ETHANOL EXTRACTS OF PROPOLIS (EEP) AGAINST LYMPHOCYTE ACTIVATION CELLS IN HEALTHY MICE (Mus Musculus) BALB/C

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ABSTRACT

Propolis is a substance like glue formed by honey bees from resin of plant which has the ability to stimulate immune system. The purpose of this study is to determine the ethanol extract of propolis on the activity of lymphocytes in healthy Balb/c mice and to asses the optimum dose administration of EEP for lymphocyte activation in Balb/c mice. Methods: mice was aclimate for two weeks, mice control without treatment EEP and a other was treated with EEP dependent dose, dose 1 (50 mg/ kgBW), dose 2 (100 mg/kgBW), and dose 3 (200 mg/kgBW). Spleen was isolated and to find out the amount of lymphocyte we analyzed with flow cytometry. Parameter measured in this experiment is quantitative by measuring relative number of T cells that consist of CD4⁺CD62L⁺, CD4⁺CD62L⁻, CD8⁺CD62L⁺, dan CD8⁺CD62L⁻. Then data was analyzed by SPSS 16.0 software for windows with ANOVA test and advanced by Tukey test and Gomes-Howell with an interval 0.05 is used. The result showed that ethanol extract of propolis can activate CD4 T cells so that CD4 T cell lost CD62L molecule and turned into T cells CD4⁺CD62L⁻. Ethanol extract of propolis can enhance proliferation of naïve type of CD8 T cell, so that the number of T cell memory (TCM) decreased. Dose of 100 mg/kgBW of ethanolic propolis extract spatially act as immunostimulant for CD4⁺CD62L⁺ activation, while the dose of 200 mg/kgBW act as immunosuppressant in the same cells.

Key words: T cell activation, propolis, Balb/c mice.

INTRODUCTION

Indonesia is a rich country who have a greatest biodiversity. Various kinds of natural materials can be used to cure many diseases. In fact, many people use propolis as alternative drug for therapy to healing various diseases. Propolis is a substances like a glue collected by honey bees from buds and exudates of plants, processed by enzymes released by bees and mixed with wax present in the nest. Honey bees use propolis as a tool for self-defense to protect it nest from the environmental from other organisms and to prevent the development and spread of diseases caused by microbes. Propolis has a wide range of positive effects on health, as an immunomodulator, antioxidant, antibacterial, antitumor, antifungal, and anti-inflammatory (Murad et al., 2002). Bees wax is basically white but can change form the dark brown color due to contamination by contact with pollen and bees in the hive (Krell, 1996).

Based on the research propolis is able to assist homeostasis by enhancing the immune system to maintain the body's balance system. Therefore, further research is necessary to determine the mechanisms and work of propolis in the immune system, so this research needs to show for all people are more certain to use herbal medicine like a propolis with the available scientific evidence. Parameter was measure by analyzed relative number of T cells CD4⁺CD62L⁺, CD4⁺CD62L⁻, CD8⁺CD62L⁺, CD8⁺CD62L⁻ and this research is expected to improve usability herbal medicine in Indonesian has the potential in health.

METHODS

Description Treatment. Animals used in this research were Balb/C mice (Mus musculus) male, age 8 weeks, body weight ± 40 grams. This research used 4 treatments: control treatment, treatment I (dose 50 mg/BW), treatment II (dose of 100 mg/BW) treatment da III (dose 200 mg/BW) with 3 replications in each treatment.
Table 1. Group Treathment

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<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
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<tr>
<td>Mice Balb/c</td>
<td>Control (Non Oral Treatment EEP)</td>
<td>0 mg/kgBW</td>
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<tr>
<td></td>
<td>Dose (Oral Treatment EEP)</td>
<td>50 mg/kgBW</td>
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<td>100 mg/kgBW</td>
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**Extraction Propolis.** Preparation extract consists of 2 stages include extraction and evaporation processes. Extraction process start from 200 grams of dried sample, put in two glass erlenmeyer then soaked with ethanol until volume 1 L and allowed to precipitate. Furthermore, the solution was filtered using filter paper to separate the ethanol from the sample with the active substance. Then evaporated the extracted solution 1 L evaporation flask mounted on the evaporator. Water bath filled with water until full and then in pairs all series tools including rotary evaporator, water bath heater (set temperature 90°C). Ethanol solution was allowed to separate the active substance in the flask.

**Preparation Extracts and Oral Treatment in Mice.** Preparation extracts depends on the average weight of mice. Total extracts were weighed then dilution use aquades with ratio 1:10. Furthermore, mice in groups of oral treatment with ethanol extract of propolis according to the dose in each group until 2 weeks.

