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Bandung, 11 - 12 Nov 2016
Holiday Inn Pasteur

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PDGI-FDI CONTINUING EDUCATION 2017

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PREFACE

FDI Continuing Dental Education is an international forum for worldwide dentists t gather, share and exchange the latests science in dentistry. The speaker in this forum will present the update information in dental science. This FDI Continuing Dental Education is an excellence program in developing dental science. Threfore, this program has become our priority and need especially for dentist to develop and increase our knowledge.

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CONTENT

The Effect Of Combination Sticopus Hermaniiand Hyperbaric Oxygen On Osteoblast And Osteoclast In Periodontal Tissue Diabetic Rat

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ABSTRACT

Objective : To examine the effect Combination *Sticopus hermanii* and Hyperbaric Oxygen Therapy 2,4 ATA on osteoblast and osteoclast in periodontal tissues diabetic rats. **Materials and methods** : A total of 45 male Wistar rats were equally divided in healthy controls (group-1), diabetic + periodontitis (group-2), diabetic -periodontitis + SH (group-3), diabetic-periodontis + HBOT (group-4), and diabetic -periodontitis+SH+HBOT (group-5). Experimental diabetic was induced by single dose 65 mg/kg of BW intraperitoneal. The SH gel 3% by topical application gave in sulcus gingiva for 7 days. HBOT 2,4 ATA 3x30 minutes interval 5 minute for 7 days and the animals were decapitated. The measurement osteoblast, osteoclast after 50 days was examined using histopathology method.**Results** :It was found that combination SH and HBOT in osteoblast can increased significantly p=0,003 (28.47 ± 3.20) after 7 sessions HBOT than DM-periodontitis (9.22 ± 4,95) and osteoclast (3,50 ± ,957) decreased significantly p=0,000 than without DM-periodontitis (5,00±0,816) can increased. **Conclusions** : The combination of *Sticopus hermanii* and HBOT can increase osteoblast, reduces osteoclast in hyperglycemia periodontal tissue diabetic rat.

Keywords : *osteoblast, osteoclast, diabetic, hyperbaric oxygen*

INTRODUCTION

Periodontal disease is second most common oral disease after dental caries in Indonesia with the prevalence of 96.58% in all stage of ages. In hyperglycemia condition, the susceptibility of periodontitis increased three times. Diabetes is the sixth complication disease and the most common in oral cavity. [1] The severity and prevalence of periodontal disease in diabetic patient related to the increasing of blood glucose level and cause *advanced glycation end* products (AGEs), lead to the increase of IL-1β and TNF-α.[2,3] The increasing level of lypopolysaccaride and TNF-α also lead to insulin resistance and the development of diabetes.[3,4] Management of periodontitis with diabetes could be pointed at local factors but systemic factor should be considered as the control of blood glucose level. Proper treatment of periodontitis potentially raised the general condition of the patient.[5] Local periodontal therapy as scaling and root planning histologically do not showed the attachment of new connective tissue[6] thus adjuvant therapy should be considered in management of periodontitis with diabetes .

Sea cucumber is marine biota with medical benefit bioactive compound and has been used as traditional food and folk medicine since ancient time. Golden sea cucumber *(Stichopus hermanii)* has been consumed as food rich with content of *polyanion* related to compound of sulfated gycosaminoglycans (GAGs) which affect physiologic activity function including wound healing.[7] Several studies stated that sulfated GAGs i.e chondroitinsulphate and heparin sulphate has the important role in wound healing.[8] *Stichopus hermanii* alsocontain protein, vitamin C, flavonoid, Superoxide Dismutase(*SOD*), *and kolagen which* have the influence in inflammation phase of healing.

