Conference Paper

# Propolis Potential Toward the Amount of Limphoblast and Spleen Diameter of Male Mice *(Mus musculus)*

**Werstant Adhityananda Rinaldhi[1], Eka Pramyrtha Hestianah[2], Sri Mumpuni Sosiawati[3], and Lita Rakhma Yustinasari[4]**

[1]Magister student of Faculty of Veterinary Medicine of Universitas Airlangga
[2]Department of Veterinary Anatomy of Veterinary Medicine of Universitas Airlangga
[3,4]Department of Veterinary Parasitology of Faculty of Veterinary Medicine Universitas Airlangga, Mulyorejo C Campus, Surabaya 60115, Indonesia

## Abstract

The aim of this research was to find out the effect of propolis in the amount of lymphoblast and the correlation between the treatment of propoliswith spleen diameter each of white male mice (*Mus musculus*). This research used 25 male white mices which are 12 weeks old has 25-35 gram of body weight. They were divided randomly into five groups. (Po) as control was given 0,5 ml aquades/day, and other group were given propolis for 0.4 mg/0.03kg/day (P1), 0.8 mg/0.03kg/day (P2), 1.6 mg/0.03kg/day (P3), and 3.2 mg/0.03kg/day (P4). After two weeks treatment, 25 mices were sacrificed their spleen were then used in histological preparation with HE staining. The data were analyzed by ANOVA method based on Completely Randomized Design and further analyzed by Duncan's Multiple Range Test. The result from statistical analysis showed that treatment with propolis increased the amount of lymphoblast also the spleen diameter of white male mice ($p<0,05$) and show positive correlation amount of lymphoblast and white pulp spleen diameter.

**Keywords:** propolis, lymphoblast, white pulp, spleen.

Corresponding Author:
Werstant Adhityananda Rinaldhi

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## 1. Introduction

Health is the most basic need for every living being. One the causes of the body susceptible to diseases is a decrease in the immune system, it makes the body to defend itself against the factor of pathogen entry, including the proliferation and differentiation of lymphocyte so that it becomes cells which can destroy the pathogen. Realizing the importance of the body's defense system, the veterinary world always tries to learn different ways to increase the immune system response by giving some stimulus or an immune stimulant to overcome some infectious diseases in animals

[1]. Propolis is one of them. The result of Missima *et al.* (2009) propolis can stimulate the growth of spleen, thymus and the production of lymphocytes associated with the immune system, whose function is to fight against infection and inflammation [2].The process of antigen entry into the body can be through the skin, gastrointestinal epithelial, and the respiratory system. Skin, epithelium, and parenchyma organ contain many lymphatic vessels that have lymph canal starting from the entry point of the antigen to the lymph nodes. The epithelium has dendritic cells that will bind the antigen and carry it out to the primary and secondary follicle. Antigen in the circulation will pass through the spleen and be captured by macrophages in the marginal zone and the sinusoid in red pulp. Marginal Zone has a major role in the immunological activity of the spleen [3, 4]. Macrophages will bring the antigen to primary follicles in the white pulp and after a few days B cells will migrate to marginal zone to produce antibodies. After receiving the stimulation or exposure of these antigens, a change will take place, primary follicles turn into the germinal center. Thus it is called secondary follicles [5, 6]. Several studies which have been conducted generally have not specifically reported the direct effect of immune stimulant on immune system. Therefore, a study of the number of lymphoblast and diameter of white pulp of mice's spleen is carried out after the administration of propolis.

## 2. Materials and Methods

### 2.1. Animals

Animals in this study were 25 male mice (Mus musculus) Balb/C strain, aged approximately 84 days and weighted 25-35g. Twentyfive animals were randomly divided into five groups. One was the control group (n=5) receiving 0.5 ml of distilled water. Four treatment groups received 0.4, 0.8, 1.6, and 3.2 mg/0.03kg/day extracted propolis for 14 days. The animals were housed in plastic cages and received standard pellet food and *ad libitum* distilled water.

