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**Submission date:** 02-May-2023 09:06AM (UTC+0700)

**Submission ID:** 2081560667

**File name:** Proceeding\_Icream\_2017\_Hematologi\_Profile\_Melva\_P\_Maulim.pdf (931.03K)

**Word count:** 3509

**Character count:** 18126

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Cite as: AIP Conference Proceedings **1868**, 090008 (2017); <https://doi.org/10.1063/1.4995200>

Published Online: 04 August 2017

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# Haematological Profile Of Rats (*Rattus norvegicus*) Induced BCG and Provided Leaf Extract of *Plectranthus amboinicus* Lour Spreng)

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**Abstract.** *Plectranthus amboinicus* Lour Spreng is a medicinal plant that has many benefits, such as an antioxidant, hepatoprotective and immunostimulant. Immune status can be seen from hematological profile. This study aims to investigate hematology profile on rats induced BCG and leaf extract of *Plectranthus amboinicus*. 24 male rats aged 3 months and weighing between 140-200 grams divided equally into six groups, P0, P1, P2, P3, P4 and P5. P0 as control was given aquadest. The P1, P2, P3, P4 and P5 treatment groups were given 19 g / kg AEP + BCG, 31.5 g / kg AEP + BCG, 19g / kg AEP, 31.5 g / kg AEP and BCG consecutively. The BCG were used as antigen. The AEP was administered orally for 30 days and 100 µl BCG were intramuscularly administered on day 14<sup>th</sup> and day 21. On day 31<sup>st</sup>, the rats were decapitated and their blood was collected for hematology (leucocyte (WBC), Erythrocyte (RBC), thrombocyte (PLT) count, Haemoglobin (Hb), erythrocyte sedimentation rate (ESR), MCV, MHC, and MHCH analysis. Data were analyzed with ANOVA. WBC increased significantly in treatment AEP 31.5 g / kg bw, 31.5 g AEP / kg bw + BCG and so were only given BCG. RBC tend to increase in all AEP treatment but tends to increase again when given a BCG. Hb increased in treatment P1, P2, T3 and P4, but the improvement was significant only in treatment P1. While PLT increase significantly in all treatments compared to the controls. HCT did not show significant differences but all of them were in the normal range. EAP without BCG and with the addition of BCG lowered ESR significantly, whereas BCG alone increased the ESR significantly. MCV increased significantly only in the treatment of P1 and show the same pattern with the MHC and MHCH. The conclusion that *Plectranthus amboinicus* Lour a positive impact on blood profiles with and without BCG. *Plectranthus amboinicus* Lour managed blood profile when administered together with BCG

## INTRODUCTION

*Plectranthus amboinicus* Lour (Spreng) (*Coleus amboinicus*, *Coleus aromaticus*), commonly known as Indian borage, country borage is a dicotyledonous plant belonging to Lamiaceae family. In Indonesia *Plectranthus amboinicus* is a traditional food used in soup to stimulate lactation for the month or so following childbirth. In Cambodia juice from the leaves is sweetened and then given to children as protection from colds; and leaves are applied to the lips. *Plectranthus amboinicus* is a medicinal plant that has many benefits, such as an antioxidant, hepatoprotective and immunostimulant. Hematological is one of the parameters observed in the immune system.

The immunostimulatory activity can also be measured through serological tests and hematology[1]. The following describes the involvement of hematologic values in the immune system. In vertebrates erythrocytes were able to give a functional response to viral infection. Other pathogens such as bacteria directly target the erythrocytes [2]. Calculation of the accompanying characteristics of average size and content of erythrocytes can be done by measuring several parameters such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular Hemoglobin Concentration (MCHC). Platelets are cell fragments that not only stop bleeding in damaged blood vessels but also is a key component in the body's innate immune system. This is supported by the toll-like receptors (TLRs) on platelets [3]. White blood cells are used to help the body fight various infectious diseases as part of the immune system. The Erythrocyte Sedimentation rate (ESR) is a simple and inexpensive