Hyperbaric oxygen therapy (HBOT) is a breath therapy using 100% pure oxygen in a igh pressure chamber more than 1 ATA (Atmosphere Absolute).[9] Our previous study stated that HBOT in periodontal disease resulted in healing effect by the decreasing level of malondialdehyde (MDA) in periodontitis with diabetes mellitus.[10] Another research in human osteoblast culture were compared the control group without therapy with the group received HBOT. Result showed the number of osteoblast in group received HBOT 2,4 ATA for 30 minute on the sixth day after exposure (2000-2500 cells) were higher than control group without therapy (1500-2000 cells).[11]

Osteoblast are fibroblast undergo modification and same as fibroblast originated from mesenchymal cells.[12] Alveolar bone loss in periodontitis caused by the increasing of bone resorption by osteoclast or the decreasing bone formation by osteoblast or both of those. Osteoblast has the role on collagen synthesis to form osteoid as basic bone material.[12]

Osteoclast is big motile branch cells with multiple nucleus.[13] In the area of bone resorption osteoclast reside in the crypt formed by enzyme on the matrix known as lakuna howship. Osteoclast caused the excessive resorption on the bone. [1,5,13]

This study aimed to examine the effect of combination of *Sticophus hermanii* and HBOT to osteoblast and osteoclast of alveolar periodontitis rat induced by *Porphyromonas gingivalis* with diabetes mellitus with the dose of 2,4 ATA 100% O_2 3x30 minute and 5 minute interval for 7 days. The dose was refered by the previous research of the same treatment resulted in the decreasing of blood glucose level effectively compared to 10 days of treatment.[15]

MATERIAL AND METHOD

This study is true experimental with post test only control group design. Thirty male wistar rats age 8 weeks were randomly arrange into 5 groups. K0 were normal control group while K1-K4 was periodontitis and diabetes rats.

Diabetes condition was induced by streptozotocin 50 mg/ kg BB single dose gingiva along molar to molar regio and anal regio. The administration of P. gingivalis were done 3 times in 4 days.[17] After 2 weeks periodontitis condition was achieved and were started to get the treatment.

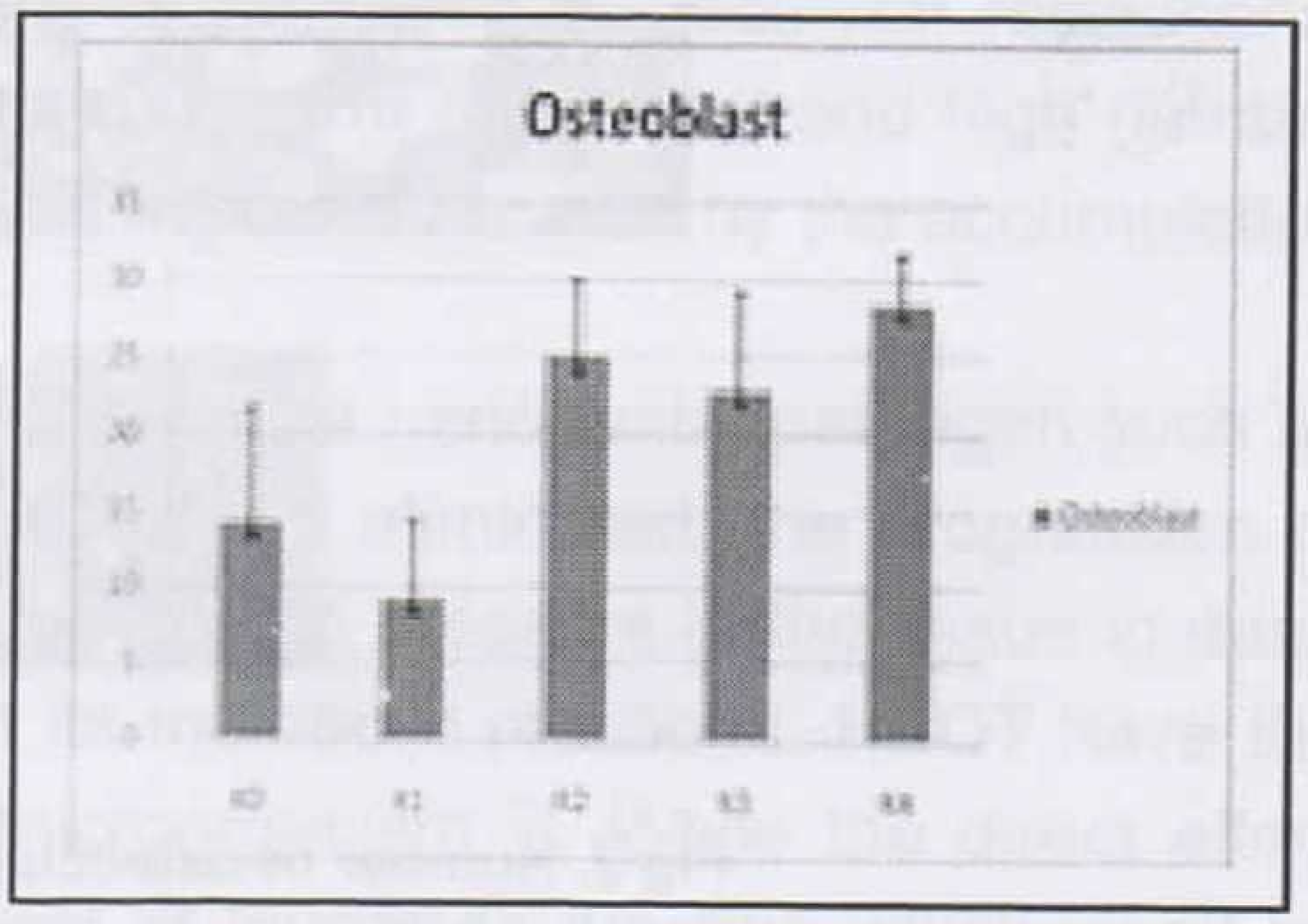
K1 group received no treatment as positive control, K2 were given HBOT 2,4 ATA 3x30 minute and 5 minute interval for 7 days, K3 were given *Sticophus hermanii* gel on gingival sulcus for 5 days, K4 were given combination of HBOT and *Sticophus hermanii*. After treatment done, rat was sacrificed and mandibular section was performed hematoxylin eosin staining. Data were analyzed by Anova and LSD.

