examined by immunohistochemistry. Data were analyzed with One-way ANOVA and LSD-test.* ***Results:*** *The expression of TLR-2 were increased in candidiasis group ($p<0.05$) while no significant different in IL-17 expression compare to normal ($p>0.05$). Treatment with Sticophus hermanii and Nystatin both increased TLR-2 and IL-17 expression compare to candidasis group ($p<0.05$).* ***Conclusion:*** *Stichopus hermanii ethanolic extract of could increase the expression of TLR-2 and IL-17 in rats with oral Candidiasis immunosupressed model.*

Keywords : *immunosupressed, oral candidiasis, TLR-2, IL-17, Stichopus hermanii*

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BACKGROUND

Candida species are the most common fungal pathogens of humans and can cause life threatening infections in immunocompromised individuals.[1,2] *Candida* infections common in patients with HIV/AIDS, diabetes mellitus, HIV disease, chronic systemic corticosteroid usage, or chemotherapy-induced neutropenia are predisposed to cutaneous candidiasis, mucosal diseases in the elderly and edentulous individuals, such as *Candida*-associated denture stomatitis.[1,3] *Candida albicans* as the common caused is a polymorphic fungus that present as a commensal of mucosal tissues in approximately 40–80% of individuals.[4]

Immune responses directed against C. albicans, as host defense against this pathogen involves complex coordination of both innate and acquired immune responses. Pattern recognition of organisms via TLRs (Toll like Receptors) have significantly contributed to the complex mechanism recognition of microorganisms by the innate immune system. TLR-2 and TLR-4 known as the main TLRs involved in the signalling cascades induced by *C. Albicans.*[3,5] TLR2 signaling pathways activation in antigen-presenting cells (APCs) by ligation of *C. albicans* cell-wall components toward production of cytokines, such as tumour necrosis factor (TNF), interleukin (IL)-1β and IL-10.[5] TNF-alpha and interleukin (IL) plays important role in mucosal defence as pro-inflammatory cytokines, whereas IL-10 is a potent inhibitor in the immune defense against C. albicans.[6,7]

Candida species recognition by TLRs activates intracellular signaling pathways that might contribute to the polarization lead to proinflammatory TH17 response.[8] Th17 responses constitute essential components of immunity to the commensal fungus Candida albicans.[9] Th17 cells produce cytokines that is IL-17A and IL-22, which mediate protection from candida infection at mucocutaneous surfaces. The role of IL-17 is recruit neutrophil accumulation by stimulating production of cytokines and chemokines from nonhematopoietic cells, while IL-22 stimulates proliferation and antimicrobial peptide production by epithelial cells.[3,9]

Sea cucumbers (Holothuria sp.) has immunostimulatory effect. Its plays a role in improving leukocyte count and differential leukocyte carp namely monocytes and Neutrofil.[10] Several studies indicate that sea cucumber extract has several therapeutic properties such as a promoters of soft tissue healing and as an antibacterial, antifungal, antitumour, antianaphylactic, anti-inflammatory, antinociceptive and antioxidant. Sea cucumbers species extract also affects viability or proliferation of human fibroblasts and osteoclast cells.[11] The presence of sulfate GAG in particular of sea cucumbers can accelerate healing through a positive effect on the acceleration of the percentage of wound contraction, epithelialization increasing migration, fibroblast proliferation, angiogenesis processes, and organization of collagen.[12]

One of sea cucumber's family, Stichopus hermanii, has potential therapeutic properties in oral cavity diseases. Several studies have shown that the metabolites of saponin in the sea cucumber Stichopus hermanii can be used as an antibacterial and

antifungal. As antibacterial, this extract inhibit the growth of caries bacteria such as Enterococcus faecalis and Streptococcus mutans. While as antifungal, it can inhibit the growth of Candida Albicans.[13-15] Revianti dkk[16] found that Stichopus hermanii is not toxic at concentration of 2.5% wherease in concentrations of 5% it is toxic. Increased fibroblasts are found in the teeth with orthodontic treatment were treated with golden sea cucumber 3% in order to prevent relapse.[17]

Based on the description above and anticandida potency of sea cucumber (Stichopus hermanii) is necessary to do research on the effects of *Stichopus hermanii* ethanolic extract on TLR-2 and IL-17 expression in rats with oral candidiasis immunosupressed model. The purpose of this study was To find out the effect of stichopus hermanii ethanolic extract on the expression of TLR-2 and IL-17 in rats with oral candidiasis immunosupressed models.

