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The Effect of Sticopus Hermanii-Hyperbaric Oxygen Therapy to Inflammatory Response of Diabetic Periodontitis

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Abstract. Periodontitis is a major risk factor in the oral cavity in chronic diabetes mellitus. The level of IL-1β as inflammatory cytokine increased in patients with type 2 diabetes and periodontitis, while IL-10 is an anti-inflammatory cytokine, may mediate periodontitis in diabetes. Stichopus hermanii (SH) have been known to have anti-inflammation property. Hyperbaric Oxygen Therapy (HBOT) thas been used as adjuvant therapy in diabetes wound healing. **Purpose**: To examine the effect of combination SH and HBOT to the expression of inflammatory response of IL-1β and IL-10 on diabetic periodontitis rats. **Methods** :Thirty male Wistar rats were divided to normal (K-0), diabetic-periodontitis (K-1), and treatment group of SH (K-2), HBOT (K-3), and SH-HBOT (K-4).Experimental diabetic was induced by single dose 65 mg/kg of BW intraperitoneal. The Sticopus hermanii gel 3% by topical application in sulcus gingiva and HBOT 2,4 ATA 3x30 minutes interval 5 minute for 7 days. After 52 days the animals were decapitated and the expression of IL-1β and IL-10 were examined by immunohistochemistry. Data were analyzed with ANOVA and LSD. **Results** : The expression of IL-1β were raised in K-1 group (10.68±1.50) ($p< 0.05$), while IL-10 were not raised (8.00 ±1.95) compare to normal group (7.25 ± 0.85) ($p>0.05$). Treatment with SH in K-2 group resulted in decreased of IL-1β expression (10.00± 1.09) while the expression of IL-10 were raised (8.33 ± 2.60) compare to K-1 (7.25 ± 0.85) ($p<0.05$). Treatment with HBOT in K-3 group resulted in decreased of IL-1β expression (8.333±0,81) while the expression of IL-10 were raised (10.67±1.03) compare to K-1 ($p<0.05$). Treatment with SH-HBOT in K-4 group showed the most decreasing expression of IL-1β (6.50±2.44) and increasing expression of IL-10 (14.83±1.47) compare to K-1 ($p<0.05$). **Conclusions** : Combination of SH-HBOT decreased the expression of IL-1β and increased the expression of IL-10 on diabetic periodontitis diabetes rats

Keywords : IL-1β**,** IL-10, Sticophus hermanii, hyperbaric oxygen, diabetic-periodontitis

1. Introduction

Periodontitis is a chronic inflammation in supporting tissue of gingival with some characteristics like the inflammation in gingival supporting tissues, formation of periodontal pockets, loss of connective tissue attachment, alveolar bone desorption, and even loose of tooth [1,2,3]. The main cause is plaque bacteria which is the mix of negative gram bacteria and their progression result from host response to the occurrence of periodontal tissue inflammation [1,2,4]. Diabetes is a disease caused by metabolic disorders due to insulin insufficiency or insulin resistance which is resulting in hyperglycemia. Diabetes is a health problem faced all over the world with a high mortality rate [1,5]. The prevalence in 2025 is estimated to increase around 7.3% in diabetics worldwide. Metabolic disorders alter cellular micro-environment, caused undesirable effects such as atherosclerosis and periodontitis [1,3]. Inflammation is a central feature of periodontitis and diabetes which has a mutually influencing relationship [1,2]. Inflammatory cells involved in macrophages, lymphocytes, neutrophils, eosinophils and dendritic cells [3]. Proinflammatory cytokines IL-1-β, IL-6, TNF-α increase in diabetics and patient with periodontitis [3,4,5]. Inflammatory cytokines triggers vascular permeability of blood vessels and migration of leukocytes to the endothelium, which then changes the vascularization response and facilitates thrombus formation that lead to formation of thrombus by inducing procoagulant activity and inhibit anticoagulants [3,6]. Chen reported that periodontitis and gingivitis

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increases thromboxane B2 and 6-keto-prostaglandin F1a. It is related with an increase in the number of inflammatory cells and vascular endothelial cells that will create inflammatory cytokines and then will cause in periodontal tissue damage directly or indirectly [3,7,8]. In diabetics, there is an increase in serum C-reactive protein (CRP), IL-6, IL-1β, TNF-α and fibrinogen. Hyperglycemia will increase oxidative stress, intracellular Ca, mitochondrial dysfunction, changes in fatty acid metabolism, mitogen activation, and phosporilation proteinkinase damage. Another study on cells reported that hyperglycemia triggers activation of NF-kB, COX-2 expression, increase production of PGE2 and apoptosis [6,7,8]. Meanwhile, therapy for periodontitis will cause the increase of anti-inflammatory cytokines IL-4, IL-10, IL-12 and IFN-γ which plays a role in inhibiting periodontal tissue damage [4].

