The Effect of Plectranthus amboinicus Lour Spreng Ethanolic Extract on Relative Organ, Body Weights Changes, and Hematology Profile in Wistar Rats Treated with 7,12Dimethylbenz(a)anthracene

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The Effect of Plectranthus amboinicus Lour Spreng Ethanolic Extract on Relative Organ, Body Weights Changes, and Hematology Profile in Wistar Rats Treated with 7,12Dimethylbenz(a)anthracene

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Abstract. This study aims to examine the protective properties of ethanol extract *Plectranthus amboinicus* (EEP) leaves on body weight gain, the relative weight of liver, kidney, lung, spleen, thymus and haematological profile of rats induced by 7,12Dimethylbenz(a)anthracene (DMBA). Used 25 female rats, divided into five groups namely NC (given Carboxymethyl cellulose (CMC) 1%), PC (given DMBA 20 mg/kg body weight), T1, T2, and T3 were each given DMBA 20 mg/kg body weight once every four days for 32 days and EEP 175, 350, and 700 mg/kg body weight given every day from day 33 to 59. Both the DMBA and EEP are given orally using a gastric gavage. On day 60, rats were killed by neck dislocation, bood collected in EDTA tubes for hematological analysis, rats are dissected to obtain liver, kidney, lung, thymus, and spleen organs. Data were analyzed with one way Analisys of varians (ANOVA). The results of this study indicate that EEP in T1, T2 and T3 treatments has no effect on weight loss compared to PC. There was no effect of EEP on the relative weight of the liver, kidneys, spleen, thymus and lungs. EEP increased the number of erythrocytes, leukocytes and platelets in rats that had DMBA

1. Introduction

7,12-Dimethylbenz(a)anthracene (DMBA) is an aromatic polycyclic hydrocarbon (PAH). It is carcinogenic compound, environmental pollutants, and pyrolysis products from oil and biological materials. Produced by cigarette smoke, vehicle smoke, and incomplete combustion from coal and petroleum fuels. It is known that DMBA as a specific carcinogenic compound for experimental breast and skin cancer [1] in experimental animals. The entry of DMBA into the body, in addition to causing breast and skin cancer, can also cause damage to the liver, kidneys, spleen, lungs, thymus, and blood. Therefore, these organs must be protected from the effects of the entry of toxic substances such as DMBA.

Organ weight is one of the most sensitive drug toxicity indicators, and its changes often precede morphological changes. Organ weight can be the most sensitive indicator of an effect of drug toxicity, as significant differences in organ weight between treated and control animals may occur in the absence of any morphological change. The role of the liver and kidney in the maintenance of life cannot be overemphasized. As the largest internal and major organ in the body, the liver metabolizes and detoxifies substances and also helps in the regeneration of body cells. The kidney, on the other hand, maintains health via its role in the elimination of waste materials such as urea, creatinine, water, etc and maintenance of body electrolytes. Failure by these organs in part or full to perform these life

functions impairs metabolic activities and causes accumulation of waste materials in the body, causing toxicity effects including and death [2,3,4]. The spleen, lungs, and thymus are also important organs that must be protected from damage due to toxic substances or carcinogens. DMBA administration also decreased the hematological profile significantly compared to the normal group. DMBA will attach hemoglobin to form hemoglobin adduct [5].

Because of the importance of the organs mentioned above, it is essential to find alternatives to protect them from the possible effects of toxic substances such as DMBA. Some effects of toxic substances in the body can be observed through body weight, relative weight, and morphology of these organs. Potential preventive to body organs can be observed through measurements of body weight and relative weight of organs [6].

Plectranthus amboinicus (PA) is a vegetable that has been investigated for its use as a cure for several diseases. PA functions as an antioxidant [7] and hepatoprotective [8]. As an antioxidant PA acts to protect the body against toxic substances so as not to damage the body's organs. The purpose of this study was to examine the effect of ethanol extract of PA leaves on body weight gain, relative weight of liver, kidneys, lungs, spleen and thymus of rats exposed to DMBA. The hematology profile and morphological changes of these organs were also examined as a result of exposure to DMBA and ethanol extract of PA.