MATERIAL AND METHODS

This research was a true experimental research with post test only control group design. Sixteen Male Ratus Novergicus Wistar strain, aged 8-16 weeks, 200-300 gram weight and healthy were immunosupressed with dexamethasone and tetracycline orally and induced by C.albicans $6x10^8$ (2 Mc.Farland) on the tongue of rats for 14 days. Divided into four groups, healthy group (K1), candidiasis group (K2), treatment group 1 (P1): candidiasis treated with ethanol extract Stichopus hermanii 3% and treatment group 2 (P2): candidiasis treated with Nystatin.

The instruments used in this study were a box cage rat, disposable syringes, analytical balance, glass boxes, portable device Glucoce test (Glucosure), scalpel, cotton bud, scissors, test tubes, tube centrifuge, pipette, erlenmeyer, tweezers, glass beaker, microscopes, cameras. The tools necessary for the preparation of the erythrocyte preparation is scalpels, scissors, tweezers, syringes, cotton, bottles, test tubes, centrifuges, refrigerators. Materials of this study were immunohistochemistry kit, Candida albicans ATCC10231, Saburoud Agar, Saburoud broth, PBS, TLR-2 and IL-17 marker.

Sea cucumber were used in this study is the golden sea cucumber (Stichopus hermanii) adults, weighs 100-250 grams from karimunjawa. The extraction method is using maceration method by soaking dried Stichopus hermanii in ethanol for 24 hours and then separated filtrate and residue. To get dried sea cucumber, gold Sea cucumbers (Stichopus hermanii) cleared later in the freeze dryer at a temperature of -85 ° C and then crushed into a powder and solvent extracted with ethanol 96%. After that added with 0.2% Na-CMC up to concentration of 3%.

Healthy Rats which were not immunosupressed and given tetracycline orally. In this group, Rats were given 1mL 0.2% CMC-Na and PBS per day. Whereas candidiasis and treatment group, rats were previously immunosupressed by providing oral dexamethasone for 1 week at a dose of 0.5mg / day / mice and supplemented with tetracycline 1mg / day / rat orally and then induced with Candida albicans ATCC10231 containing $6x10^8$ on the entire surface of the tounge three times/week for 2 weeks.

During the induction to treatment, the rats were given tetracycline orally at a dose of 0.1 mg / day / rat.

Therapy for this study was given stichopus hermanii 3% and nistatin topically for 14 days after Rats undergoing oral candidiasis, after that were sacrificed. Samples that is rat's tounge are processed and stored in paraffin blocks and ready to do the cutting. Results pieces and placed in glass objects made by immunohistochemical staining and by TLR-2 and IL-17 marker. Measurement of the amount of antibody made by observing in the microscope with 400x magnification. Data were analyzed with One-way ANOVA and LSD-test.

RESULT

Expression of TLR-2 and IL-17 showed a dark brown color (Figure 1). The results of this study indicate the amount of both TLR-2 and IL-17 expression in the group was given therapy candidiasis with stichopus hermanii (P2) has the highest number compared to other groups. While in the healthy group (K1), the number of TLR-2 and IL-17 expression at least compared to the others. TLR-2 and IL-17 expression in Candidiasis groups has compared to both therapy groups has the lowest number (Table.1).

There were significant different between groups in expression of TLR-2 ($p<0.05$). The expression of TLR-2 and IL-17 were decreased in candidiasis group ($p<0.05$). Treatment with Sticophus hermanii and Nystatin both increased TLR-2 and IL-17 expression compare to candidasis group ($p<0.05$). TLR-2 expression showed significance different between K1 and K2 ($p<0.05$). Whereas There were no significant different between healthy (K1) and candidiasis (K2) groups in expression of IL-17 ($p>0.05$) (Figure 2).

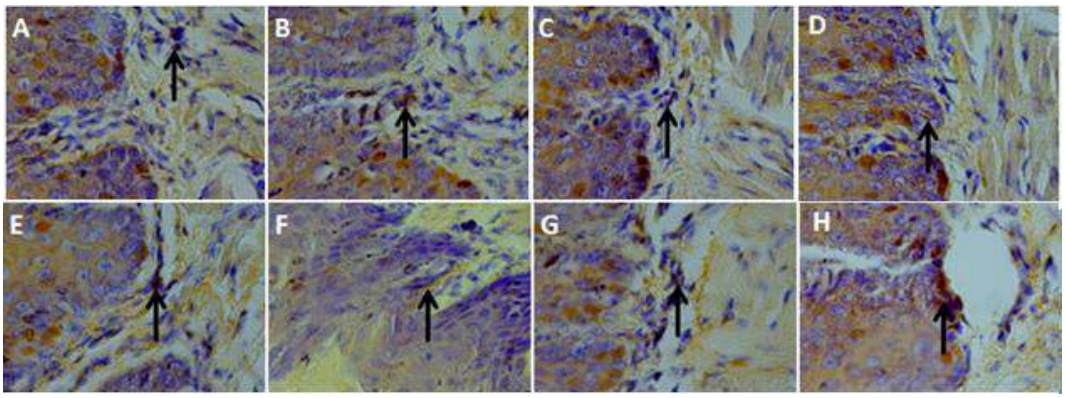

Figure 1. Expression of TLR-2 and IL-17 in the tongue epithelium of Wistar rats with oral candidiasis immunosupressed model