Golden sea cucumber (Stichopus hermanii), a holothuroidea class, is an invertebrate marine biota that is found in deep sea waters throughout the world [8]. Stichopus hermanii has high nutrition such as vitamin A, B, minerals Ca, Mg, Fe, Zc and contains bioactive ingredients such as saponins, glycosaminoglycan, chondroitin sulfate, sterols, lectins, peptides, glycoproteins and essential fatty acids EPA and DHA. Stichopus hermanii is also able to act as anti-angiogenic, anticoagulant, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, anti-hypertension, anticancer, antitumor and wound healing [8,9].

Hyperbaric Oxygen Therapy is a therapy by breathing using 100% pure oxygen in a high pressure room of more than 1 ATA (absolute atmosphere) [10,11]. The use of HBO doses of 2.4 ATA 3x30 minutes at 5 minute intervals for 7 consecutive days in diabetic rats able to reduce blood sugar levels compared to day 1, day 3 and day 5 [10]. Hyperbaric oxygen in the treatment of diabetics has been shown to improve hypoxic tissue, regulate blood sugar levels, increase blood vessel growth and nerve endings, so that it can speed up the healing process of wounds. A studied is proved that administration of high pressure oxygenation at a dose of 2.4 ATA 100% O_2 3x30 minutes with a 5 minute interval inhaling ordinary air which was carried out for 5 consecutive days was able to increase the number of osteoblasts in alveolar bone mice induced by Porphyromonas gingivalis with diabetes mellitus [11, 12].

2. Experimental Method

This research is an experimental laboratory research with post test only control group design. Thirty male wistar rats aged 12-16 weeks were divided equally into 5 groups. Experimental diabetics was induced by single dose 65 mg / kg of intraperitoneally, after administration of nicotinamide 230 mg / kg 15 minutes previously. Overnight after the induction, the animal were given 10% dextrose to avoid sudden hypoglycemic post injection. Diabetics condition was stated when blood glucose levels reach 230 mg / dL [13]. Periodontitis conditions was performed by administration 2 ml bacterial suspension of *Porphyromonas gingivalis* ATCC 33277 in PBS (10^9 CFU / ml) via nasogastric tube, swabbed in buccal / labial-palatal gingiva along molar to molar region and anal region. The application of *P. gingivalis* were given 3 times in 4 days.

Treatment agent *Sticophus hermanii* (SH) were prepared from the freshly body cuts of golden sea cucumber *Sticophus hermanii* freeze dried at a temperature of -80ºC with a pressure of 5 Torr, crushed into powder with regular blender, mixed to be 3% gel by dissolving with CMC-Na 2%. Hyperbaric oxygen therapy (HBOT) were performed in hyperbaric animal chamber of 2.4 ATA

Control groups were K0 (normal healthy) and K1 groups (diabetic periodontitis, no treatment) Treatment groups were given topical application of 3% *Sticophus hermanii* (SH) gel in the molar region of gingival sulcus for 7 consecutive days (K2 group), given HBOT in 2.4 ATA Hyperbaric Oxygen 3x30 minutes, each with 5 minute interval, for 7 days (K3 groups) and given the combination of SH dan HBO treatment (K4 groups) [15]. Rats were then terminated and mandibular section was performed immunohistochemistry staining for the exmanination of IL-1β and IL-10 expression. Data were analyzed by Anova and LSD test.

3. Results

The data obtained were analyzed using the one way ANOVA statistical test with a significant level of 95% ($p < 0.05$) and processed with IBM SPSS version 20.