2. Material and Methods

2.1. Animals

Male, wistar strain albino rats weighing about 170–190 g were obtained from Faculty of Pharmacy North Sumatera University Medan. The animals were housed in cages under proper environmental conditions and were fed with a commercial pelletted diet . The animals had free access to water. All the experiments were designed and conducted according to the ethical norms approved by North Sumatera University Biology Department Ethics Committee guidelines (No: 0453/KEPH-FMIPA/2019).

2.2. Source of Chemical

7,12Dimethylbenz(a)anthracene (DMBA) from D3254 Sigma-Aldrich used to induce damage to body organs. Another chemical, ethanol 96%, is obtained from Merck. All other chemicals used were of analytical grade.

2.3. Experimental Desain

This study uses a complete random design. The experimental animals were divided into five groups, each groups comprising of five animals.

Group 1 (NC) : Normal control rats fed with standard diet and pure drinking water
Group 2 (PC) : Rats were induced with providing 20g/kg of body weight DMBA through oral every four days for 59 days
Group 3 (T1) : Rats were induced with providing 20g/kg of body weight DMBA through oral every four day for 32 days, on the day 33 the EEP was given 175 g/kg body weight until the 59th day
Group 4 (T2) : Rats were induced with providing 20g/kg of body weight DMBA through oral every four day for 32 days, on the day 33 the EEP was given 350 g/kg body weight until the 59th day
Group 5 (T3) : Rats were induced with providing 20g/kg of body weight DMBA through oral every four day for 32 days, on the day 33 the EEP was given 700 g/kg body weight until the 59th day

After the experimental period, the rats were fasted overnight, anaesthetized with diethyl ether, and then killed by cervical decapitation.

2.4. Evaluation of Body Weight gains and Relative Organ Weight

Before being anesthetized, all rats were weighed. Weight gain is obtained from the initial and final weight differences. After the rat is killed, the liver, kidney, lung and thymus organs are separated from the body and then weighed using analytical scales. To get data on organ relative weight, was used formula …1

$$RW = \frac{W}{BW} 100 \text{ g} \quad .....1)$$

Were RW = relative weight of organ
W = weight of organ
BW = body weight

2.5. Data Analysis

Means and standard deviations of body gains and organ weights in each group of rats were calculated and differences between group means determined using the 2-way analysis of variance (ANOVA).

3. Result and Discussion

3.1 Relative Weight Organ

It is a common practice to present organ weight data relative to the animal's body weight since this will help to remove bias doe to differences in body weight, hence relative organ weights (ROW) was evaluated in this work. As can we seen from Figure 1, the ROW of the liver increases with exposure to DMBA. The increased of liver ROW is the same as the results of previous studies which explained that DMBA significantly increased the ROW when compared with the control group [9]. This increase was significant compared to the control and relative weight of the liver in all EEP treatments. Giving EEP after exposure to DMBA decreases the relative weight of the liver close to normal (Fig. 1).

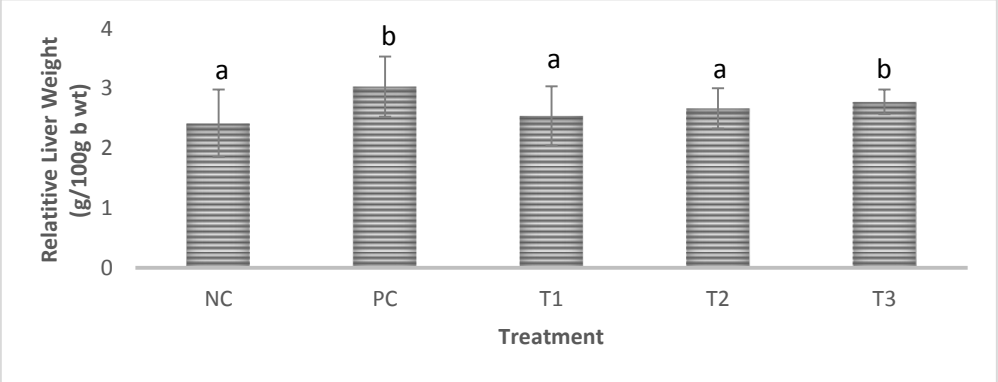

Figure 1. Relative weight liver in control and experimental groups of rats. Results are expressed as mean ± S.D for six rats in each group. Statistical significance at $p<0.05$ compared with NC group, PC group T1, T2 and T3 group. Relative Liver weight is expressed in grams