TLR-2 and IL-17 expression appointed by arrow: TLR-2 expression in Healthy group (A), TLR-2 expression in candidiasis group (B), TLR-2 expression in the group treated with Stichopus hermanii 3% (C) and group treated with Nystatin (D). IL-17 expression in Healthy group (E), TLR-2 expression in candidiasis group (F), TLR-2 expression in the group treated

with Stichopus hermanii 3% (G) and group treated with Nystatin (H) (magnification 400x).

Table 1. Number of TLR-2 and IL-17 in a group of healthy mice, candidiasis, treatment with stichopus hermanii extract 3% and Nystatin.

GROUPS	n	MEANS±SD	
		TLR-2	IL-17
HEALTHY	4	1,75 ± 0,5	1,75 ± 0,95
CANDIDIASIS	4	6,25± 2,5	3,5± 1,29
STICHOPUS HERMANII	4	15,5 ± 3,5	15 ± 1,41
NYSTATIN	4	11,25 ± 0,95	11,5 ± 1,29

n = Replication, SD= standard deviation

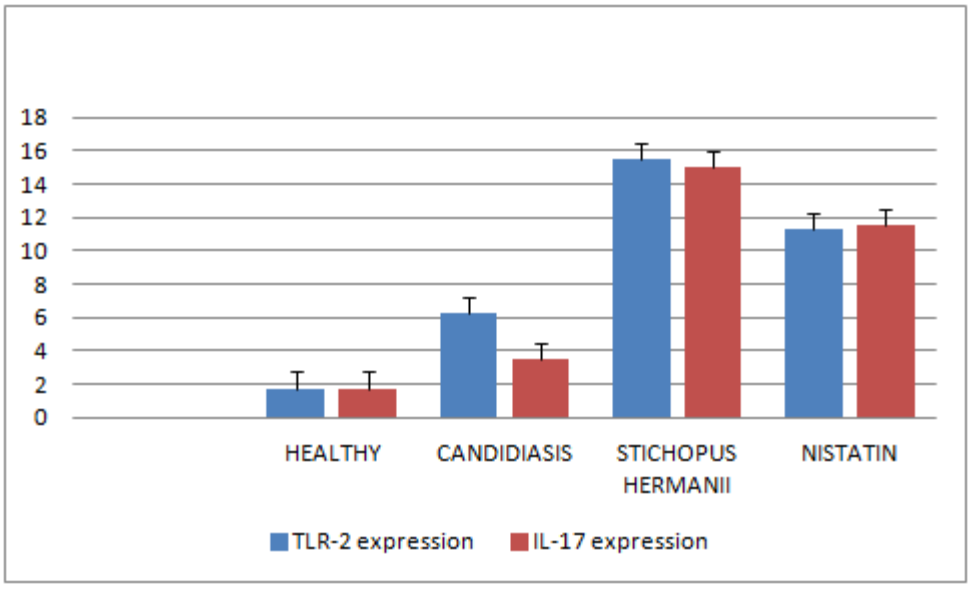

Figure 2. Variation of means TLR-2 and IL-17 expression in the tongue epithelium of Wistar rats with oral candidiasis immunosupressed model

DISCUSSION

The used of systemic immunosuppressive medication and broad-spectrum antibiotics hopefully will increase susceptibility candidal infection. Neutrophils and macrophages, as control both yeast and short filamentous forms of Candida albicans, were no longer respond effectively to Candida albicans infection in immunocompromised patients apparently due to an impaired immune status.[18] The used of broad-spectrum antibiotics alters the oral microflora. Imbalance of the normal bacterial population favours yeast growth, since it decreases the competition for nutrition and cell adhesion.[19] Therefore immunosuppressed condition and the use of broad-spectrum antibiotics is a well known predisposing factor for oral candidiasis.

C. albicans can invade the superficial layers of the oral epithelium, and cause proteolytic breakdown of E-cadherin which is important structural protein in the oral cavity, responsible for epithelium continuity, and a barrier against harmful substances.[19] Recognition of spesific antigens by T-cells in the adaptive immune response, are recognised by PRRs as part of the innate immune response.

