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Modeling Dimensional Alterations Induced in Blood Platelets and Plateletcrit of Rats by Administration of Ethanolic Extract of Plectranthus amboinicus Lour

[1]Melva Silitonga, [2]Syafruddin Ilyas, [2]Salomo Hutahaean, [1]Herbert Sipahutar

[1]Biology Departmant, Faculty of Mathematic and Natural Sciences, State University of Medan, Medan Indonesia
[2]Biology Department Faculty of Mathematic and Natural Sciences, Universityof North Sumatera, Medan Indonesia

ABSTRACT

Plectranthus amboinicus Lour is a medicinal plant that has many benefits, such as an antioxidant andimmunostimulant. The purpose of this study is examine the effect of the ethanolic extract of *Plectranthus amboinicus* L (EEP) on the dimensions alteration of PLT, PCT, PDW and MPVin rats. Twenty four male wistar rats, 3 months in age was used in this study. Rats were divided into 4 groups, and each group consisted of six animals. Group I as a control was given 1% CMC, Group II was given 500 mg EEP/kg bw, group III was given 500 mg EEP/kg bw +SRBC and GroupIV was given SRBC only. EEP was given for 30 days, and SRBC was given on days 8 and 15. On the day 31, blood was collected by decapitation for hematology analysis. Data were analyzed by ANOVA using SPSS20. PLT and PCT wereincreased significantly on rats that was given EEP and EEP+SRBC. EEP does not have a significant influence on the MPV and PDW. Conclusion, dimentional alteration of PLT and PCT with administration EEP are the same increased model in treated with EEP and also EEP+SRBC simultaneously.

Keywords:*Plectranthus amboinicus* Lour, PLT, PCT, PDW,MPV

Corresponding Author: MelvaSilitonga, Biology Departmant, Faculty of Mathematic and Natural Sciences, State University of Medan, Medan Indonesia. E-mail: melvasilitonga@unimed.ac.id

INTRODUCTION

Immunostimulant are substances that can increase the body's resistance to infections. Various immunomodulators have been reported to enhance non-specific immunity. Immunomodulator is a way to restore and repair the compromised immune system and suppress excessive immune function [1]. Immunomodulatory agents that origin from plants and animals can increase the immune responsiveness of the body against pathogens by activating primarily the non-specific and specific immune system, example stimulation function and efficiency of the platelet and plateletcrit. However the drugs should be subjected to systematic studies to substantiate the therapeutic claims with regard to their clinical utility.

The uses of medicinal plants in traditional medicine are widespread and still serve as leads for the development of novel pharmacological agents. Many such medicinal plants have hepatoprotective, neuroprotective, anti-inflammatory and also antioxidant or radical-scavenging properties [2]. *Plectranthus amboinicus Lour*,(Spreng) (*Coleus amboinicus*, *Coleus aromaticus*) (Fig. 1) commonly known as Indian borage, country borage is a dicotyledonous plant belonging to Lamiaceae family. The content of the *Plectranthus amboinicus* leaves that serve as immunostimulant namely vitamin C [3]particular classes of flavonols .The benefits of *Plectranthus amboinicus* leaves as immunostimulatory will be assessed by measuring immunoglobulin [4] which acts as an antibody to kill the antigen enters

the body. In *Plectranthus amboinicus* leaves, there are also vitamin B1, vitamin B12, beta carotene, niacin, carvacrol, calcium, fatty acids, oxalic acid, and fiber[5]. These compounds have potential for a variety of biological activities, including as immunostimulan. *Plectranthus amboinicus* leaves as immunostimulatory to the fish[6]. *Plectranthus amboinicus* has many benefits, as an antipyretic, analgesic, wound medicine, cough, and thrush, antioxidant, antitumor, anticancer, and hypotensive [7]. Usually, drugs that having multifunction has receptors on the target organ limforetikular systems that perform immune functions. Bio-active substances which suspected in *Plectranthus amboinicus* leaves as immunostimulan are flavonoids, steroids,and polyphenols. Flavonoids are antioxidants to prevent the oxidation of low density lipoprotein (LDL) and lower risk of atherosclerosis. Antioxidants contained in food can stimulate cellular immunity and help preventing oxidative damage to cellular components. However there is no report on the immunostimulatory activity of *Plectranthus amboinicus Lour*.