### 2.2. Propolis Extracted

The sample materials used were raw propolis of local strains, *Apis mellifera,* obtained from bee breeding of Rimba Raya Lawang in Malang, East Java. The propolis was extracted by means of maceration method with 96% ethanol. The extraction was made by immersing 500 grams of raw propolis in 850 ml of 96% ethanol for four days with shaking it for an hour and soaking it for three days. The filtrate was decanted,

the residue was extracted again with 850 ml of 96% ethanol, shaken for one hour at a speed of 120 rpm, and the filtrate was decanted. The extraction of residue was repeated up to five times. The total solvent used was 4250 ml and the total maceration was seven days. The filtrate was collected and concentrated by means of a rotary evaporator so that the extraction formed would be ready for use.

## 2.3. Preparation of Spleen

One day after the end of the treatment period, male mice were sacrificed by means of dislocation cervical, the spleen was removed and divided into two parts, one part for the preparation of loading method by infiltrating it with 0.5 to 1 ml of physiological saline, massaging it gently and applying it on the object glass. After that, it was stained by Giemsa to measure the lymphoblast by means of light microscope (Olympus CX21) at magnification of 1000x. The other part was for histological slide with H E staining, used to measure the diameter of white pulp by means of 40x magnification.

## 2.4. Examination lymphoblast

Lymphoblast counting was done by counting the number of lymphoblast in 200 lymphocytes that was obtained from homogenous area of the spleen. The lymphoblast amount obtained from the treatment groups was compared with the control group.

## 2.5. Examination Diameter White Pulp of Spleen

The diameter of the white pulp of the spleen was measured by means of Olympus microscope connected to Optilab plus viewer that had been calibrated with raster image software 3.0. The white pulp had round shape. The diameter of the white pulp of the spleen was measured by dragging from one side to another side. The measuring was performed twice on each white pulp, the results was added and divided by two. The magnification used was 10x objective, 10x eye piece. The diameter of white pulp was calculated as follows [7]:

$$\frac{(\text{diameter transverse max} + \text{diameter perpendicularly max})}{2}$$

Each micro slide was observed in five fields. The diameter of the five white pulps in each field was measured (magnification 100x). The entire collection of data, covering lymphoblast cells number and diameter of white pulp of male mice's spleen, was analyzed by means of ANOVA at 5% confidence level. If significant differences were

Table 1: Mean of lymphoblast cells.

| Treatment Group | Mean + SD |
|---|---|
| Po (Control) | $45.00^{a}$ + 11.46 |
| P1 (Propolis 0.4 mg/0.03kg/day) | $61.00^{ab}$ + 15.16 |
| P2 (Propolis 0.8 mg/0.03kg/day) | $81.60^{b}$ + 11.61 |
| P3 (Propolis 1.6 mg/0.03kg/day) | $66.00^{ab}$ + 12.94 |
| P4 (Propolis 3.2 mg/0.03kg/day) | $74.54^{c}$ + 30.81 |
| Mean with differing superscript within row were significantly different at the p<0.05 based on ANOVA and Duncan's test. | |

Table 2: Mean of the diameter of the white pulp of the spleen.

| Treatment group | Mean + SD |
|---|---|
| Po (control) | $334.64^{a}$ + 15.47 |
| P1 (Propolis 0.4 mg/0.03kg/day) | $347.83^{a}$ + 46.65 |
| P2 (Propolis 0.8 mg/0.03kg/day) | $423.31^{b}$ + 32.38 |
| P3 (Propolis 1.6mg/0.03kg/day) | $363.12^{a}$ + 36.73 |
| P4 (Propolis 3.2mg/0.03kg/day) | $505.52^{c}$ + 51.25 |
| Mean with differing superscript within row were significantly different at the p<0.05 based on ANOVA and Duncan's test | |

found among treatments, it was continued by Duncan's test (5%). ANOVA and Duncan's test were performed by means of SPSS version 16.0 for windows.