One of PRR family that play a role in fungal recognition is TLR2. TLR2 activation induces cytokine production.[1]

Analysis of candida albicans colonization in the rat's tongue by TLR-2 expression showed significant different between healthy group and candidiasis group ($p<0.05$). This means the amount of candida in rats with candidiasis immunosupressed model were increased. Expression of TLRs might be associated with the progression of infection in these sites. Recognition of Candida albicans through TLRs activates intracellular signaling pathways that might contribute to the polarization toward a proinflammatory TH17/IL17 response where as essential components of immunity to Candida albicans.[8,9]

TLR-2 was involved in the induction of pro-inflammatory cytokine production.[20,21] However, some data suggest that TLR-2 is able to suppress immunity against Candida albicans through induction of IL-10 and regulatory T cells.[22,23] Which means that TLR-2 has two mechanism in immunity agains candida albicans. C. albicans may be able to induce both pro- and anti-inflammatory responses when recognized by macrophages depend on TLR-2 signaling.[23] In this study, IL-17 expression were increased in candidiasis groups but no significant compared with healthy groups ($p>0.05$). It is possibly due to a balance response of TLR-2 signaling.

Both treatment group with Sticophus hermanii and Nystatin were increased TLR-2 and IL-17 expression compare to candidasis group ($p<0.05$). Nystatin is an antifungal agent widely used for treatment of superficial mycoses with potent proinflammatory properties.[24,25] Razonable *et al*[25] found that nystatin induces cytokine secretion in TLR2-expressing but not TLR2-deficient cells. Nystatin reported could induce pro-inflamatory cytokine.[24] In this sudy, Nystatin could increased IL-17 that may be important in immune responses to candida infections.

Unbalanced Th17 and Treg responses during candidiasis due to cytokine, positively correlate with increasing severity in candidiasis.[9] IL-17 play a role in Epithelial cells and neutrophils as a bridge between the adaptive and innate immune responses, include induction of antimicrobial peptides, MMPs, and other inflammatory mediators. T*h*17 cells also been shown to drive antibody responses at mucosal surfaces, in particular secretory IgA (sIgA).[1] IgA known play an important role by causing fungal aggregation and prevnting adherence to mucosal surface.[26]

Stichopus hermanii ethanolic extract can increase TLR-2 and IL-17 expression compare to candidasis group since its has bioactive component. Flavonoids component in this extract modulated signaling pathways. The other component such Glikosaminoglican play role in wound healing demonstrated through increased fibroblast proliferation,

angiogenesis process and collagen formation.[27] Amino acids identified in powdered golden sea cucumber (Stichopus hermanii) such as Aspatat acid and glutamic acid have indirect role in the activation and proliferation of NK cells (Natural Killer). While arginine can increase the body's immune system by stimulate the activation and proliferation of T cells, which in turn triggers the activation of antibodies. Glycine can stimulate production and expenditure of IL-12 and cell B. B cells stimulated by candida will proliferate, differentiate and develop into plasma cells that produce antibodies.[28,29]

CONCLUSSION

Stichopus hermanii ethanolic extract has potency in inhibiting Candida albicans and promote immune system againts Candida albicans . Stichopus hermanii ethanolic extract could increase the expression of TLR-2 and IL-17 in rats with oral Candidiasis immunosupressed model.

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RESEARCH ARTICLE

TGF-β1 Expression on Traumatic Ulcer Healing Process Treated with Water Extract Gold Sea Cucumber

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ABSTRACT

Background: *Ulcers are the most common oral soft tissue lesions that can cause discomfort or pain which interferes with daily social activities. Glycosaminoglycan in gold sea cucumber proven have a positive effect on the wound healing, by regulate the activity of TGF-β1 process. TGF-β1 functionates to stimulate fibroblast proliferation which plays a role on wound healing* ***Purpose :*** *The aim of this study was to determine level of TGF-β1 expressionon oral traumatic healing process Wistar rats treated with gel of extract water gold sea cucumber.* ***Materials & Methods:*** *This study using Rattus Novergicus strain Wistar male, aged 3 months, were divided into 4 group. The group are gold sea cucumber extract concentration 20%, 40%, 80% and a negative control group. Ulcer was made with heated burnisher on lower lips of Wistar rats, The level of TGF-β1 was measured with immunohistochemistry and analyze with ANOVA* ***Result:*** *There is significant difference between negative group and treatment group (p<0,05). The most significant difference in group concentration 40% compared to all group (p<0,05) Animals treated with water extract gold sea cucumber showed the highest results regarding the level of TGF-β1 after 4 days, especially in concentration 40%.* ***Discussion:*** *Glycosaminoglycans (GAGs) in gold sea cucumber bind to TGF-β1 in ECM so this growth factor more stable. Poliferation and Migration of fibroblasts mainly triggered by transforming growth factor-β (TGF-β). Omega 3 in gold sea cucumber also can leading to neutrophil clearance and release of anti-inflammatory and reparative cytokines such as TGF-β1.* ***Conclusion:*** *It can be conclude that water extract gold sea cucumber could be used to enhance the oral traumatic healing procces by increasing level of TGF-β1*