Figure 1. *Plectranthus amboinicus* Lour Spreng

Platelets are anucleate cells that are crucial mediators of haemostasis and have inflammatory functions and can influence both innate and adaptive immune responses. Platelets are normally thought to be the primary cellular mediators of hemostasis and can encounter a variety of inflammatory processes. For years, however, data has been accumulating that platelets may not only be exposed to inflammation but may also work to mediate it directly[8]. For example, platelets contain and secrete several biological mediators that have no obvious role in hemostasis but significantly affect local innate immune responses by, for example, attracting neutrophils to sites of inflammation. In addition, platelets may directly regulate adaptive humoral immune responses by the expression and secretion of molecules such as CD40/CD40L. Platelets also avidly bind to microorganisms by expressingToll-like receptors (TLR). It appears now that platelets may act as circulating sentinel cells that encounter blood borne infectious products for presentation and activation of innate immune responses. However, there has been a gradual realization that platelets have important roles in modulating innate and adaptive immune responses. For example, platelets have been shown to have roles in the initiation of inflammation, angiogenesis, atherosclerosis, lymphatic development and tumour growth. How platelets multitask and perform such diverse

immune-related functions is still an enigma, but various lines of evidence suggest that many distinct facets of platelet biology are important. These include the unique origin and structure of platelets, their expression of immunomodulator molecules and cytokines and their ability to interact with various cells of the immune system. Interestingly, like erythrocytes, platelets are confined to the circulation and do not enter the lymphatic, so they primarily interact with leukocyte populations in the spleen or liver. Whether this attribute is important in how platelets may modulate the immune response is unknown.

Platelet indices consist of Mean platelet volume (MPV) (fL), Platelet volume distribution width (PDW) (%), Plateletcrit (PCT) (%), Mean platelet component (MPC) (g/dL), Mean platelet mass (MPM) (pg), Platelet component distribution width (PCDW) (g/dL), Platelet larger cell ratio (P-LCR) (%) and Immature platelet fraction (IPF) [9]. Among these platelet indices, plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW) are a group of platelet parameters determined together in automatic CBC profiles; they are related to platelets' morphology and proliferation kinetics Platelet parameters, which include the plateletcrit (PCT), platelet distribution width (PDW) and mean platelet volume (MPV), have been available in the laboratory routine using blood cell counters for several years [10].

Plateletcrit (PCT) is another platelet parameter, which is a reliable measurement of platelet biomass, because it combines the MPV with the absolute platelet count [11]. Platelet indices (PCT, PDW and MPV) are considered markers of platelet activation and are altered in different inflammatory diseases, such as inflammatory bowel disease [12], rheumatoid arthritis [13], familial Mediterranean fever [14] and PFAPA syndrome [15].

According to researches and opinions above, this research conducted to know dimensional alterations induced in blood platelets, plateletcrit, MPV and PDW of rats by administration of ethanolic extract of Plectranthus amboinicus Lour leaves as immunostimulan on rats. As antigen in this study was given Sheep Red Blood Cells (SRBC).

MATERIAL AND METHODS

Animals: Male rats 3 months and between 140-180 g in body weight) were used in the study. The animals were maintained at room temperature on 12 h light – 12 h dark cycle. 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 materials: The leaves from the plants (Fig. 1) were collected during the months of April and May of the year, cleaned with water, shade-dried using an oven with 40^{o} C temperature. The dried leaves were powdered mechanically and extracted 95% ethanol by using soxhlet extractor. After 72h extract was concentrated under reduced pressure (22-26 mmHg) at 50-60^{o}C (yield 6.2%). The liquid extract was then cooled and concentrated by

evaporation [16]. Ethanolic extract of *Plectranthus amboinicus* (EEP) was prepared at 500 mg / kg of body weight in dose [17] [18].EEP was dissolved in 1 % CMC [18] .