Figure 1 shows the relative liver weight of negative control and experimental group of animals. In group PC animals, rats that exposed DMBA increased significantly in the relative liver weight when compared with group NC and T1 rats. No obvious changes were observed between the negative control and EEP treatment. The liver is an important organ that functions to store all the nutritional products of digestion. So if it's possible to be exposed to DMBA one of the organs that is also exposed is the liver. Increases the relative weight of the liver in this research, because liver can adapt easily to changes in feed and the environment. Being at the center of a number of digestive, metabolic ad productive activities, it is esential to have a better understanding of this organ and the factors affecting

liver fungtionality [10]. Besides PA which has hepatoprotective activity [7] so it is able to protect the liver from toxic substances such as DMBA.

Lungs are important organs in the respiratory system and are very vulnerable to toxic substances in the environment. In this study DMBA significantly increased the lung weight of mice compared to controls. The same research has been reported by previous researchers [9] who found an increase in ROW of lungs due to DMBA exposure. However, in this study, if the rats exposed to DMBA were given EEP, the relative weight of the lung again decreased even though it was toward the normal relative lungs weight as in the NC group. Flavonoids are cytoprotective compounds that are present in dietary plants and vegetables [11]. *P amboinicus* contains flavonoids [12]. The anti-inflammatory and antioxidant properties of flavonoids make them likely candidates for evaluation for the treatment of inflammatory diseases including pulmonary diseases. So that the presence of flavonoids contained in P amboinicus heart can be protected from the influence of toxic substances, and the relative weight of these organs as described in this study becomes normal.

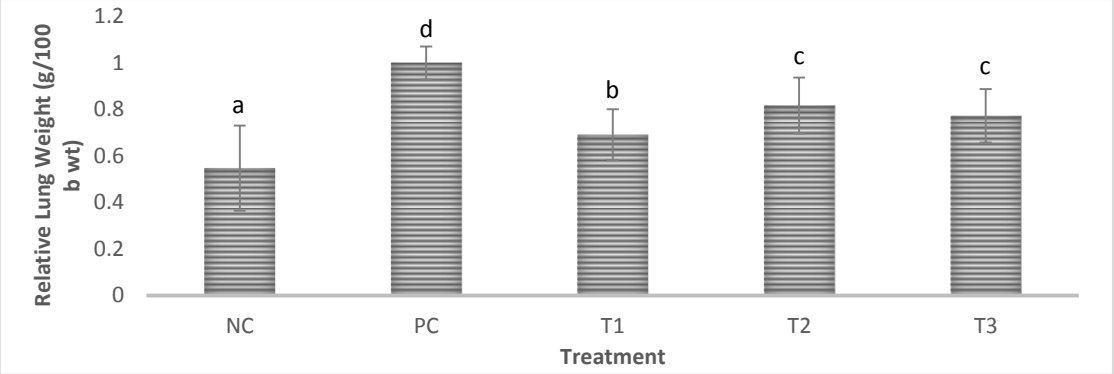

Figure 2. Relative weight Lung in control and experimental groups of rats. Results are axpressed as mean ± S.D for six rats in each group.Statistical significance at $p<0.05$ compared with NC group,
PC group T1, T2 and T3 group.

The kidney is an excretory organ that removes metabolised and non metabolised toxic materials from the body [8], hence this organ would be exposed to high concentrations of the noxious materials that could have caused the lesions. In this study the ROW of the kidney can be seen in Figure 3. DMBA does not have a significant effect on the relative weight of the kidney, but there is a slight increase in the relative weight of the kidney at T1, T2 and T3. *P amboinicus* is nephroprotective that can protect the kidneys from oxidizing agents or those that are clastogenic [13]. Thus even though exposed to DMBA, EEP can maintain it well so that it does not affect organ weight. *P amboinicus* components that are thought to play a role in this case are the flavonoid groups such as quercetin, luteolin and apigenin.