Keys words: *Traumatic Ulcer, Healing TGF-β1.Gold sea cucumber*

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BACKGROUND

Ulcers are the most common oral soft tissue lesions, most are caused by simple mechanical trauma. Many are a result of accidental trauma and generally appear in regions that are readily trapped or abraded between the teeth, such as the lower lip, tongue, and buccal mucosa.[1] Ulcer is a lesion eroding epithelial tissue with clear border. Ulcer can happen spontaneously and reccurently. Traumatic ulcer on the mucous membranes of the oral cavity is a clinical appearance of inflammation indicated by an area with exudat and surrounded by connective tissue. Inflammation.[2] Oral health is important to the quality of life of all individuals. Oral lesions can cause discomfort or pain that interferes with mastication, swallowing, and speech, which interfere with daily social activities, so it need a medication to improve the healing process.[3]

Damage to any tissue triggers a cascade of events that leads to rapid repair of the wound. Wound healing is a specific biological process related to the general phenomenon of growth and tissue regeneration. It is characterized by the formation of a granulation tissue consisting of inflammatory cells, newly formed blood vessels and fibroblasts embedded in a loose collageneous extracellular matrix. Re-epithelization, angiogenesis and matrix deposition are critical events controlling this process.[4] Platelets are the first cells recruited at sites of injury, as a result of the coagulation process. Platelets have generally been believed to be important in the wound- healing cascade, both as initiators of coagulation and through the release of growth factors such as platelet- derived growth factor (PDGF) and transforming growth factor b (TGFb) at the site of injury, thus initiating activation of fibroblasts and other mesenchymal cells Numerous studies have shown that macrophages make a variety of cytokines, growth factors and mediators of inflammation that regulate both fibroblasts and microvascular blood vessels, key players in fibrosis and scar formation. TGF-β, a growth factor produced by numerous cells types within wounds, is one of the more potent of the chemoattractants for monocytes and other cell types. Topical application of TGF-β to wounds results in increased inflammation, angiogenesis and fibrosis, with increased matrix deposition.[5]

Traumatic ulcer, furthermore, can be treated with certain medical therapies, namely topical corticosteroid, sodium bicarbonate with water, or mouthwash with antiseptics, such as 0.2% chlorhexidine gluconate or benzydamin hydrochloride. Unfortunetely, the side effect of using chlorhexidine in a long term is discolouration of tooth.[2]

Indonesia is a country with the biggest sea cucumber potential in the world. Empirically gold sea cucumber has been widely used for the treatment wound healing in community.[6] Sea cucumber has been known as poly-anion rich food, due to the presence of glycosaminoglycans (GAGs).[7] Glycosaminoglycan in gold sea cucumber proven have a positive effect on the wound healing process.[2]

Research using sulfated GAGs (glycosaminoglycans) of extract gold sea cucumber showed an increase in contraction through enhanced collagen synthesis.[7]

Many previous researches show that glycosaminoglycans (GAG) sulphate, such as chondroitin sulphate and heparan sulphate, have a positive effect on the wound healing process.[2] Proteoglycans are composed of several glikosamonoglikan can modulate the ability of heparin-binding growth factors, such as vascular endothelial growth factor (VEGF), and FGF. Proteoglycan regulate the activity of TGF-β1 and the preparation of collagen fibrils in type I and III. The release of TGF-β1 led to an increase in collagen synthesis.[8] TGF-β functionates to stimulate fibroblast proliferation which plays a role on wound healing. Many previous researches show that the water extract of gold sea cucumber could increase the number of fibroblast cells with optimal concentration of water extract from gold sea cucumber as much as 40% on the traumatic ulcer of Wistar rats.[2] The process of wound healing will occurs faster.

Another content of gold sea cucumbers that are suspected to have an influence on wound healing is omega 3. EPA and DHA is known can accelerating wound healing procces. In gold sea cucumbers the content of EPA and DHA are relatively high.[6]

Indonesia as the biggest producer should harness the potential of gold sea cucumber. Based on all of the research above this study is aim to determine the level of TGF-β1 expression on oral traumatic healing process Wistar rats treated with gel of extract water gold sea cucumber.

MATERIAL AND METHODS

All experiments were approved by the Faculty of dentistry Animal Care Committee and performed in accordance with the guidelines of the Airlangga Council on Animal Care**.** Extract water of gold sea cucumber was made using freeze dried method. Water extract gold sea cucumber gel 80%, 40% and 20% (m/v) was made with diluted of water extract gold sea cucumber powder in PEG 400:4000 base. PEG base was added little by little until it reaches the desired concentration. For concentration 20%, 20 gram of powder mix with 100 ml PEG base.[9]

Twenty Wistar rats male, weighing 200-300 g and aged 3 months, were divided into 4 group.namely group 1 which given water extract gold sea cucumber gel concentration 80%, group II which given water extract gold sea cucumber gel concentration 40%, group 3 given water extract gold sea cucumber gel concentration 20%, and group 4 as negative control which were not given any treatment.