## 3. Result

The results of ANOVA test with significance level of 5% showed that the extracted propolis affected the increased number of the lymphoblast of the spleen of *Mus musculus* male mice ($p < 0.05$). This was indicated by the value of $p = 0.002$, indicating that the result was significant. The further test to determine differences among treatment groups used Duncan's test. The result showed that the treatment group was significantly different, i.e. P4 to Po, P1, P2, and P3, while P1 and P3 were not significantly different. P2 was significantly different from Po. The average of the lymphoblast can be seen in Table 1. The average result of the research of the diameter white pulp of the spleen can be seen in Table 2.

Results of ANOVA with significance level of 5% shows that the extract of propolis affects the increased diameter of white pulp of male mice's spleen, this is indicated by the value of $p = 0.000$ ($p<0.05$). This suggests that the results are significantly

different and meaningful. It means the administration of propolis can increase the diameter of the white pulp of male mice's spleen. The results of calculation of the average diameter of white pulp of the spleen can be seen in Table 2. The results of further test with Duncan, P0, P1 and P3, are not significantly different. P2 shows a clear difference with P0, P1 and P3, while P4 shows a highly significant difference with the rest of the treatment. P4 group has a real difference to the whole treatment, while the P0, P1 and P3 do not show a significant difference.

## 4. Discussion

The products of the honeybee (Apis mellifera) are used to maintain healthy body. Propolis is another excellent product beside honey. Propolis has a composition very complex substance and consists of over 300 components including aldehydes, polyphenols, sequiterpene quinines coumarins, steroids, amino acids, and inorganic compounds [8, 9]. Propolis has potential as an antioxidant that can maintain cellular Gluthatione (GSH) and the integrity biomembrane [1]. A discussion of propolis in overcoming the disease cannot be separated from the body's immune system because it is important to the effort of defending itself against the entry of pathogens such as by the proliferation and differentiation of lymphocytes into cells that resist pathogen agent [10–12].

### 4.1. Lymphoblast

The results of the lymphoblast of P1 (Propolis 0.4 mg/0.03kg/day) and P3 (Propolis 1.6 mg/0.03kg/day) had the same high value, both were not significant. P2 shows significant results from P0 (control). P4 (Propolis 3.2 mg/0.03kg/day) shows significant differences with all existing treatments. The treatment group showing a higher value than the treatment group of P2 (Propolis 0.8 mg/0.03kg/day) was P4 (Propolis 3.2 mg/0.03kg/day), which was not the best result of the increased number of the lymphoblast. This was due to an increase in the number of the lymphoblast, not only by the provision of food, instead by the immune response towards immunogens and proliferation, which are prevalent in the area of the germinal center of the white pulp of the spleen. The results of the calculation of the group P3 (Propolis 1.6 mg/0.03kg/day) shows a decline when compared with the amount of lymphoblast group P2 (Propolis 0.8 mg/0.03kg/day), which is very significantly different from the P3 (Propolis 1.6 mg/0.03kg/day), P1 (Propolis 0.4 mg/0.03kg/day) and P0 (control). It is possible because the dose P3 (Propolis 1.6 mg/0.03kg/day) has not been able

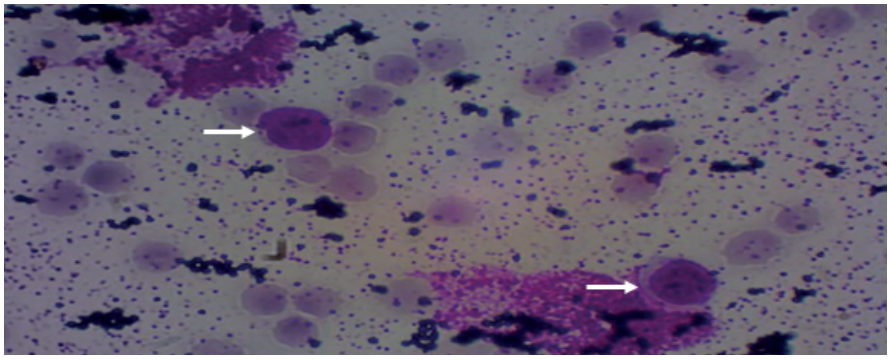

**Figure** 1: The lymphoblast (white arrows) of the spleen appears with loading method (Giemsa staining, 100x).

to proliferation lymphoblast, so it takes a higher dose as the dose P4 (Propolis 3.2 mg/0.03kg/day).