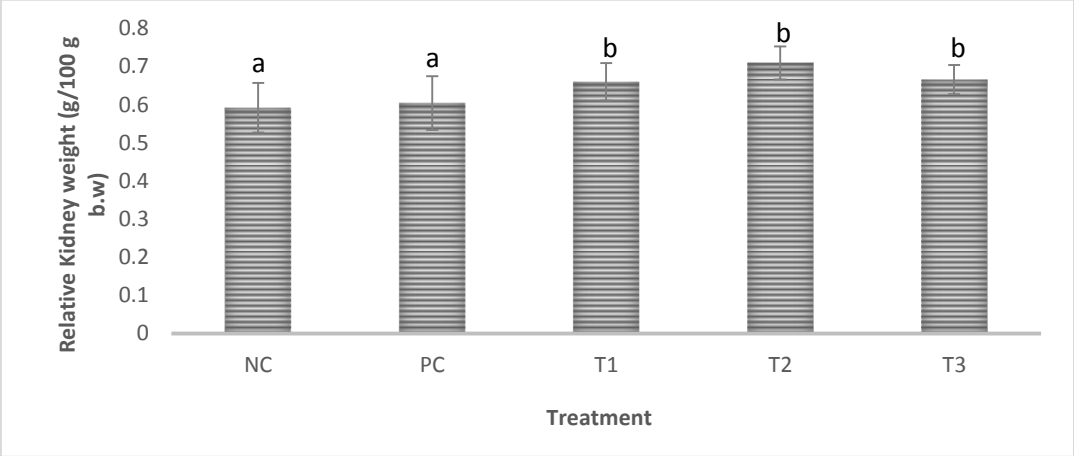

Figure 3. Relative weight Kidney in control and experimental groups of rats. Results are expressed as mean ± S.D for six rats in each group. Different letters to indicate significance at ($p<0,05$) compared with a group NC, PC group T1, T2 and T3 group.

The spleen plays multiple supporting roles in the body. It acts as a filter for blood as part of the immune system. Old red blood cells are recycled in the spleen, and platelets and white blood cells are stored there. The spleen also helps fight certain kinds of bacteria that cause pneumonia and meningitis. As a result of toxic substances the spleen can change in size or structure. DMBA in this research is toxic or even carcinogenic especially to mammary glands and skin. DMBA is also toxic to the spleen which can result in enlargement of sizes[14]. This can be seen in the results of this study (Figure 4) DMBA increases the relative weight of spleen organs. T1 is a group of rats exposed to DMBA 20 mg/kg body weight and given EEP 175 mg/kg body weight turned out to reduce the ROW of the spleen to normal. *P amboinicus* is non-toxic to the body and organs, but acts as an antioxidant that can protect the body [15]. The ROW of the spleen in the T2 and T3 groups showed an increase in the relative spleen weight. As an organ of the immune system the ROW of the spleen can increase due to immunostimulators affect that it as the results of previous studies. In this case the immunostimulator is *P amboinicus* [16]

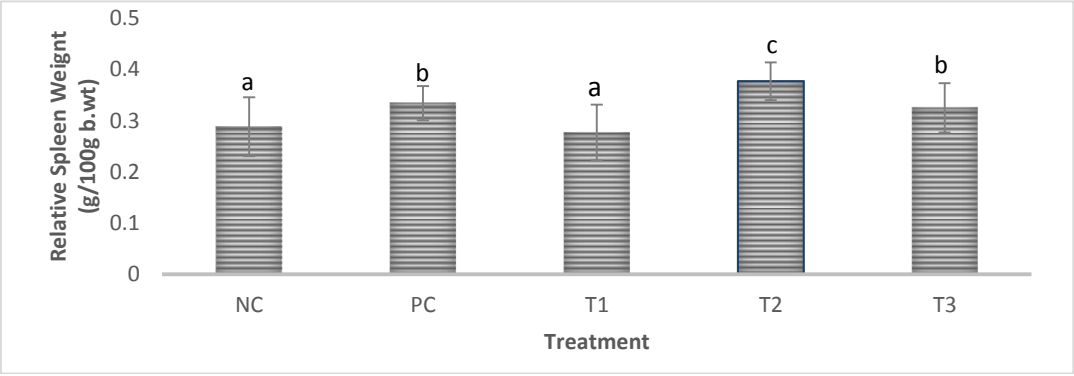

Figure 4. Relative weight Spleen in control and experimental groups of rats. Results are expressed as mean ± S.D for six rats in each group. Statistical significance at $P<0.05$ compared with with a group NC, PC group T1, T2 and T3 group. Group NC and T1 statistically different from group PC, T2 and T3 ($P<0.05$). Group T2 statistically different from NC, PC, T1, T2 and T3 ($P<0.05$).