Ulcer was made but first Rats was anastized by inhalation anesthesia. Wistar rat's lower lip mucous was wounded by no 4 burnisher with 2 mm diameter that had been heated for 1 minute, and it was then touched to Wistar rat's lip mucous for 1 second.

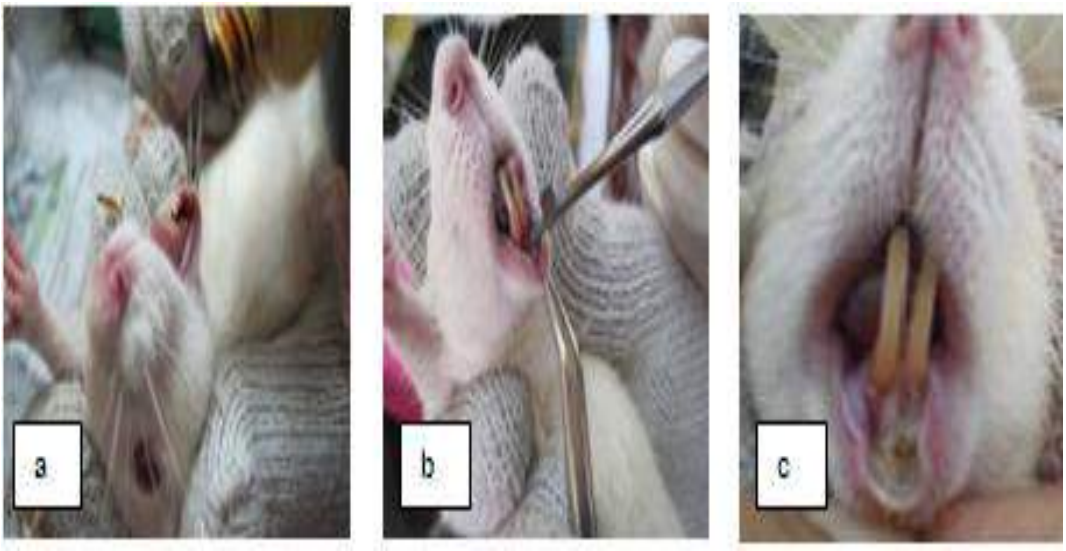

Figure 1. a). Lower lip mucosa before injured with a burnisher was smeared using clorhexidine digluconate 0.12%. (B). Lower lip mucosa injured with a burnisher No. 4. That has been heated. (C). traumatic ulcers Wistar rats appeared, on day 3 after injured.

The ulcer first seen on day 3 after injury. Water extract gold sea cucumber gel were applied into the ulcer on day 3 when the ulcer first time can be observed apply once a day. The preparation of immunohistochemical preparations begins by cutting the lower lip mucosa of rats were sacrificed on day 4, by including the normal tissue of rats. Then proceed with paraffin method, after that lips tissue was stained with antibody secunder TGF-β1.[10]

TGF-β1 expression was count with modification of Brandacher methods. Histometric using Olympus CX-22 and optilab program, magnificent at 400x. Slide were divided into 3 field of view and score using proportion score multiplied with intensity score technic. Proportion score (1(≤25%), 2 (26%-50%), 3 (51%-74%), and 4 (≥75%), of the cells were detectable) and intensity score (1 = no staining/background of negative controls; 2 = weak staining detectable above background; 3 = moderate staining; 4 = intense staining). [11] The measuring result were analyzed using ANOVA test. It analyzed the comparison between water extract gold sea cucumber gel tretead group and negative control group ($p<0,05$).

RESULT

The effect of water extract gold sea cucumber histopathology shown on figure 2, which in group two or 40% concentration showing the most intensive staining with immunohistochemestry.