laboratory test for assessing the inflammatory or acute response[4]. While the role of acute phase reactants and cytokines in inflammatory responses is well-established. The ESR has also been found to be of clinical significance in the follow-up and prognosis of non-inflammatory conditions such as prostate cancer, coronary artery disease, and stroke. Therefore, the ESR is important in the diagnosis of inflammatory conditions and in the prognosis of non-inflammatory conditions, making this old test far from obsolete in either the near or distant future. This study examined the profile of hematology by administration of *Plectranthus* at the time of entry of the antigen into the body.

## METHODS

### Animals

Male rats, 2-3 months old (*Rattus norvegicus*) weighing 140 to 200g, were supplied by the Animal House, Faculty of Pharmacy, Nort Sumatera University. The animals were maintained at room temperature on 12 h light – 12 h dark cycle[5]. They were fed with standar pellet diet, and tap water ad libitum. Rats were placed in plastic cages measuring 40 x 25 x 20 cm, at the top of the cage is equipped with a wire cover. Each cage is filled with chaff as the base and then placed three rats per cage. Acclimatization is done for 7 days.

### Plant Material

A total of 500 grams of fresh leaves sliced into small pieces and fresh juice obtained by adding 30 ml of water, with the aid of a mixer. Juice fresh filtered and deposited into dry ingredients using a vacuum destillasi. Dry residue obtained as much as 16 g [6]. AEP dose for rats is determined based on a daily consumption of Bataksneece[7], namely 150 g / 50 kg bw, then converted into rats. Conversion is done by looking at the dose conversion table[8] which is determined on the human body weight and mice 70.Kg 200.g

### Experiment Design

Complete randomized design was used in this research. Rats were divided into six groups of four rats each. The first treatment group as control (*P1*) was given *aquadest*. The second (*P2*), third (*T3*), fourth (*P4*), fifth (*T5*) and the sixth (*T6*) treatment groups were given 19 g / kg AEP + BCG, 31.5 g / kg AEP + BCG, 19g / kg AEP, 31.5 g / kg AEP and BCG consecutively. The BCG were used as antigen. The AEP was administered orally for 30 days and 100 µl BCG were intramuscularly administered on day 14 th and day 21. *Hematology* parameters that included in this research were *erythrocyte (RBC)*, *Leucocyte (WBC)*, thrombocyte (PLT) count, Haemoglobin (Hb), erythrocyte sedimentation rate (*ESR*), *MCV*, *MHC*, and *MHCH*. On day 31st, the rats were decapitated and their blood were collected. Hematological data obtained using standard tools (ABX Micros 60). Data were analyzed using one way Anova.

## RESULT AND DISCUSSION

### Red Blood Cells (RBC) and Hemoglobin

Assessment of red and white blood cell count, hematocrit and hemoglobin concentration is useful in determining the effect of some chemical substances on hematopoietic system. The number of RBC increased significantly in the treatment of P1, P3 and P4 compared with the controls (Table 1). P2 and also P5 treatment did not increase in RBC count but just same as the control. Hemoglobin values in all of the treatment significantly higher ( $p < 0.05$ ) compared to the control treatment (P1). There is a tendency of increase in hemoglobin at treatment P2, P4 and P4. Hematology value (blood profile) is useful to assess the health condition and as a reference value of the initial (baseline) or a control in a study. The presence of metabolic disorders, diseases, damage to the structure and / or function organ, the influence of agents / drugs, and stress can be seen from the changes in blood profile [9]. Erythrocytes play a role in maintaining the immune system, when erythrocytes undergo lysis by pathogens or bacteria, hemoglobin releases free radicals that function to kill pathogen cell membranes [10]. The components of *Plectranthus* that play a role to

support erythropoiesis are Fe and Vitamin B12. With its contribution in maintaining the normal erythrocyte profile it can be said *Plectranthus* has potential as immunostimulant.