Sheep Red Blood Cells (SRBCs): SRBC antigen used is according to research conducted by [19]. Preparation is done at Veterinary Laboratories Medan, North Sumatra. Fresh blood was collected from sheep jugularry vein. Blood was washed with solution pH of 7.4 koliner diluent then centrifuged for 15 minutes at 2000 rpm. Sheep blood was taken and added to 5 ml as much as 5 ml alcevier with a ratio of 1:1. The results of centrifuges storage in the refrigerator -4oC. Sheep Red Blood Cells (SRBCs) was washed three times in normal saline and adjusted to a concentration of 0,1 ml containing 1 x 10^8 cells for immunization and challenge

Experiment design: Twenty four rats were taken and divided into four groups, each consisting of six rats. Group I served as rats treated control were given 1 % of CMC orally every day. Group II (EEP) served as rats treated with the extract 500 mg EEB/kg. Group III served as rats treated with 500 mg + SRBCs (EEP+SRBCs). Group IV served as rats treated with 0.1 ml SRBC. TheEEP was given orally every day for 30 days. SRBCs was given intramusculary on the 8 and 15 day of treatments.

Evaluation of platelet (PLT), plateltcrit (PCt), MPV and PDW: At the end of thirty one days again the blood samples were collected by decapitation in EDTA tube for PLT, PCT, MPV and PDW. Hematological parameters were evaluated with an automated hematological analyzer system analysis used ABX Micros 60

Data analysis: Data were analyzed using SPSS 20 software and the data were statistically analyzed using ANOVA program and the means evaluation was done using LSD test. A value of $p<0.05$ was considered as statistically significant. Results are presented as mean ±SD.

RESULTS AND DISCUSSION

Platelet:EEP and EEP+SRBC significantly increased PLt. The results are consistent with the results of research [2] which showed a tendency to increase platelets in mice with methanol extract of Plectranthus leaves. In this study platelet value was 650.00 ± 704.67 ± 125.4 and 93.5 respectively in the treatment of EEP and EEP + SRBC. This value was still within the normal range is 638 - 1177 (103 / mL) [20] and 500-1000 (103/ mL) [21]. Platelets, or thrombocytes, or often called pieces of blood is one of haematological parameters are often used to determine the occurrence of agglutination and attack foreign substances. This shows the potential of Pletranthus leaves as immunostimulatory by stimulating the synthesis of platelet. It is possible role in increasing platelet calcium and iron are contained in Plectranthus leaves.It has become clear that platelets are not simply cell fragments that plug the leak in a damaged blood vessel; they are, in fact, also key components in the innate immune system, which is supported by the presence of Toll-like receptors (TLRs) on platelets [22].In this study platelets in all of treatment groups were within the normal range is 500 - 1000 x 10^3 / µl of blood [21]. In this study the PLT and PCT concentrations increased significantly ($p<0.05$) not

only at 500 mg EEP/kg and also EEP+SRBC treatment (Fig. 2 and Fig.3). There is no significantly in MPV and PDW concentration in compare to the control.

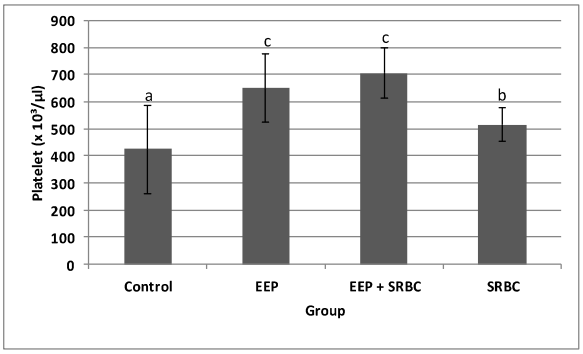

Figure 2. Alteration of Platelet

Plateletcrit: Plateletcrit (PCT) is another platelet parameter, which is a reliable measurement of platelet biomass, because it combines the MPV with the absolute platelet count [11]. In this research EEP and EEP+SRBC altered plateletcrit increased significantly in treatment (Fig. 3). The increase in plateletcrit (PCT) may be due to overproduction of hematopoietic regulatory elements such as colonystimulating factors, erythropoietin and thrombopoietin by the stromal cells and macrophages in the bone marrow [23], thus providing the local environment for hematopoiesis [24;26. From the observed significant increase in the values of WBC, it was clear that an increase in the number of WBC is a normal reaction of rats to foreign substances, which alter their normal physiological processes.