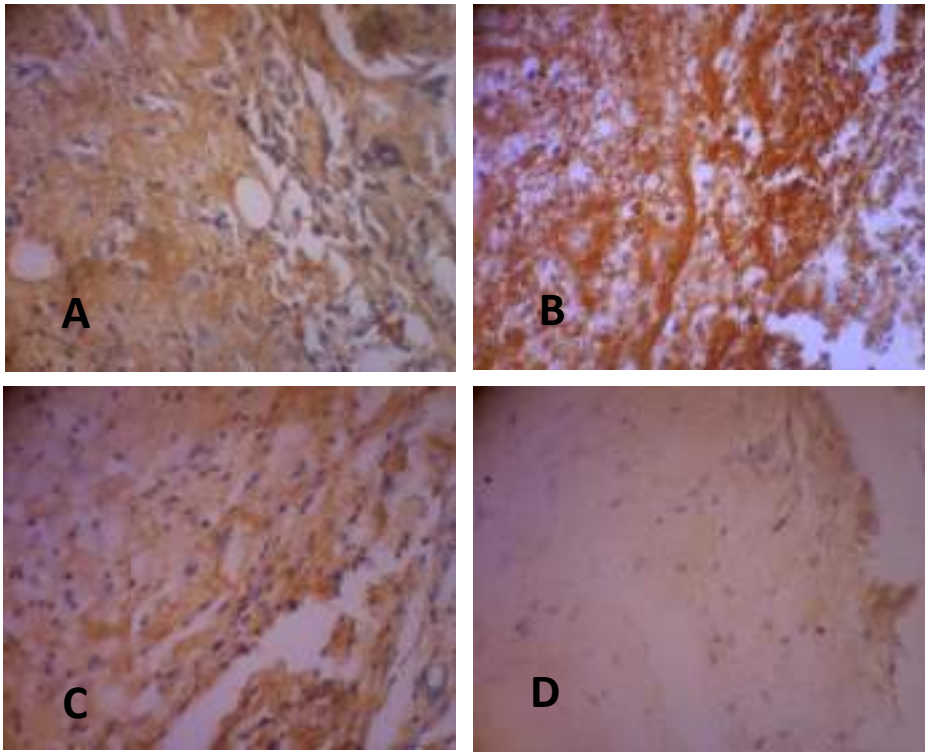

Figure 2. A. Immunohistochemestry image of group 1 with treatment water extract gold sea cucumber concentration 80%. B. Immunohistochemestry image of group 2 with treatment water extract gold sea cucumber concentration 40%. Showing the most intensive colour. C. Immunohistochemestry image of group 3 with treatment water extract gold sea cucumber concentration 20%. D. Immunohistochemestry image of group 4 with no treatment or negative control.

Table 1. Median and mode score TGF-β1 between groups

	Concentration 80%	Concentration 40%	Concentration 20%	Negative control
Median	4	6	6	2
Mode	4	6	4	2

Result of experimental study using twenty wistar rats with water extract gold sea cucumber shown in table 1. It shown that the best result on group concentration 40% which median score and mode score 6.

Table 1 shows us median and mode score, because it shows the median value of the center then it shows the distribution of scores is highest in the group of 40%, the next in the group, 80%, 20% and the negative control group. Meanwhile mode shows the value score that often appear in groups, the highest score that appears most frequently is on the group concentration of 40%. it can be conclude the best scoring on group concentration of 40%. Distribution of scoring can also be seen in diagram figure 3.

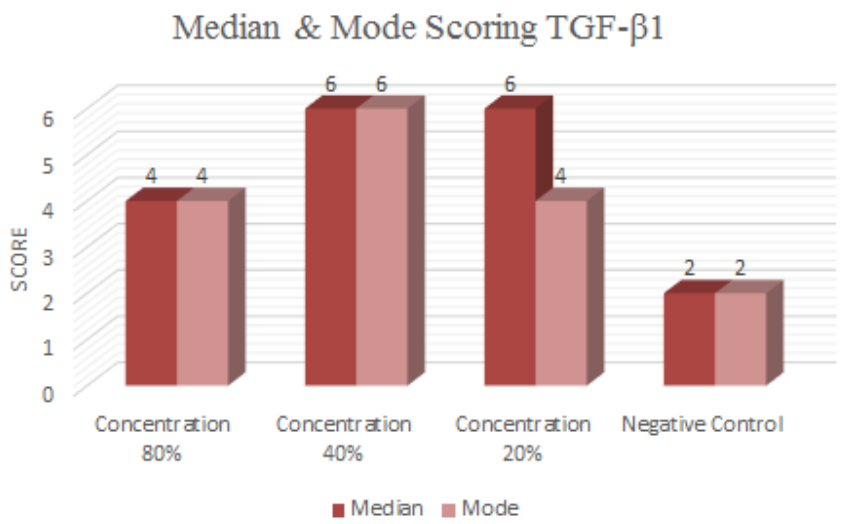

Figure 3. Diagram of median and mode scoring TGF-β1

The data was collected and analyzed using Shapiro-wilk statatistical test because sample test less then 50. The analyze showed distribution not normal ($p<0.05$) which not fulfill the requirement of parametric test, so analyze continue with non parametric test kruskal wallis. Test showed there were significant difference ($p<0.05$) between groups. Analyzed continue with mann whitney shown that in group gold sea cucumber and negative control there are significant difference ($p<0,05$), and the comparation between treatment group concentration 80% and 40% there is significant difference, also in group; concentration 40% and 20% showing significant difference ($p<0,05$), mean while the comparation between group concentration 80% and 20% not to significant ($p>0,05$) (table 2).

