Macrophages Analysis on Gingival Tissue of Diabetic Rats after Insulin Leaf Extract Administration

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Abstract

Periodontitis is an infection of periodontal tissue which generally commonly associated with diabetes mellitus. A. actinomycetemcomitans is a major cause of aggressive periodontitis. Macrophages and monocytes are increased in inflammation caused by bacteria. Macrophages are involved in the process of phagocytosis. Insulin leaf(Smallanthus sonchifolia) contains anti-diabetic and anti-microbial activities that can inhibit or kill bacteria.

To determine the effect of insulin leaf extract to decrease the number of macrophages in the gingival tissues of diabetic rats that induced A. actinomycetemcomitans.

This study was laboratory experimental with post-test only. Samples were divided into two groups;treatment group and control group. The control group rats were given alloxan and A. actinomycetemcomitans, while experimental group were given alloxan, A. actinomycetemcomitans, and the insulin leaf extract. Examination of macrophages is done by making histological preparations with Hematoxin-eosin staining.

The mean of number of macophages in control group was 6.04±2.133, while in the treatment group was 2.62±0.517. Statistical analytic using Paired T test showed a significant decrease of the number of macrophages ($p<0.049$).

There was a decrease in the number of macrophages in the gingival tissues of diabetic rats induced by A. actinomycetemcomitans after giving insulin leaf extract.

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Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by high blood glucose levels that occur due to abnormalities of insulin secretion, insulin performance, or both.[1] Glucose unable to be utilized and therefore resulting in elevated blood sugar levels and urinary glucose secretion. In 2003, an estimated 194 million people suffered from DM worldwide, reaching 5.1% of the world's population. This number is expected to increase annualy by 333 million, or 6.3% of the world population by 2025.[2]

Diabetic patient who has uncontrolled high glucose level tend to have high risk of systemic and oral complication such as: periodontal disease (periodontitis & ginginivitis), salivary disfunction which affect; salivary flow reduction; salivary composition changes; and taste dysfuction.[1] DM is widely recognized worldwide but the periodontal disease associated with DM not well known. Individual who has diabetic mellitus have higher risk to suffering periodontal disease because they are suspectible to bacterial infection and the decrease of host's immune ability fight gingival infection. [3]

Periodontitis is a pathologic condition which characterized by inflammed tissue which surround the tooth. This happened progressively and gradually continued until loss of alveolar bone around the tooth, and if not treated immediately will lead to loss of tooth. [4,5,6] The function of some cells that play a role in the inflammatory response such as neutrophils, monocytes, and macrophages changed in DM patient. These cells are the innate immunity. Increased immune-inflammatory responses of DM individuals (i.e macrophages and monocytes)

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increase the production of proinflammatory cytokines and Tumor Necrosis Factor-α (TNF-α) in periodontitis. Uncontrolled DM is capable to increase Advanced Glycation End Products (AGEs) in the periodontal tissue. AGEs increase immuno-inflammatory responses periodontitis. AGEs are an active bond between proteins and excessive glucose levels.[4,5]

Macrophages are precursor cells in haemopoietic organs and the respiratory tract through the blood and lymph nodes. Macrophages play the role of most cells in the process of phagocytosis.[8]

Macrophages are divided into 2 groups based on their function, namely as phagocytes and antigen presenting cells (APC) that serves as antigen against lymphocytes. Macrophages release enzymes and granule content out of cells with cytokines such as TNF to kill pathogens and act as phagocytes to destroy antigens inside phagolysozyme.[8]

Traditional plants for DM therapy were well known such as insulin plant. Insulin plant has been shown to have active chemicals component such as fructooligosacharride and flavonoids that caused decreased glucose level. The insulin leafcontained phenol components. Phenol components such as chlorogenic, caffeic, and ferulic that can improve pancreatic β cells thus increase insulin secretion.[9]

The insulin leaf also contains Smallanthaditepepic acids A, B, C, and D that show edanti-diabetic activities. This suggested insulin leaf can be used as a new hypoglycemia agent. In addition, flavonoids contained in the insulin leaf are also anti-microbial that can inhibit or kill bacteria growth.[10]

The aim of this research was to determine the effect of insulin leaf extract (*Smallanthus sonchifolia)* to the amount of macrophages in gingival tissue of diabetic rat induced *Actinobacillus actinomycetemcomitans.*

Materials and methods

This study had been approved with ethical cleareance from Committee of Ethical Clearance of Health Research, Faculty of Dental Medicine, Universitas Airlangga Indonesia (No. 197/KKEPK.FKG/VIII/2016). This study was done in Biochemistry Laboratorium Faculty of Medicine, Universitas Airlangga, on September until November 2016. Insulin leaf extract was made in Integrated Services Unit(ISU) Materia Medika, Batu, Malang usingmaseration technique.

This study was experimental laboratory using 150-200 gram weight, 3 months-old, male Wistar rat as a sample.The rats were adapted for one week. The control group and treatment group were induced with alloxan. Samples were injected alloxan with dosage of 120 mg/kg BW intraperitoneally 0.3 ml. The blood glucose level of samples were examined on the 3rd day after alloxan injection using Accu Chek® Active Kit (Roche, Indianapolis, IN). Blood was taken from rat's tail.[11]

Elevation of blood glucose level (>200mg/dL) was detected on the 3rd day after alloxan administration. The control group were induced by *A. actinomycetemcomitans* bacteria with volume of 100 µl and 0.5 ml of insulin leaves extract with 300mg/kgBB dosage using micropipette in gingival sulcus of mandibular incisor every day for 7th days.[11] Samples were fed sufficiently after induction and treatment, general health also monitored during the experiment.