**Isolation of Lymphoid Organs.** Neck of mice was dislocation. Sprayed with alcohol 70% and cut ventral using sectio sets. Spleen organs were taken and put in a petri dish containing sterile PBS, then using base of the syringe crushed with rotated clockwise. Homogenates pipetted until mix, then filtered using a sterile wire. Propylene tubes inserted and filter again until the volume reaches 10 ml and then centrifuged.

**Analysis of Changes Number of T Cells Subset Using Flow Cytometry.** Supernatant was discarded, the result of pellet 1 centrifugation were resuspended with 1 ml of sterile PBS and pipetting. Take 70 mL resuspension and incorporated in mikrotube containing 500 ml of sterile PBS and pipeting. Then centrifuged and the supernatant discarded. Pellet 2 obtained was added 50 ml of each preparation antibodiBD Science™antimouse CD4 FITC conjugated, BD Science™antimouse CD4 FITC conjugated, PE-Cy™ 7 Rat antimouse CD8 and PE-Cy™ 7 Rat antimouse CD62L for 15 minutes. Pipeting and incubated for 15 min in dark conditions. Added 300 mL of sterile PBS. Resuspended and transferred to a cuvette flow cytometer. Cuvette mounted in nozzle Calibur™flow BD FACS cytometer. The computer was set with BD Cells Quest software Pro™ and made connections with a flow cytometer (acquiring mode).

**Data Analysis.** The parameters measured the relative number of T cells include CD4^+CD62L^+, CD4^+CD62L^−, CD8^+CD62L^+, CD8^+CD62L^−, then data was analyzed using SPSS 16.0 for Windows. ANOVA to test normality and homogeneity, the data homogeneous advanced Tukey test, while the data are not homogeneous test using Games-Howell with the confidence interval of 0.05.

**RESULTS AND DISCUSSION**

Effect of ethanol extract of propolis in healthy mice Balb/C by analyzing relative number of T cells, namely CD4^+CD62L^+, CD4^+CD62L^−, CD8^+CD62L^+, CD8^+CD62L^−.

**Population T Cells CD4^+CD62L^+ dan CD4^+CD62L^− on Spleen Organ.** The results of flow cytometry analysis showed that control treatment the relative number of T cells CD4^+CD62L^+ is 2.26%. Dose 1 is 2.24%. Dose 2 is 1.07%. The relative number of cells increased again at dose 3 that is 2.1% (figure 1). Dose 3 activator molecules bound by other molecules so percentage was increases. Based on the analysis of Gomes-Howell, relative number of T cells CD4^+CD62L^+ (Figure 2) control treatment showed no significant difference with dose 1 and dose 3. Dose 1 and dose 3 showed no significant between treatments, but control treatment had significantly different on dose 2. The relative number of T cells CD4^+CD62L^+ (figure 1) control treatment amounted to 5.96%. Dose 1 have a similar value when compared to control as 5.6%. Dose 2 showed the relative number of cells is highest among the other treatment equal to 14.18%. The relative number of CD4^+CD62L^+ cells decreased back at dose 3 is equal to 2.5%. Based on the analysis of Gomes-Howell relative number of T cells CD4^+CD62L (figure 3) control treatment no significant difference with a dose 1, but there is...
a significant difference with dose 2 and 3. Dose 2 was very significant value with dose 3.

Figure 1. Profile Relative Number of T Cells CD4$^+$CD62L$^+$ dan CD4$^+$CD62L$^-$ in Spleen Organ.

Comparison the number of T cells CD4$^+$CD62L$^+$ dan CD4$^+$CD62L$^-$ after therapeutically ethanol extract of propolis show that number of CD4$^+$CD62L$^+$ less then T cells CD4$^+$CD62L$^-$. CD62L is a molecule can mediate naïve T cells to peripheral lymphoid organs, this happens initiation of immune response that decreased expression of CD62L, also lead to the decline in the number of naïve cells (Rifa’i, 2011). It shows that the ethanol extract of propolis can activated T cells CD4$^+$CD62L$^+$, so T cells CD4$^+$CD62L$^+$ lose CD62L molecules and change become T cells CD4$^+$CD62L$^-$. Increasing the number of memory cells happens at dose 2 shows that immunostimulatory effects on T cells CD4$^+$. When the number of memory T cells increased the number of naïve T cells become less and the opposite. Taheri et al., (2005) explain that propolis can respond the immune system, for example can increase the activity of macrophages, increasing IL-1, IL-2 and IL-4. Dose 100 mg/kg BW can increases proliferation T lymphocyte. Gu (2005) explain that propolis can enhance the number of T lymphocyte. T cells CD4$^+$ produce cytokines to activated T cells CD8$^+$, so T cells CD8$^+$ differentiate into cytotoxic T cells effector and memory cells. Increased proliferation of T cells CD4$^+$ can be triggered by saponin compounds, because saponins has the ability to increase cytokine IFNγ (Cheeke, 2010). IFNγ can stimulate the

Figure 2. Changes of Propolis Extract Ethanol Treatment Toward the Relative Number Of T Cells CD4$^+$CD62L$^+$; K=Control; D1=Dose 1 50 mg/kgBW; D2=Dose 2 100 mg/kgBW; D3=Dose 3 200 mg/kgBW

Figure 3. Changes of Propolis Extract Ethanol Treatment Toward the Relative Number Of T Cells CD4$^+$CD62L$^+$; K=Control; D1=Dose 1 50 mg/kgBW; D2=Dose 2 100 mg/kgBW; D3=Dose 3 200 mg/kgBW
up-regulation of MHC II expression, it cause T cells deferentiated become T cells CD4⁺ (Lee et al., 2008 and Shi et al., 2008).