### White Blood Cells (WBC)

The significant increased in the levels of WBC observed in the P5, P4 and P3 treatment (Table 1). The number of WBC in the three treatments is significantly higher ( $P < 0.05$ ) compared to the controls. WBC on P1 and P2 treatments almost same as control. Increased in total WBC count has been suggested to be due to stimulated lymphopoiesis and/or enhanced release of lymphocytes from lymph myeloid tissue [11]. The profile of the WBC count reflects the balance between the rate of granulocyte production and that of WBC. The raised WBC count may also reflect low-grade inflammation.

In this study, treatment of AEP and AEP + BCG increasing significantly WBC count. The increased WBC count believed to be the cause of trigger lymphopoiesis and or increase the release of lymphocytes from the lymph glands myeloid tissue [12]. Treatment with ethanol extract of 2500 mg *Plectranthus* / kg bw significantly increase of leukocytes in rats [13]. The results of this study are also consistent with research conducted by [10] in which the administration of 200 mg of methanol extract of leaves of *Plectranthus* / kg bw mice significantly increase the number of leukocytes and the increased leukocytes may indicate the influence of the extract in improving the immune system of test animals. In those studies have not explained what components are important role in the increase, but explained that vitamin B12 is one of the nutrients in *Plectranthus* that play a role in the synthesis of leukocytes. Vitamin B12 plays an important role in the production of white blood cells, which are crucial components of the immune system [11]. Vitamin B12 deficiency has been shown to cause a decrease in the numbers of white blood cells, which can lead to increased susceptibility to infection and disease. White blood cells (leukocytes) are the active units of the body's defense system, functions provide fast and powerful defenses against any infectious agent that exists. The total number of WBC is one of the parameters which are measured as the impact of giving immunostimulant. With the increasing number of leukocytes then AEP potential as immunostimulant. *Plectranthus* contain chemical substances apigenin as much 0.0236 ng / ml [15]. Apigenin is caused *Plectranthus* their potential as an immunostimulant and anticancer. The results of this study can be explained that AEP can maintain hematological profile can thus support the body's defense against antigens. The active biological principles such as alkaloids, cardiac glycosides, saponins, tannins, terpenoids, phenols and steroids contained in the extract, may be responsible for its hematopoietic effects.

**Table 1.** The effect of Aqueous Extract of *Plectranthus amboinicus* Lour Spreng on Hematology

Treatment	Erythrocyte (million/ $\mu$ L)	Leucocytes(WBC) (x 10 <sup>6</sup> /mm <sup>3</sup> )	Hemoglobin (g/dL)	Platelet (x 10 <sup>3</sup> / $\mu$ L)
Controle (PO)	6.12 $\pm$ 0.57	8.28 $\pm$ 1.74	12.05 $\pm$ 0.57	338.67 $\pm$ 137.59
19 g AEP +BCG (P1)	7.31 $\pm$ 0.53*	7.30 $\pm$ 1.20	14.33 $\pm$ 2.12*	670.00 $\pm$ 119.62**
31.5 g AEP+ BCG (P2)	6.69 $\pm$ 0.27	7.38 $\pm$ 1.05	13.55 $\pm$ 1.15	804.00 $\pm$ 146.37**
19 g AEP (P3)	7.48 $\pm$ 0.25*	13.15 $\pm$ 0.47*	13.75 $\pm$ 0.25	887.50 $\pm$ 147.79**
31.5 g AEP (P4)	7.35 $\pm$ 0.56*	12.08 $\pm$ 1.60*	13.60 $\pm$ 0.23	838.00 $\pm$ 236.65**
Aquadest + BCG (P5)	6.75 $\pm$ 0.10	14.45 $\pm$ 0.30*	12.80 $\pm$ 1.21	968.00 $\pm$ 212.44**

\* significant (p,0.05)

\*\* significant (p,0.01)