RESULT

Result on osteoblast examination showed the decreasing amount in K1 group compare to normal. All treatment in group K2-K4 raised the number of osteoblast significantly but the highest number was on group K4 ($p<0.05$) which receive the combination of *Sticophus hermanii* and HBO. Result on osteoclast examination showed the increasing amount in K1 group compare to normal. All treatment in group K2-K4 decreased the number of osteoblast significantly but the lowest number was on group K4 ($p<0.05$) which receive the combination of *Sticophus hermanii* and HBOT.

Table 1. Mean of osteoblast number

Group	Mean of Osteoblast
K0	14.12 ± 7.54
K1	9.22 ± 4.95
K2	24.97 ± 4.99
K3	22.80 ± 6.45
K4	28.47 ± 3.20



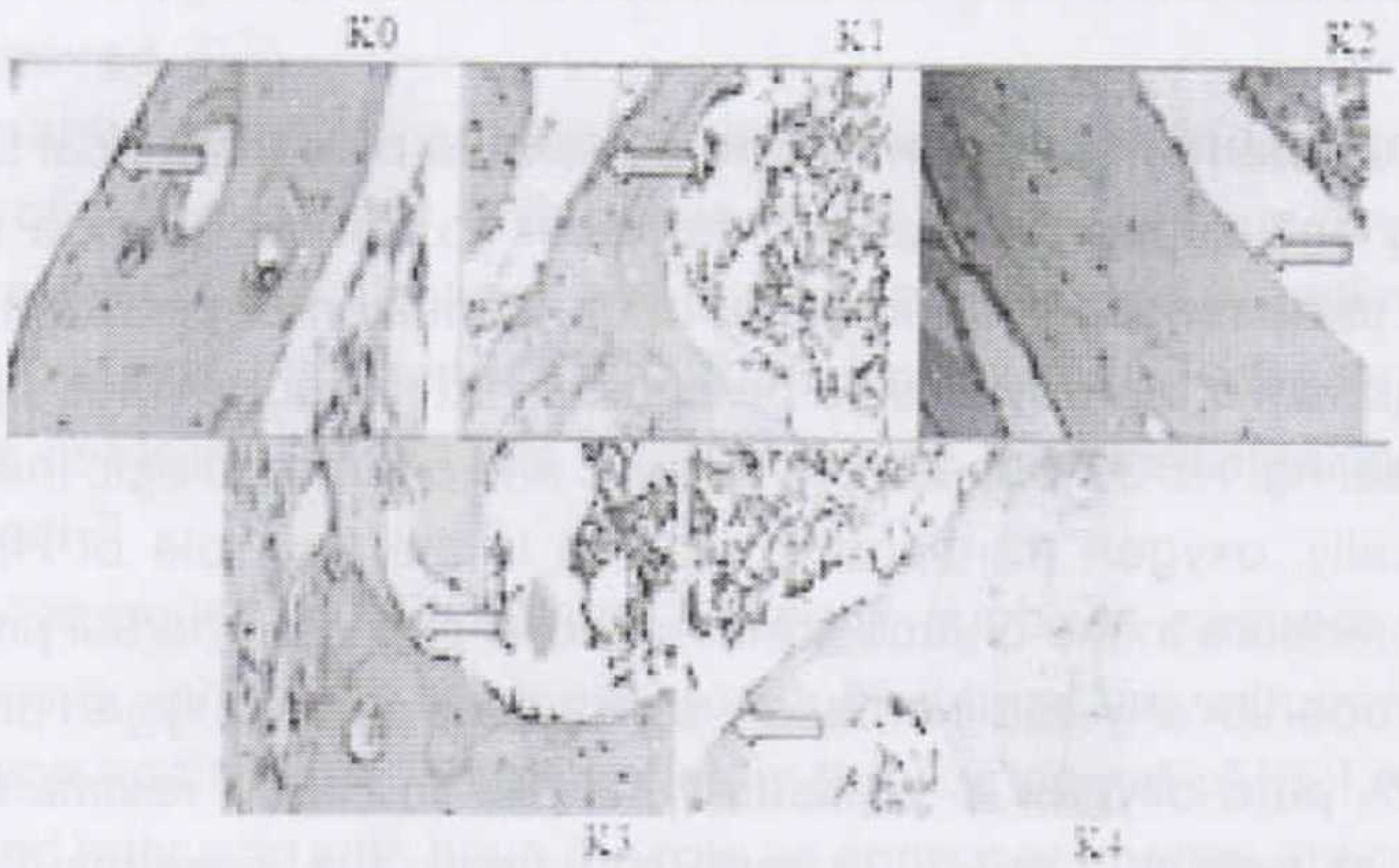
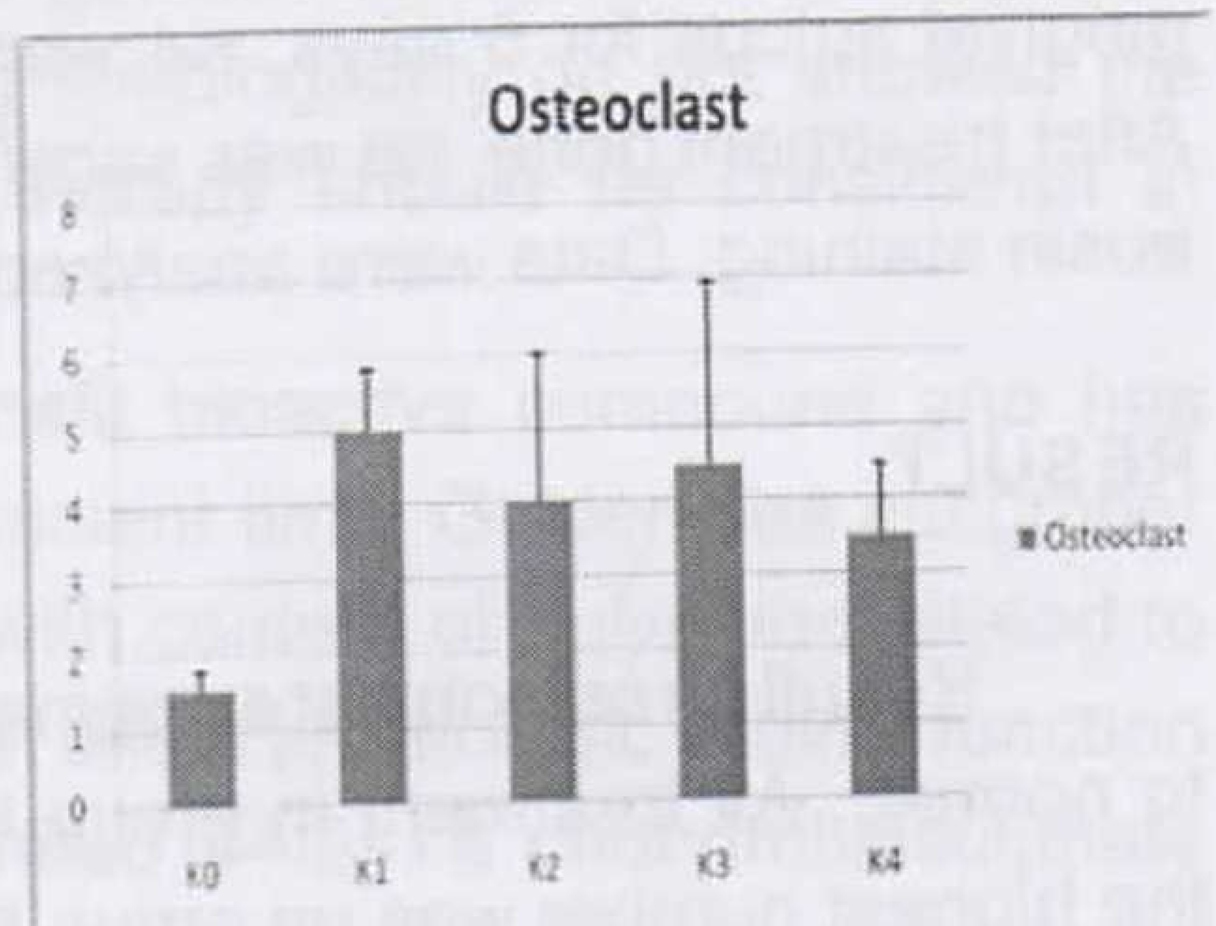