Extracted propolis can increase the production of lymphocytes activating factor cytokine IL-1 by increasing the proliferation of T cells and B cells [13]. Flavonoids give a lot of influence on the activation of the immune system by increasing macrophage phagocytosis index as well as the increase in the number of lymphocytes. According to this study the increased lymphoblast in propolis is found to act both as immune stimulant and immunosuppressant. The existence of cytotoxic and immunosuppressive effects may create barriers on cell proliferation lymphoblast at certain dose [14].

That is why there is not any significant difference between the groups P1 and P3 with the control group Po. The result of the same study conducted by Syaifulhaq (2009), in which propolis dose is gradually given, shows significantly different result of lymphocyte proliferation between treatment and control groups [15].

## 4.2. Diameter of white pulp

Further results of Duncan's test Po (control), P1 (Propolis 0.4 mg/0.03kg/day), and P3 (Propolis 1.6 mg/0.03kg/day) are not significantly different, while P4 (Propolis 3.2 mg/0.03kg/day) shows very significant difference to the whole treatment. This difference is consistent with the results of research conducted by Underwood in 1999, which stated that white pulp is the largest place in producing antibodies and forms *germinal center* if it gets stimulus. The stimulus which was meant in this study was the provision of extracted propolis in five different doses.

Regional growth in *germinal center* seen under light microscope was visible with empty space and young cells. This empty space is required for the growth of young cells lymphocytes derived from lymphoblast development. The diameter of white pulp

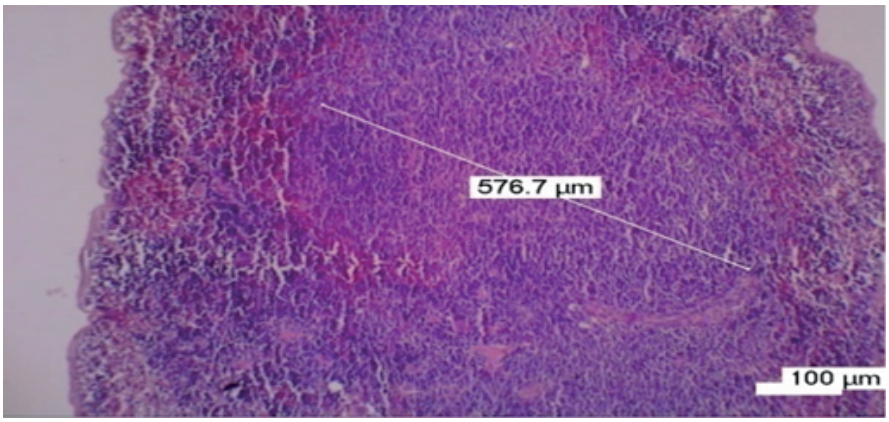


**Figure** 2: The diameter of the white pulp of the spleen (HE staining, 100x).

and *germinal center* is larger than that of treatment groups when compared with diameter of the control group.

## 4.3. Lymphoblast and Diameter of white pulp

Pearson correlation statistical test result shows that there is a relationship between the increase in the number of lymphoblast with an increased diameter of the white pulp of the spleen ($P < 0.05$). The correlation coefficient (r) indicates positive value, which means that the higher the amount of lymphoblast the bigger the diameter of white pulp lymph is with value of 0.527. The antigens known in the bloodstream will be bound by the Antigen Presenting Cell (APC). The reacting antigen can stimulate immune called immunogen. Molecular of Major Histocompatbility Complex (MHC) class I is for T cell interactions CD-$4^+$, and MHC class II is for T cell interactions CD-$8^+$. This activation will produce IFN$\partial$ as an indicator of an increase in the immune system, both cellular and humoral. [7]

Lymphoblast proliferation becomes T lymphocytes in the Peri Arterial Lymphoid Sheet (PALS), and B lymphocytes in the germinal center stimulated by Interleukin 2 (IL-2).

