Toll-Like Receptor 3-4 Expression Decrease in BALB/ c Diabetic Mouse Models

[1*] Muhaimin Rifa'i, [1] Aris Soewondo, and [1] M. Sasmito Djati

[1] Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang 65145, Indonesia

** Corresponding Author*: rifa123@ub.ac.id

Abstract
In this study, we observed the effect of propolis extract on immunological function in diabetic mouse models with the aim of highlighting the dynamics of immunological status in type-2 diabetes. In this study we tested the ability of propolis to normalize homeostasis. Here we showed that propolis improve homeostasis by slightly increasing the level of TLR expression. The results of this study differ from previous findings which reported that TLR expression increased in diabetes mellitus and most treatment is intended to suppress the expression of TLR. In this study we found that TLR-3 and TLR-4 expression decreased in mouse models of STZ induced diabetes mellitus. Furthermore, we found that administration of propolis showed an increase of red blood cell precursors (TER-119) and improve the ratio of CD4:CD8 dependent manner.
Key words: Diabetes mellitus, propolis, imunomodulator, Toll-like receptor

Introduction

Currently about 348 million people worldwide suffer from diabetes mellitus (DM). DM is the most deadly diseases and has penetrated all countries. It is currently known, 4.6% of the total Indonesian population suffer from diabetes mellitus, and this will increase to 7.8% in 2030. During this time, the treatment of various diseases including diabetes really dependent on synthetic drugs. On the other hand, Indonesia has abundant of natural resources with the potential to cure various diseases. Propolis is one of Indonesia's pride traditional medicine which is often claimed by the community with ability to cure diabetes mellitus. But unfortunately there have not enough research been done on this existing claims. On the other hand, patients with diabetes mellitus want to get the right medicine to improve the quality of their life. In general, the severity of diabetes is triggered by inflammation factors known as proinflammatory cytokines. Administration of propolis to diabetic mice results in a significant decrease of blood sugar levels and increases the number of naïve T cells. When propolis was given to normal or diabetic mice, it can increase the number of regulatory T cells. In mouse models of diabetes mellitus the development of effector cells can be suppressed after propolis administration. In individual with diabetes mellitus propolis tends to suppress IFN-γ but rather increased TLR production.

Materials and Methods

Procedure

Preparations of Ethanol Extract of Propolis.

Propolis extract preaparation consisted of three phases including drying, extracting, and evaporating. The drying process began by washing the sample, cutting it into small pieces, and putting them in the oven with 40-60ºC degree. Samples were dried and crushed by a blender. As much as 100 grams of dry samples were weighed and put in 1 L Erlenmeyer glass, soaked with ethanol to the volume of 1 L before the extraction process. Sample in ethanol was stirred for ± 30 minutes and allowed to stand overnight to settle. Supernatant was removed and filtered. The extract of propolis was dried by using an evaporator and weighed. Propolis powder was then dissolved in distilled water for oral treatment. Type 2 diabetes mice models were then treated with the ethanol extract of propolis for 7 weeks. Mice were maintained in pathogen free facility. Mice were fed with pellets BR1 by 5-10 grams per day and drink was provided ad libitum.

Induction of Type 2 diabetes mellitus with STZ.

Streptozotonin (STZ) dissolved in 0:01 M citrate buffer, pH 4.5 and always prepared in a fresh condition. STZ was intraperitoneally injected to mice in the age of 5 days (100 mg/kg BW). Five weeks after STZ injection, diabetic condition is confirmed by measuring the glucose levels using a glucometer (GlucoCare Ultima).

Results and Discussion

In this study we showed that TLR-3 expression in diabetic mice decreased 6 times lower than normal. The suppression of TLR-3 expression is suspected to affect the quality of homeostasis because TLR-3 normally functions as an activator of innate immunity. The low expression of TLR-3 in turn make difficulty for eliminating infectious agents in patient with diabetes mellitus. So that in many cases people with diabetes mellitus often wounds and difficult to be cured. However, the increase of TLR-3 expression far beyond normal will bring bad impacts and also aggravate diabetic patient health conditions.

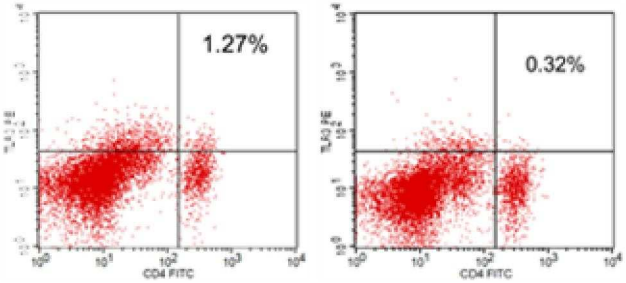

Figure 1. In mice models of diabetes mellitus induced by STZ, expression level of TLR-3 decreased

Left panel showed TLR-3 expression on CD4 cells in normal mice. Right panel is an expression of TLR-3 on CD4 T cells in mouse models of DM. Cells were isolated from spleen and analyzed by flow cytometry. CD4 positive cells expressing TLR-3 ($CD4^+TLR\text{-}3^+$) were shown in each panel.

Observations on CD4 T cells clearly showed that the expression of TLR-3 decreased in mouse model of diabetes. In this experiment we cannot explain the site effect of TLR decline on T lymphocytes. However, there is a little clue that the decline of TLR expression may correlate with the failure of patient in eliminating foreign antigen specially bacteria and virus. TLR-3 has been known to be associated with the emergence of signal transduction related to virus elimination. The decline of TLR-3 expression in DM will lead to the emergence of disorders associated with TLR-3 function.

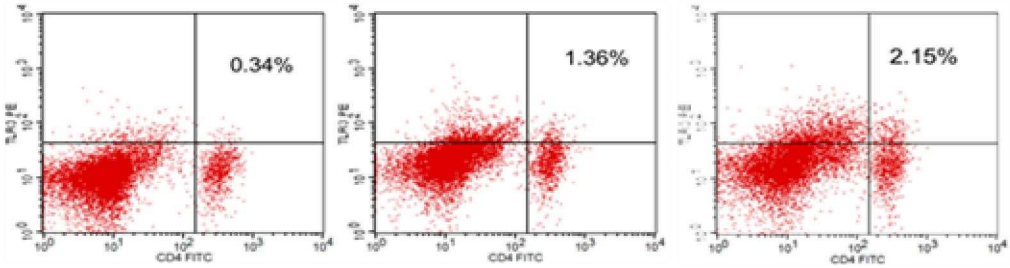

Figure 2. Administration of propolis in mice model of diabetes increase the expression of TLR-3 on CD4 cells approaching normal conditions

From left panel to right is propolis treatment at a dose of 50, 100, and 200 mg/kg BW recpectively. The cells were isolated from spleen and analyzed by flow cytometry. CD4 positive cells expressing TLR-3 ($CD4^+TLR\text{-}3^+$) were shown in each panel.

Conclusions

TLR-3 expression in diabetic mouse models decrease on CD4 T cells. Propolis can normalize homeostasis by slightly increasing the level of TLR expression. In general, diabetic mice models that had received propolis develop into healthy signed by the decline in glucose levels and also physiologic improvements. The ratio of T lymphocytes seem to be normalized after consuming propolis for two weeks. Interestingly, propolis has ability to increase the precursor of red blood cells.

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