In this study DMBA decreased the ROW of the thymus (Figure 5). The reduction in the ROW of the thymus in this study is in line with the results of previous studies [17] which explains that DMBA

decreases the weight ratio of thymic organs. In addition to decreasing the weight ratio of the pulmonary organs, DMBA also damages the thymus histology.

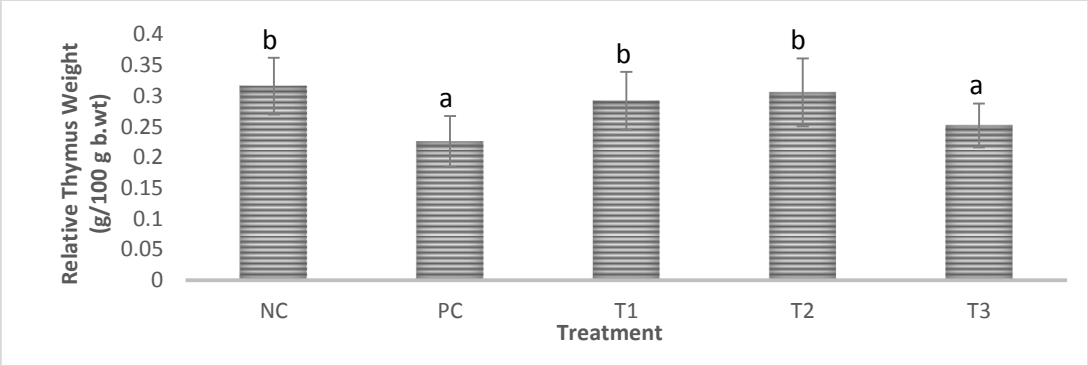

Figure 5. Relative weight Thymus in control and experimental groups of rats. Results are expressed as mean ± S.D for six rats in each group. Statistical significance at $p<0.05$ compared with with a group NC, PC group T1, T2 and T3 group. Group PC and T3 statistically different from group NC, T1 and T2 ($p<0.05$).

The administration of EEP in rats exposed to DMBA in this study significantly increased the ROW of the thymus compared to those only exposed to DMBA. Enhancing the relative weight of the thymus is almost reaching the relative weight of the control thymus. *P amboinicus* contains various flavonoids such as quercetin. Quercetin is an antioxidant [18] which can protect the body from oxidizing agents such as DMBA.

3.2 Body Weight Gain

Body weight is one important parameter that must be considered in testing a drug or other treatment. The toxic effects of a substance will also affect body weight. In this study weight gain was measured before and after the treatment ended. Weight gain was obtained from the difference in final weight with the start of treatment. In Figure 6 can be seen the weight gain of mice for all groups.

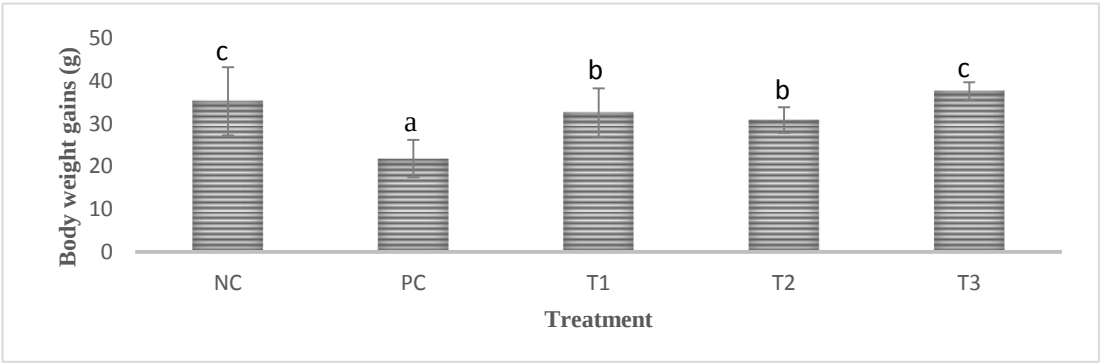

Figure 6. Effect of EEP on body weight gains in experimental group of animals. Body weight gains is expressed in grams, all the values are expressed as mean ± S.D (n=6). Statistical significance was determined by one way ANOVA followed by Tukey post hoc test; diffrent letters to indicate significant on $p<0.05$ compared with group NC, PC group T1, T2 and T3 group. Group PC statistically different from all group ($P<0.05$). group NC and T3 statistically different from group T1 and T2 ($p<0.05$).