**Population T Cells CD8⁺CD62L⁻ in Spleen Organ.** The results of the analysis using flow cytometry showed relative numbers of T cells control treatment is 1.97%. Treatment dose 1 is 1.81%. Dose 2 showed the highest relative number of cells is equal to 3.03%. Dose 3 is 2.05%, relative number of cells decreased again at dose 2 (figure 4). Based on the analysis of Gomes-Howell (figure 5) the relative number of CD8⁺CD62L⁻ showed that there was no significant between control with dose 1 and 3. But dose 2 are significantly different with all treatments. Ethanol extract of propolis with 4 different dose give different results on the activity of CD8⁺CD62L⁻ (Figure 6). Relative number T cells CD8⁺CD62L⁻ in control treatment is 11.84%. Dose 1 is 9.32%, dose 2 is 7.30%, and dose 3 is 16.34%. Based on Tukey analysis (figure 6), relative number of T cells CD8⁺CD62L⁻ showed that there was no significant difference between control, dose 1, dose 2, but dose 3 has significantly value from the other. Based on the relative number T cells CD8⁺CD62L⁻ known that ethanol extract of propolis can increase the relative number of CD8⁺CD62L⁻ at dose 2 that is 100 mg/kg BW. This indicates that there is a mechanism of cells homeostasis, because the activated CD4⁺ produce cytokines IFNγ and IL-2, which cytokines were used CD8⁺ cells to proliferate. Increase of T cells CD4⁺ influence the activation of T cells CD8⁺.

**Figure 4.** Profile Relative Number of Cells T CD8⁺CD62L⁻ dan CD8⁺CD62L⁺ in Spleen Organ

**Figure 5.** Changes of Propolis Extract Ethanol Treatment Toward the Relative Number Of T Cells CD8⁺CD62L⁻; K=Control; D₁=Dose 1 50 mg/kgBW; D₂=Dose 2 100 mg/kgBW; D₃=Dose 3 200 mg/kgBW

**Figure 6.** Changes of Propolis Extract Ethanol Treatment Toward the Relative Number Of T Cells CD8⁺CD62L⁻; K=Control; D₁=Dose 1 50 mg/kgBW; D₂=Dose 2 100 mg/kgBW; D₃=Dose 3 200 mg/kgBW

T cells CD8⁺ can increase also influenced by the presence of CD4⁺ that have been activated. Activated T cells CD4⁺ had been previously can activated Th1 deferentiated, Th1 produce cytokine IFNγ and IL-2 (Rifa'i et al., 2008). By utilizing IL-2 CD8⁺ have a higher affinity than the affinity of CD4⁺ (Rifa'i et al., 2008). IL-2 produced from CD4⁺ addition is used to regulate itself and also used by CD8⁺ as...
a stimulant for cells proliferate. T cells CD8+ are activated due to the stimulus of IL-2, which defferentiated become T cells killer. T cells killer used to lyse a variety of cells that have been exposed antigen by lysing perforin. Perforin is a protein that causes lysis of target cells by forming pores on target cells membrane. After lysing the cells that have been infected with the antigen, then CD8+ cells will lyse other cells infected by a similar antigen (Rifa’i, 2004).

Most of T cells that developed into memory T cells can survive in a long time. Memory cells is off and circulate in the body, it can be respond quickly in the event of recurring exposure to microbes. After the T cells effector successfully overcome the infection, the stimulus that triggers the expansion and differentiation of T cells also stopped anyway. T cells clones that have been formed will die and then returned to the basal state. This occurs in T cells CD4+ and CD8+, but there is difference in function of efektor (Abbas, 2005).

CONCLUSIONS

Based on the results of the discussion can be concluded that the ethanol extract of propolis is able to increase the activation of T cells memory CD4+CD62L+. Ethanol extract of propolis can decrease the proliferation of T cells memory CD8+CD62L+. Dose of 100 mg/kgBW of ethanol extract of propolis are immunostimulatory the activation of T cells memory CD4+CD62L+, whereas at dose 200 mg/kgBW are immunosuppressants on proliferation of T cells memory CD8+CD62L-.

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REFERENCES