Fig 1. Number of osteoclast on alveolar mandible of all groups

Table 2. Mean of osteoclast number

Group	Mean of Osteoblast
K0	1.50 ± 1.291
K1	5.00 ±.816
K2	4.00 ± 2.062
K3	4.50± 2.449
K4	3.50± .957



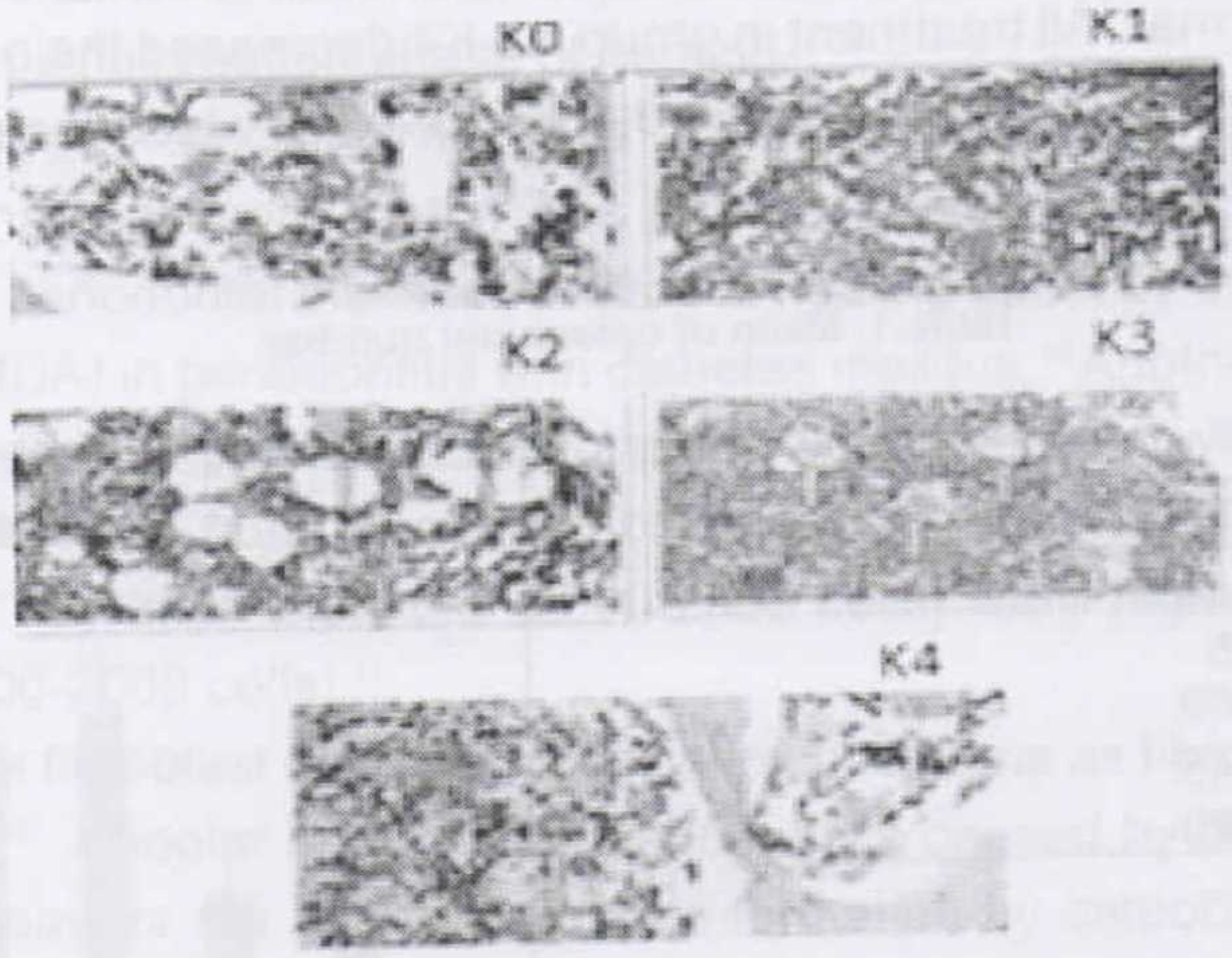


Fig 2. Number of osteoclast on alveolar mandible of all groups

DISCUSSION

Standard treatment of periodontitis are performed by nonsurgical therapy as scaling and root planning and supportive periodontal recall to remove plaque bacterial as main etiologic factor of periodontitis [6]. Concerning to the condition of periodontitis with diabetes mellitus (DM), adjunctive therapy should be considered to control blood glucose level and improve wound healing. HBOT posses' physiologic and pharmacologic therapeutic effects.[18]

Physiologically, oxygen transport in plasma is the key role of HBOT effect in the tissue. When air pressure in the chamber increased, the oxygen partial pressure in alveolar will be enhance proportionally and increase the dissolved partial oxygen pressure in plasma. Inhalation of 100% pure oxygen in pressure 2-3 ATA in HBOT resulted in arterial partial oxygen pressure of sekitar 1200-2000 mmHg, lead to the increasing of subcutaneous oxygen diffusion between 30-50 mmHg compare to regular breathing in pressure of 1 ATA. HBOT increase blood oxygen concentration to regulate various enzymatic reaction and cell respiration in normal tissue.[19]

Therapeutic effect of HBOT affected the modulation of NO production, modify the growth factor and cytokine effect by regulating the level or its receptors, stimulate protein membrane which influence ion formation, enhancing cell proliferation, rapid collagen depositioin, stimulate capillary building and arboriation, antimicrobial effect¸ immune response modulation, provide oxygen supply to the tissue, rapid wound healing, eliminate toxic substance and reduce its effect, swelling reduction in healing[11,19,20].