Table 1. Mean and standard deviation expression of IL-1β and IL-10

Group	IL-1 β	IL-10
	X ± SD	X ± SD
K0	3.00 ± 0.89	7.25 ± 0.85
K1	10.68 ± 1.50	4.5 ± 0.96
K2	10.00 ± 1.09	8.33 ± 1.04
K3	8.34 ± 0.82	7.5 ± 0.96
K4	6.50 ± 2.44	11.5 ± 1 .04

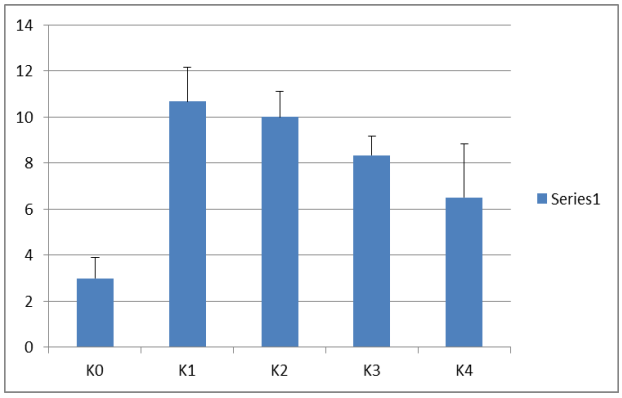

Figure 1. Bar Chart the expression of IL-1β in each group on the ligament Periodontal

Table 2. Tests of One Way Anova Expression of IL-1β

Variabel	*Sig.*
Ekspresi IL-1β	.000

Table 3. Test Results of IL-1β LSD Post-Hoc in each group

Group	K0	K1	K2	K3	K4
K0		0.000*	0.000*	0.000*	0.000*
K1			0.432	0.010*	0.000*
K2				0.057	0.000*
K3					0.037*

Description: *P<0.05 (there are significance differences)

Based on Table 3. there was a significant decrease in IL-1β in the K2, K3, K4 groups compared to the K1 group but the decrease was not the same as K0.

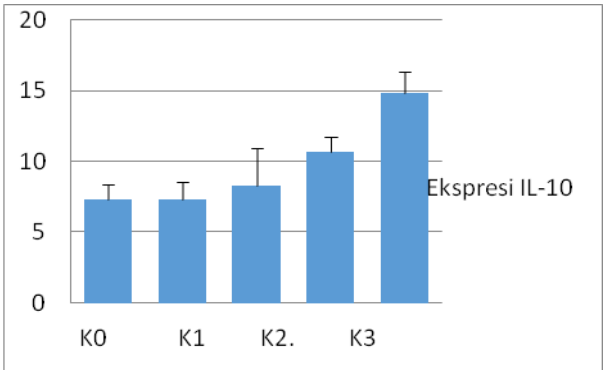

Figure 2. Bar Chart The expression of IL-10 in each group on the ligament Periodontal

Table 4. One-way Annova test results on IL-10 expression

Variabel	Nilai *Sig.*
Ekspresi IL-10	0.000

Table 5. Result of *Post Hoc LSD on* IL-10 expression

	K-0	K1	K2	K3	K4
K0		**1.000**	**0.283**	**0.001***	**0.000***
K1			**0.283**	**0.001***	**0.000***
K2				**0.017***	**0.000***
K3					**0.000***
K4					

Description: * p <0.05 (there are significant differences)

Based on Table 5, there was a significant increase in IL-10 expression in the K3, K4 groups compared to the K1 group. While K2 does not have a significant increase in IL-10 expression compared to K1 group.

4. Discussion

Interleukin-1β (IL-1β) is a cytokine pro-inflammatory which is induced during the course of an inflammation response and is associated with its onset and progression periodontitis and diabetes. Interleukin-10 (IL-10) is an anti-inflammatory cytokines block inflammation or at least suppress the intensity of the cascade and suppress the production of IL-1 β, TNF, chemokines. In periodontal disease, the balance between pro- and anti-inflammation is directed towards proinflammatory activity. IL-1β and PGE2 are important mediators in the periodontal inflammation and stimulate bone loss. [3, 5,14]. Interleukin-10 is a regulatory cytokine, which on the one hand limits inflammatory responses by inhibiting the expression IL-1β and upregulates the recruitment and activation of B cells. [3, 4, 5,14]. It down regulates the T helper1 response, and by controlling the B-cell lesion. It is suggested to play a role in controlling the progression of periodontal disease. They are secreted by a variety of cell types comprising monocytes, macrophages, dentritic cells, epithelial cells, keratinocytes and fibroblasts. [5,14].