### Platelet

Platelets are significantly higher in all treatments compared with controls. Platelet highest value at T5 treatment. AEP significantly increased platelets. Methanol extract of *Plectranthus amboinicus* leaves increase platelet in mice [10]. Platelet value of this research is still in the normal range is 638 - 1177 (10<sup>3</sup> / mL) [11] and 500-1000 (10<sup>3</sup> mL) [12]. Platelets, or thrombocytes is one of the haematological parameters are often used to determine the occurrence of agglutination and attack foreign substances. Platelets contribute to innate immunity in various ways, namely [1] the platelets have a basic activity as antibacterial and fagositotik and show interactions with bacteria, viruses and parasites. Bacterial interactions with platelets induces platelet activation and secrete antimicrobial peptides, [2] platelet containing several proinflammatory cytokines (eg IL-1) that modulate the inflammatory response / immune [13]. This shows the good functioning AEP in maintaining platelet function, in which case a very large increase in

the MVP can cause some diseases such as a coronary artery, acute inflammatory diseases, stroke and so on [14].The significant increase in platelets indicates that the plant compounds present in the extract promote blood clotting.

### Hematocrit, MCV, MCH, MCHC and ESR

Hematocrit percentage is the ratio of erythrocytes to the total blood volume. The percentage of hematocrit between 45-47%[8].Hematocrit and MCHC, did not show significant difference at all doses of administration (Fig 1).MCV increasing significantly in treatment P1, whereas the other treatments did not different significantly as compare with the controle (Figure 2). MCH or mean corpuscular hemoglobin was to measure the color indexes erythrocytes in the blood. In this study there were a significant increase MCH in treatment P1, P2, P3 and P4 compared to the control and P5 (Figure 3).Provision of 200 and 400 g / kg methanolic extract in mice no significant effect on MCV, MCH and MCHC [10]. But AEP significantly increase levels of MCH in this study. There was no significant difference in the levels of MCHC, although there was a trend increase in treatment P1, P2, P3, P4, and P5. MCHC levels was highest in treatment P1 (Fig 4).

Normally MCHC in male rats aged 17 months or more was 35.1 g / dl [11] whereas in this study the levels of MCHC mice treated EEP, the EEP + SDMD and SDMD are respectively 38.60, 37.67 and 36.00 g / dl.

Erythrocyte Sediment Rate (ESR) of blood is a non-specific indicator that can be used to diagnose several diseases with a wide spectrum. ESR tends to be associated with the presence of inflammation or infection, but may also help monitoring immune abnormalities, diabetes, tuberculosis, anemia, and even cancer [17]. In this study there is a significant difference between ESR in AEP treatment and control. AEP decreased ESR significantly with compare to the control so ESR profile profile is maintained.

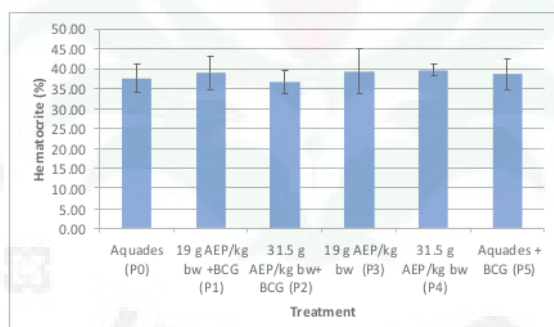


FIGURE 1. Hematocyte profile of rats by administration of aqueous extract of *Plectranthus amboinicus* Lour

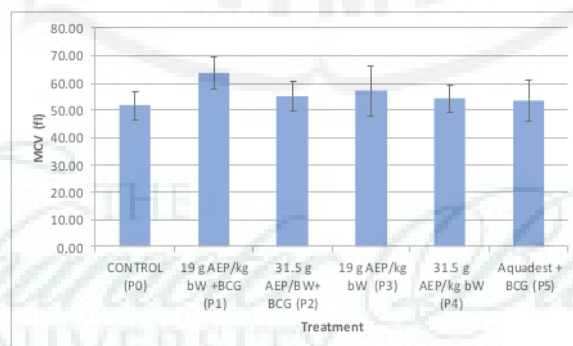


FIGURE 2. MCV profile of rats by administration of aqueous extract of *Plectranthus amboinicus* Lour