Figure 6 showed the body weight gains of negative (NC) and positive control (PC) and also treated etanolic *P amboinicus* Lour group of rats. In PC group rats induced of DMBA, there is a significant decrease in the body weight gain when compared with group negative control, T1 and T2. In T3 group

there is significant increased in body weight gain if compare with PC group. The ethanolic extract of *P amboinicus*-treated groups T1, T2 and T3 showed a significant increase in the gain body weight when compared with group PC animals. During the course of the experiment, all rats showed the greater tolerance to treatment with EEP.

P amboinicus contains flavonoids which in its metabolism in the body will enter the body's organs such as liver, kidney, lung and other organs. Flavonoids in food will enter the small intestine in the form of rings [19]. From the small intestine flavonoids will be absorbed and then enter the liver, from the liver will be distributed to the kidneys and other organs. Not all of it is absorbed into the liver, some flavonoids enter the colon and from the colon enters the heart again in the form of broken rings. With the entry of flavonoids into the organs of the body, these organs are protected from the attack of toxic substances such as DMBA in this study.

3.3 Hematology Profile

A complete hematology test is a complete blood examination that includes leukocytes, erythrocytes and platelets. In this study DMBA reduced erythrocytes, leukocytes and platelets in mice. The results of this study have been reported by previous researchers [20, 21]. In rats exposed to DMBA and given *P amboinicus* extract, blood profile was restored to normal levels (Table 1).The role of P amboinicus ethanol extract in this case is to regulate the blood profile to remain normal [16,22] like the results of previous studies [15] leukocytes in this study increased significantly in mice exposed to DMBA and were given ethanol extract P amboinicus. Increase in WBC may indicate the impact of PA in boosting the immune system of treated groups.

Table 1. Hematology Profile of rats induced DMBA and treatment EEP

Group	Erytrocyte	Leucocyte	Trombocyte
CN	$7{,}40 \pm 0{,}58^{b}$	$5{,}62 \pm 1{,}71^{b}$	$697{,}33 \pm 218{,}02^{b}$
PC	$6{,}71 \pm 0{,}50^{a}$	$4{,}62 \pm 1{,}29^{a}$	$636{,}75 \pm 218{,}55^{b}$
T1	$6{,}89 \pm 0{,}52^{b}$	$6{,}82 \pm 2{,}45^{c}$	$775{,}50 \pm 104{,}55^{c}$
T2	$7{,}06 \pm 0{,}69^{b}$	$7{,}10 \pm 1{,}84^{c}$	$462{,}75 \pm 96{,}87^{a}$
T3	$7{,}84 \pm 0{,}29^{b}$	$10{,}97 \pm 1{,}10^{d}$	$932{,}60 \pm 219{,}55^{d}$

All are expressed as mean ± SD for groups of six rats in each. One-way ANOVA followed by Tukey post hoc test. $p < 0.05$ as compared with control animals. DMBA decreases the levels of erytrocyte, group PC statistifically different from all group. DMBA decreases the levels of leucocyte, group PC statistifically different from all group, group T1 and T2 statistifically different from group CN, PC and T3. DMBA decreases the levels of trombocyte, group CN and PC statistifically different from group T1, T2 and T3 ($p < 0.05$).

4. Conclution

Decreased body weight gain by DMBA is significantly if compare with the negative control group. Administration of ethanolic extract of *P amboinicus* in rats exposed to DMBA restored weight that had decreased due to exposure to DMBA that it became normal again. Relative weight of liver, lungs and spleen was increased significantly in DMBA treatment, but etanolic extract of *P amboinicus* Plectranthus ethanol extract every day for 27 days to restore the relative weight of the liver and lungs to be the same as normal. There is no effect of amboinicus on kidney ratio. DMBA significantly decreases the relative weight of thymus compared to controls, but etanolic extract of *P amboinicus* increase the relative organ weight significantly if compare with DMBA group. DMBA decreases the levels of erythrocytes, leukocytes and platelets. amboinicus ethanol extract has a significant effect on the number of erythrocytes, leukocytes and platelets.

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