The condition of periodontitis with diabetes in this study showed an increase in IL-1β expression and decreased expression of IL-10 compared to the group of healthy rats. This is in lined with previous researchers [1,2,5]. In general, the classic mechanism of diabetes and microvascular complications of diabetes is the same as that which occurs in periodontitis. The presence of hyperglycemia results in an increase in AGEs in the blood which triggers an increase in ROS, resulting in elevated levels of IL-1β, TNF-α in monocytes and macrophages [3]. Lipolisaccharide in bacteria will be released into the tissues and result in increased ROS which causes oxidative stress in

the body which triggers an increase in inflammatory cytokines which activate NF-kB and subsequently induce secretion of inflammatory cytokines and hyperglycemia.[1,4]. AGEs and insulin move from the cytoplasm to the nucleus to activate gene transcription by NF-kB [16,17]. Hyperglycemia can trigger NF-kB activation and expression of COX-2, PGE2 which is an inflammatory marker. Host cells produce ROS as a mechanism to fight intracellular microorganisms and as a signal mediator in cells for various cellular functions. However, persistent ROS production results in the induction of oxidative stress and can trigger an inflammatory response, interfering with tissue homeostasis sequentially and cell death. The accumulation of AGEs also affects cell adherence to the extracellular matrix and interactions between matrices and increased oxidative stress, changes in endothelial cell function, and increased activity of matrix metoproteinases [2,5]. In humans, elevated serum AGEs levels are associated with the severity of type2 diabetes periodontitis [16]. In models of diabetic mice, there was an increase in expression of receptors for advanced glycation end-product (RAGE), and therapy with soluble RAGE reduced inflammatory cytokines and inhibited alveolar bone loss.

Sticopus hermanii contains the active ingredient Saponin which functions as an anti-inflammatory, which inhibits the growth of bacteria by interacting with bacterial cells so that the bacteria will be damaged or lysed. The content of PUFA (Polyunsaturated fatty acids) in SH is unsaturated fatty acids as a source of energy, antioxidants, membrane formers and mediators of cell signal transmission. EPA (Eicosapentaenoate) and DHA (Docosahexanoate) are PUFAs that can cause PMNs, macrophages, produce ROS, and increase antimicrobial activity and play a role in the immune system that can block formation prostaglandin and inhibit proinflammatory cytokines IL-1β and IL-6, and TNF-α [9]. Sticopus hermanii possesses potent anti-inflammatory activity and as a source of potent therapeutic agents for the treatment of inflammation. Sticopus Hermanii also have a valuable nutrients such as Vitamin A, Vitamin B1, Vitamin B2, Vitamin B3, and minerals, especially calcium, magnesium, iron and zinc. Total phenolics and total flavonoids contents as antioxidant activity .[17].

Hyperbaric oxygen therapy 2.4 ATA 3x30 minutes for 7 days with a 5 minute interval inhaling environmental air can increase the amount of dissolved oxygen in the plasma, so a condition is reached where oxygen demand can be met from dissolved oxygen without using oxygen bound to hemoglobin. [10,11]. Oxygen in the dissolved form of plasma is more easily consumed by tissues through direct diffusion than oxygen bound to hemoglobin. Hyperbaric oxygen therapy can increase ROS in mitochondria, this is followed by the body's response, especially the liver, to form HSp 70 as cell protection from oxidative stress [19]. Hsp 70 as a ligand will repair insulin receptors that experience high free radical damage, as the carrier will increase anti-tumor immunity and the secretion of proinflammatory cytokines (TNF-∝) from monocytes. This secretion will stimulate anti-inflammatory factors resulting in insulin repair. [20, 21].

5. Conclusion

Combination of doses of 2.4 ATA 100% O_2 3x30 minutes with 5 minute intervals for 7 days and sea cucumber gel (Stichopus hermanii) concentration of 3% and a combination of both therapies showed a decreased the expression of IL-1β and an increased the expression of IL-10 significantly in periodontitis diabetes rats

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