Table 2. Mann whitney result between group

	Concentration 40%	**Concentration 20%**	**Negative Control**
Concentration 80%	0.005*	0.828	0.00*
Concentration 40%		0.018*	0.00*
Concentration 20%			0.001*

Table 2 shown that group concentration 40% has the most significant difference. Among all group, group concentration 40% shown the best result in increasing level of TGF-β1 (figure 3). From this analysis provable that group concentration 40% is the most effective treatment in increasing level of TGF-β1 in all group.

DISCUSSION

In the past several decades, we have learned a great deal about the biochemistry, molecular biology, and

cell physiology of the events that lead to wound healing. The physiologic mechanisms of wound healing are similar with slight variations in all tissue.[12] Oral mucosa is covered with stratified squamous epithelium, and the connective underneath the epithelium consists of fibroblasts, collagens and capillaries. The process of wound healing devide into four phase which are hemostasis, inflammation, cell proliferation and remodeling. The first phase of hemostasis begins immediately after wounding, fibrin clot formation was perform in this phase. The inflammatory phase is characterized by the sequential infiltration of neutrophil, macrophages, and lymphocytes. The proliferation phase overlaps with the inflammatory phase, and is characterized by epithelial proliferation and migration over the provisional matrix within the wound, vascularization, collagen synthesis and extracellular matrix formation. The remodeling phase involve collagen remodeling, vascular maturation and regression.[13]

Collagen is synthesized primarily by fibroblasts, beginning 3 to 5 days after the injury. Finally, there is a balance between the rates of collagen production and collagen destruction by collagenase. Age, tension, pressure, stress, and TGF-β affect the rate of collagen production. [12]. That's why this research held on day 4 after injury.

TGF-β1 is secreted in a latent form, bound to a latent TGF- β1-binding protein-1 (LTBP-1), and can be activated by plasmin and modulated by fibromodulin. The regulation of TGF- β1 activation may also play a role in modulating repair.[14] Poliferation and Migration and proliferation of fibroblasts mainly triggered by transforming growth factor-β (TGF-β) and FGF produced by macrophages.[15]

Glysomaninoglycans (GAGs) in gold sea cucumber which are sometime known as mucopolysaccharides are large complex carbohydrate molecules that interact with wide range of protein involved in physiological and pathological processes.[7] Glycosaminoglycans, linear carbohydrates such as heparan sulfate and hyaluronan, participate in a variety of biological processes including cell-matrix interactions and activation of chemokines, enzymes and growth factors.[16] There two types of GAGs, sulfated and non-sulfated GAGs, sulfated Gags include Chondroitin sulfate, dermatan sulfate, keratan sulfate, heparan sulfate, and heparin.[7] There are a variety of growth factors in the ECM, including members beta (TGF-β) superfamily, and vascular endothelial growth factor (VEGF). These growth factors stimulate cells and have been shown to improve wound healing. However, they are unstable in solution. Many growth factors, including members of the TGF- β superfamily, can bind to GAGs in the ECM (extracellular matrix) where they are protected and localized.[17]

This research proved that gold sea cucumber can accelerated TGF-β1 especially on concentration 40% which show the highest median and mode scoring in expression TGF-β1, which also shows the significant difference between group. TGF-β1 expression increase possible because GAGs contents in gold sea cucumber. Another research prooven using gold sea cucumber can accelerated wound healing in traumatic ulcers with rapid

wound closure by decrease of ulcer diameter.[18]

Pharmacological modulation of TGFβ signaling pathways that results in decreased influx of monocytes into wounds might thus be a valuable approach to reducing fibrosis and promoting basic repair mechanisms [5]. Modulation of TGF-β1 will improve traumatic ulcers wound healing. Gold sea cucumber with GAGs contents, bind to TGF-β1 in ECM so this growth factor more stable.

The contents omega 3 on gold sea cucumber also has inffluance in TGF-β1. Omega-3 polyunsaturated fatty acids, of resolvins and protectins, which critically shorten the period of neutrophil infiltration by initiating apoptosis. Consequently, apoptotic neutrophils undergo phagocytosis by macrophages, leading to neutrophil clearance and release of anti-inflammatory and reparative cytokines such as TGF-β1.[19] Shorten period of neutrophil mean shorten the inflammation. The tissue damage because inflammation can be avoided and the next phase proliferation can run and wound healing can occur more faster.

CONCLUSION

Based on the research results revealed that the treatment using water extract gold sea cucumber gel shown an increase TGF-β1 expression in traumatic ulcer healing process , where most effective improvement is achieved at concentration 40% . It can be conclude that water extract gold sea cucumber could be used to enhance the oral traumatic healing procces by increasing level of TGF-B1

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