Result showed the higher increasing amount of osteoblast in K4 group treated by the combination of *Sticophus hermanii* gel and HBOT compare to K2 group treated only with HBOT or K3 group treated by *Sticophus hermanii* gel. Induction with streptozotocin (STZ) lead to accumulation of AGEs resulted in alteration of cell and extracellular matrix component as abnormal function of endothelial cells, capillary formation and vascular proliferation then disturb the vascularization and bone healing resulted in decreasing number of osteoblast [2,4]. AGEs also cause the increasing of inflammatory immune response to periodontal pathogens by the increasing production of proinflammatory cytokine such as IL-1β and TNF-α, showed in gingival crevicular fluid of DM patients [3,4]. TNF-α elicit bone destruction marked by the increasing of osteoklact number. In the other hand, *Porphyromonas gingivalis* trigger the elevation of lipopolysaccharide serum level and TNF-α cause the insulin resistance and development of Diabetes Mellitus. Periodontitis and DM have bidirectional relationship which affect the severity each other. The reduced number of osteoblast and high number of osteoclast in group K1 might be the result of the TNF-α and worsen by the accumulation of AGEs.

HBOT also has its role on antimicrobial effect against periodontal pathogen such as *Porphyromonas gingivalis* Once main bacterial pathogen eliminated, the progression of disease could be terminated. [21] HBOT enhance the oxygen pressure in the tissue in deep level that prevent the growth of microbe by inibit its metabolic reactions. HBOT have the bacteriostatic and bactericidal effect to certain microorganism [22]. While the direct effect of HBOT in bacterial killing is by the enhancement of leucocyte function which won't be happen when tissue oxygen pressure is below 30 mmHg as in infected tissue, the neutrophil activity will be impaired. [21,22]

Collagen matrix formation, angiogenesis and wound healing process depend on the sufficient amount of oxygen supply, in this case HBOT enhance tissue oxygenation. Hypoxia wound healing on HBOT are stronger and faster and resulted in optimum stimulation of fibroblast proliferation and angiogenesis [11,19] Sufficient amount of oxygen stimulate cartilage formation, on the contrary, insufficient oxygen supply lead to failed formation of fibrous tissue by osteoblast [21]

Bioactive compound of *Sticophus hermanii* such as saponin posses the anti-inflammatory property, inhibit the bacterial growth by interaction with bacterial cells lead to bacterial destruction and lysis. [8, 18] It also contain the compound of PUFA (*Polyunsaturated fatty acid*) saturated fatty acid with have the role as source of energy, antioxidant, membrane production, and mediators of cell signal transmission[31] EPA (*Eicosapentaenoate*) dan DHA (*Docosahexanoate*) are PUFA which stimulate PMN, macrophage, produce ROS, enhance antimicrobial activity and has its main role in immune system to prevent prostaglandin formation and inhibit inflammatory cytokine (IL-6, IL-1 dan TNF-). Prostaglandin has its role in osteoclast formation and bone resorption.[8,18]

Treatment with 2,4 ATA HBOT in 3x 30 minute, each 5 minute break proved to press down the osteoclast differentiation by reducing the number of RANKL induced by positive TRAP mononuclear and multilocular osteoclast formed in human monocyte and blood perifer cells so that excessive formation of osteoclast could be prevented.[36] The increasing number of osteoblast and decreasing number of osteoclast by combination therapy of *Sticophus hermanii* gel and HBOT are related with our previous research of the effect HBOT on the reducing of periodontal ligament fibroblast MMP-8 expression in hyperglycemic rats [10]

The decreasing osteoclast number were appeared highest in K4 group treated by the combination of *Sticophus hermanii* gel and HBOT compare to K2 group treated only with HBOT or K3 group treated by *Sticophus hermanii* gel. Statistical analysis result showed that the number of both osteoblast and osteoclast in K0 group as normal control and K4 group which given combination therapy have no significant different ($p>0.05$) it means that the number of osteoclast in both groups are not different. The treatment resulted in the same osteoblast and osteoclast number as in normal periodontal tissue, thus potentially developed as significant adjunctive therapy of periodontitis with diabetes.

CONCLUSION

Combination *Sticopus hermanii* and Hyperbaric Oxygen 2,4 ATA 100% O_2 3x30 minute, 5 minute break interval for 7 consecutive days could increase the number of osteoblast and decreased the number of osteoclast of diabetic rat periodontal tissue

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