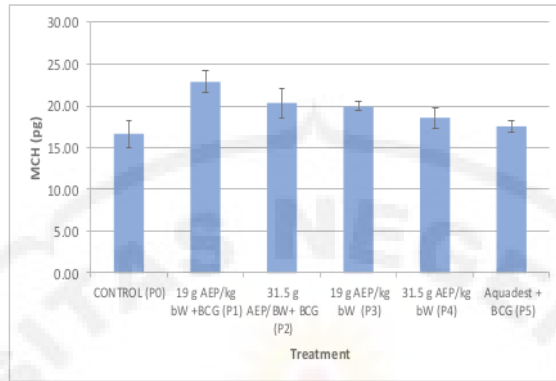


FIGURE 3. MCH profile of rats by administration of aqueous extract of *Plectranthus amboinicus* Lour

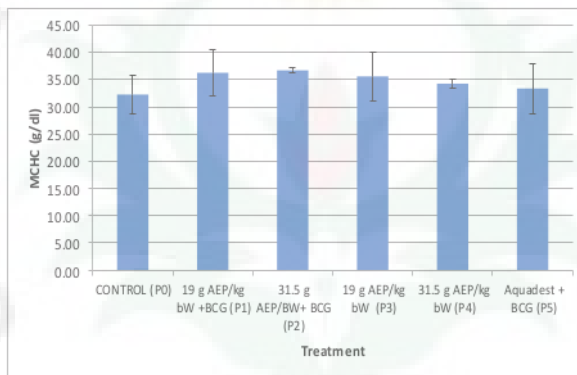


FIGURE 4. MCHC profile of rats by administration of aqueous extract of *Plectranthus amboinicus* Lour

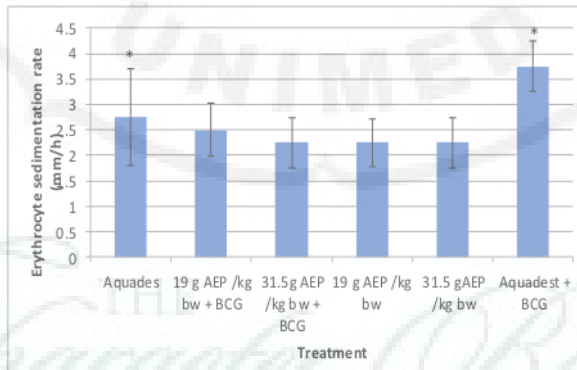


FIGURE 5. ESR profile of rats by administration of aqueous extract of *Plectranthus amboinicus* Lour

## CONCLUSION

WBC increased significantly in treatment AEP 31.5 g / kg bw, 31.5 g AEP / kg bw + BCG and so were only given BCG. RBC tend to increase in all AEP treatment but tends to increase again when given a BCG. Hb increased in treatment P1, P2, T3 and P4, but the improvement was significant only in treatment P1.

While PLT increase significantly in all treatments compared to the controls. HCT did not show significant differences but all of them were in the normal range. EAP without BCG and with the addition of BCG lowered ESR significantly, whereas BCG alone increased the ESR significantly. MCV increased significantly only in the treatment of P1 and show the same pattern with the MHC and MHCH. The conclusion that *Plectranthus amboinicus* Lour a positive impact on blood profiles with and without BCG. *Plectranthus amboinicus* Lour managed blood profile when administered together with BCG

## ACKNOWLEDGEMENT

We are grateful to the Direktorat Penelitian dan Pengabdian Kepada Masyarakat Direktorat Jenderal Pendidikan Tinggi Kementerian Pendidikan dan Kebudayaan (DRPM) which provide funding in 2016 for Fundamental Research.

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