On the 7th day after *A. actinomycetemcomitans* induction and insulin leaf extract administration, samples were sacrificed and ginigval tissue was taken for histopathological preparation with the thickness of 4µm from mandibular incisiors gingiva.

Macrophage count was examined by histopathological preparation (HPA) with Hematoxin-Eosin (HE) staining then observed using a microscope with 3 different observation fields with 400x magnification.[13]

The results of this study were tested for normality by Kolmogorov-Smirnov test using Statistical Package for the Social Sciences (SPSS) 20.0 software for Windows 8 (IBM SPSS Inc, Chicago, United States). Later, continued with the mean calculation between observation in the control group and the treatment group. The observation was continued with the normality test of control group and treatment group, followed by Paired T test to see the significance level of macrophages in the control group and the treatment group.

Results

The difference between control and treatment group can be seen in table 1. There was decreased in mean number of macrophages in the treatment group rather than the control

group at 3 times observations in 400x magnification.

The macrophage expression in HPA with HE staining that can be seen in figure 1, 2.

Group	Total Sample	Mean Number of macrophage	Standard Deviation
Control	7	6.04	2.133
Treatment	7	2.62	0.517

Table 1. The mean and standard deviation of both group.

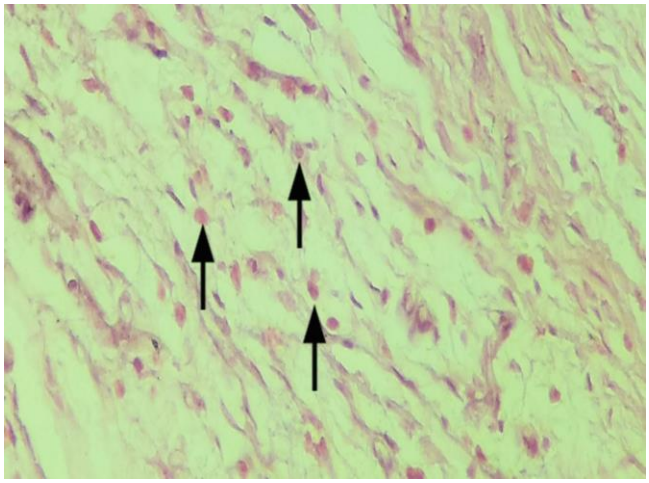

Figure 1. The macrophages expression (arrow) in control group.

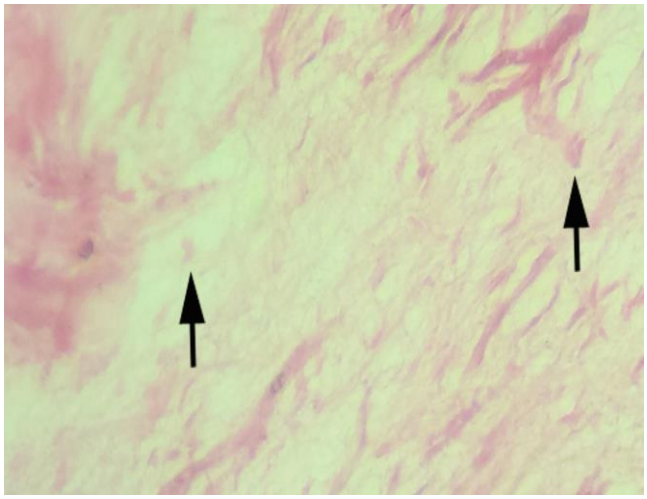

Figure 2. The expression of macrophage (black arrow) in treatment group.

The paired t test was perfomed to see different levels of macrophages in the control group and the treatment group using Statical Package of Social Science (SPSS) sofware 17.0 edititonshowed a significant decreased number of macrophages ($p<0.049$).

Discussion

The control group compared to treatment group showed insulin leaf extract decreased the number of macrophage cells. This effect occured because in the insulin leaf contains flavonoidsfrom flavonol and khalkon type that have antibacterial activity. The insulin leaf has three main principles as an antibacterial agent by destruct the cytoplasmic membrane, inhibit the synthesis of nucleic acid and inhibit metabolism of the bacteria.[14]

Flavonoid of insulin leaf can decrease *A. Actinomycetemcomitans*colony in gingival sulcus followed by a decrease number of macrophage as one of inflamation marker.[15]

In hyperglycemic conditions, AGEs as a result of hyperglycemia that can enter the tissue thus altering macrophage and other cell phenotypes via specific cell surface receptors. AGEs will convert macrophages into destructive cells thus increase pro-inflammatory cytokines that can lead severe local damage in the periodontal tissue.[2]

This study showed that insulin leaf extract was significant to decrease the number of macrophages in the gingival tissue on the 7th day. The decrease number of macrophages caused by insulin leaf contained phenol components such as chlorogenic, caffeic, and ferutic. The effects of these active components can improve pancreas β cells, hence increase insulin secretion and insulin receptor sensitivity. In addition, small anthaditepenic acids A, B, C and D contained anti-diabetic agent.[14]

The phenol and small anthaditepeps A, B, C, and D components surpressed the immune system and decrease the number of macrophages while the blood glucose level decreased. The decreased number of macrophages indicated inflammatory process and inflammatory cells decreased.

The active component of insulin leaf can control the inflammatory process, inflammatory cells, and blood sugar levels. Therefore, active component of insulin leaf can eliminate the bacteria *A. actinomycetemcomitans* and decrease the number of macrophages.

Conclusions

There was a significant decreased number of macrophage in diabetic rat's gingival tissue induced by *A. Actinomycetemcomitans* after insulin leaf extract.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

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