The positive correlation meant here is that the amount of lymphoblast proliferation in the PALS and germinal center, when seen under microscope, shows an increase or the widening diameter of the white pulp of spleen.

## 5. Conclusion

Ectracted ropolis as an immunomodulator can boost the immune system by increasing the amount of lymphoblast and the widening diameter of the white pulp of spleen with the best dose of propolis of 0.8 mg /0.03kg /day.

## References

[1] H. Ichikawa, K. Satoh, T. Tobe, I. Yasuda, F. Ushio, K. Matsumoto, K. Endo, and C. Ookubo, Free Radical Scavenging Activity Of Propolis. *n* Redox Rep. 7 (5) (2002) 347-350.

[2] F. Missima, A. C. Pagliarone, C. L Orsatti., J. M. Sforcin, The Effect of Propolis on Pro-Inflammatory Cytokines Produced by Melanoma-Bearing Mice Submitted to Chronic Stress. Journal of ApiProduct & ApiMedical Science. 1 (2009) 11–15.

[3] K. A. Abbas, A. H. Lichtmant, S. Pillai, Cellular and Molecular Immunologi, $6^{th}$ ed. Philadelphia: W.B. Saunders Company, 2007.

[4] F. D. Batista, N. E. Harwood, The Who, How and Where of Antigen Presentation to B cells,. Nature Reviews Immunology, 9 (2009) 15-27.

[5] I.R. Tizard, Veterinary Immunology: An Introduction, $7^{th}$ ed, China: Saunders, 88-91, 2004.

[6] K. G. Baratawidjaya, I. Rengganis, Imunologi Dasar, $9^{th}$ ed., Jakarta, Balai Penerbit Fakultas Kedokteran Univeritas Indonesia, 2010.

[7] D. K. Yuliawan, Pengaruh Pemberian Simunox Dosis Bertingkat terhadap Proliferasi limfosit pada Mencit *Swis,* Skripsi, Fakultas Kedokteran Universitas Diponegoro, Semarang, 2010.

[8] V. Bankova, Recent Trends and Important Developments in Propolis Research, eCAM 2 (2005) 29-32.

[9] M. L. Khalil, Biological Activity of Bee Propolis in Health and Disease. Asian Pac. J. Cancer Prev. 7(1) (2006) 22-31.

[10] A. H. Banskota, Y. Tezuka, S. Kadota, Recent Progress in Pharmacological Research of Propolis. Phytoter Res 15(7) (2001) 561-571

[11] S. Suhirman, C. Winarti, Prospek dan Fungsi Tanaman Obat sebagai Imunomodulator, Buletin Perkembangan Teknologi Tanaman Rempah dan Obat 19(2) (2007) 121-133.

[12] J. M. Sforcin, G. A. Nunes F. Missima, A. Nunes, L. H. Faccioli, Effect of a Leukotriene Inhibitor (MK886) on Nitric Oxide ond Hydrogen Peroxide Production

oy Macrophages of Acutely an Chronically Stressed Mice. Journal of Pharmacy and Pharmacology 59 (2007) 1249-1254.

[13] N. Orsolic, I. Basic. Immunomodulation by Water Soluble Derivates of Propolis: Factor of Antitumor Reactivity. Journal of Etnopharmacology 23(8) (2003) 265-273.

[14] E. Middleton, C. Kandaswarni, and TC. Theoharides,. The Effect of Plant Flavonoid on Mammalian Cell: Implication For Inflammation, Heart Disease, and Cancer. Pharmacological Reviews 52(4) (2000) 673-751.

[15] M. Syaifulhaq, Pengaruh Pemberian Ekstrak Buah Mahkota Dewa Dosis Bertingkat terhadap Proliferasi Limfosit Lien pada Mencit BALB/C. Biomedika 2 (1) (2009) 33-36.