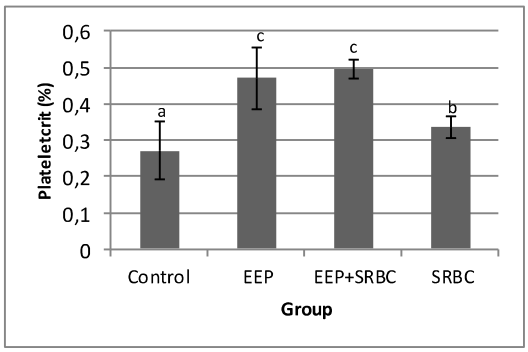

Figure 3. Alteration of Plateletcrith

Mean Platelet Volume (MPV): Mean platelet volume (MPV) is a measure of the platelet average volume commonly reported as part of the standard full blood count [9].The wider use of platelet function is the mean platelet volume (MPV), which describes the bone marrow production rate and platelet activation [24]. In this study MPV at EEP treatment, increased significantly is compare to controls but not significant on EEP + SDMD treatment. The results of this study are in line with the study of [2] in which the administration of EEP 200 mg in mice did not show significant changes in MPV levels. But with a higher dose of 2500 mg / kg bb in mice significantly increased MPV [25]. EEP administration in this study increase MPV only on EEP+SRBC treatment. This indicates a good function of EEP in

maintaining platelet function, which in the case of an enormous increase in MVP may cause some concomitants such as coronary arteries, acute inflammatory diseases, strokes and so on [26].

Platelet Distribution Width: PDW is the coefficient of platelet size variation. High levels of PDW can be found in sickle cell disease and thrombocytosis, whereas low PDW levels can show small platelets. In this study EEP administration has no significant effect on increasing PDW. PDW in this study was lower than the haematological data reported by [20].

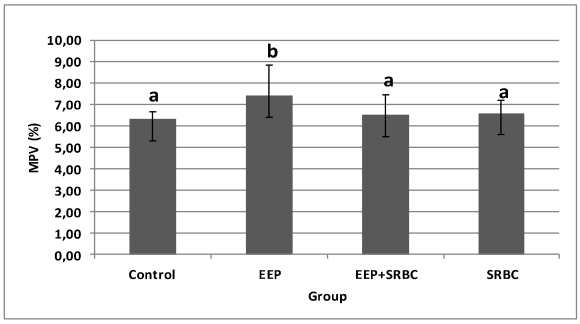

Figure 4. Alteration of MPV

The increasing PDW in this study (Fig.4) can not be explained in detail about EEP's contribution in increasing the PDW levels. But despite an increase in PDW in rats EEP treated, the EEP + SRBC values are within the range of nominal values

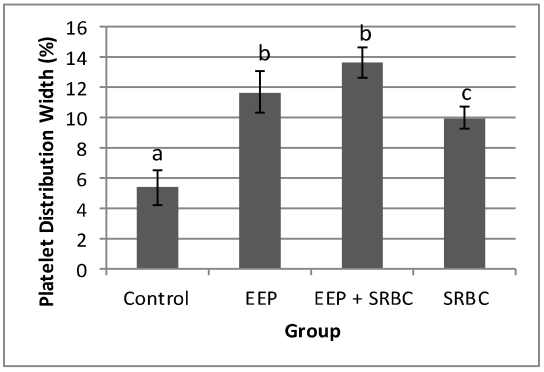

Figure 5. Alteration of PDW

In this study, EEP, EEP + SDMD no significantly effect on MPV. The results are consistent with research [2], where the provision EEP 200 mg in mice showed no significant changes in levels of MPV. Giving EEP in this study did not increase the MPV. This indicates that either EEP function in maintaining the function of platelets, in which case a very large increase in MVP can cause some diseases such as coronary artery, acute inflammatory diseases, stroke and so on [27]. So despite an increase in platelets in this study did not increase levels of MPV.

In this study, there was no significantly effect EEP improve PDW , but LSD showed PDW EEP treated mice was significantly different compared with controls. Rats PDWin this study is lower than the data reported by [20]. Increased PDW in this study cannot be explained

in detail on the contribution of EEP to increase the levels of the PDW. But despite the increase in mice treated PDW EEP, EEP + SDMD scores fall in the range of nomal value. It is well known that PDW is linearly correlated with MPV in normal individuals [28]

CONCLUSIONS

The significant increase in PLTs count might lead to thrombosis or aggregation of blood vessels. Alteration of platelet and plateletcrit are in same model. In this study EEP can increase PLT and PCT, becausePLTandPCTasone of the cellsof the immune system, then theeffectimmunostimulatEEPsignificantly improvethe PLTandPCT.

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