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ANTITHROMBOCYTOPENIA ACTIVITY OF PEANUT SHELL (*Arachis hypogea* L.) EXTRACT AND INFUSA ON HEPARIN INDUCED BALB/C MICE

Aktivitas Anti Trombositopenia Ekstrak dan Infusa Kulit Batang Ari Kacang Tanah (Arachis hypogea L.) pada Mencit Balb/c yang Diinduksi Heparin

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ABSTRACT

Certain group of people empirically use peanut shell stew in the treatment of thrombocytopenia due to dengue fever. The active compounds suspected to play a role in the activity of thrombocytopenia from peanut shell are flavonoids. Flavonoids can be extracted using heat and cold methods. This study aims to determine the antithrombocytopenia activity of peanut shell extract and infusa on heparin induced Balb/C mice. This study is an experimental research with randomized matched pre and postest control group design. Peanut shell extraction was done in a cool way by maseration using 70% ethanol solvent, and hot way by infundation using aquadest solvent. Forty-eight Balb/C mice were divided into 8 groups consisting of control group CMC Na 0.5 mL/20gBW/day, control group aquadest 0,5mL/20gBW/day, 3 groups of peanut shell ethanol extract (0.019, 0.038, and 0.076) g/20gBW/day, and 3 groups of peanut shell infusa (0.026, 0.052; 0.104) g/20gBW/day. All treatments were given orally. The decrease of platelet count in Balb/C mice using 26 UI/20gBW subcutan heparin induction. The measurement of the platelet count is performed by taking blood samples in the lateral veins of the tail. Platelet counts data before and after treatment was tested with dependent T-test. Differences in platelet increases across the groups were tested by 2 way anova and continued with a LSD test with 95% confidence level. The results showed that peanut shell ethanol extract had better antitrombocytopenia activity than peanut shell infusa.

Keywords: *Anti thrombocytopenia*, *shell*, *peanuts*, *infusa*, *extract*.

ABSTRAK

Beberapa kelompok masyarakat tertentu secara empiris menggunakan rebusan kulit ari kacang tanah (KAKT) dalam mengatasi trombositopenia akibat demam berdarah. Senyawa aktif yang diduga berperan dalam aktivitas trombositopenia dari KAKT adalah flavonoid. Flavonoid dapat diekstraksi menggunakan cara panas dan dingin. Penelitian ini bertujuan untuk menentukan aktivitas anti trombositopenia ekstrak dan infusa kulit ari kacang tanah pada mencit Balb/C yang diinduksi heparin. Penelitian ini merupakan penelitian eksperimental dengan rancangan *randomized matched pre and postest control group design*. Ekstraksi dilakukan dengan cara dingin yaitu maserasi menggunakan pelarut etanol 70%, dan cara panas yaitu infundasi menggunakan pelarut aquadest. Empat puluh delapan ekor mencit Balb/C dibagi menjadi 8 kelompok yang terdiri dari kelompok kontrol CMC Na 0,5 mL/20gBB/hari, kelompok kontrol aquadest 0,5mL/20gBB/hari, 3 kelompok ekstrak etanol (0,019; 0,038; dan 0,076) mg/20gBB/hari, dan 3 kelompok infusa (0,026; 0,052; 0,104) g/20gBB/hari. Seluruh perlakuan diberikan secara per oral. Penurunan jumlah trombosit pada mencit Balb/C menggunakan induksi heparin 26 UI/20gBB subcutan. Pengukuran jumlah

trombosit dilakukan dengan pengambilan sampel darah pada vena lateral dari ekor. Data jumlah trombosit sebelum dan sesudah perlakuan diuji dengan T-test dependent. Perbedaan peningkatan jumlah trombosit seluruh kelompok diuji Anova 2 arah dan dilanjutkan uji LSD dengan taraf kepercayaan 95 %. Hasil uji menunjukkan bahwa aktivitas antitrombositopenia ekstrak etanol kulit ari kacang tanah lebih baik daripada infusa kulit ari kacang tanah.

Kata Kunci: anti trombositopenia, kulit ari, kacang tanah, infusa, ekstrak.

INTRODUCTION

Dengue fever is an acute febrile illness that is found in most tropical and subtropical regions, especially Southeast Asia. The cause of dengue fever is dengue virus that is transmitted through *Aedes aegypti* mosquito bites. One of the symptoms of dengue fever is a plasma leak due to damage of capillary walls. Plasma leakage can cause a decrease in platelet count resulting in a thrombocytopenia state, which, if not treated promptly, can cause bleeding and shock (hypovolaemic shock), which can lead to death in most cases of dengue (Depkes RI, 2016).

The basic management of dengue fever is rehydration and antipyretic therapy (Rampengan, 2007). Treatment of dengue fever can be maximized by the provision of natural medicines as complement therapy. One of the natural medicines used empirically by the community is the peanut shell. Dewi *et al*., (2013) identified the content of peanut shell's ethanol extract, among others, tannins, polyphenols, flavonoids, alkaloids, and terpenoids. Win *et al*., (2011) proved that the content of the peanut shell is mostly a phenolic compound. Research conducted by El-Baroty *et al.* (2014) identified phenolic compounds in the methanol extract was 71.06 mg/100 g more than the water extract (41.64 g/100 g). Flavonoid compound in ethanol extract of betadin plant stem (*Jatropha mulitifida* Linn) has been proven to increase blood platelets (Sundaryono, 2011).

Flavonoid compounds in plants are vary that is polar or non polar. Polar flavonoid compounds are present in the form of glycosides in which one of the hydroxy groups is substituted with sugar, and it is soluble in water or ethanol. Ethanol 70% can attract polar and non polar compounds compared with high purity ethanol. Flavonoid compounds can be extracted by means of heat or cold (Bimakr, 2010).

Furthermore, how the flavonoids contained in peanut shell affect the thrombocytopenia is important to be explored. Empirically many people use peanut shells in the treatment of dengue by boiling 10 grams of peanut shell in 1000 mL of water (Dyah, 2016), but there has never been a study examining the effectiveness of peanut shell to elevate blood platelet levels either infusa or extract. So it needs to be studied more about the anti thrombocytopenia of infusion and maceration of peanut shell.

METHOD

This research was an experimental research with a pre and post-test controled group design. This study had approved by The Bioethics Commission of Medical/Health Research, Medical Faculty, University of Islam Sultan Agung Semarang, to be carried out on animal testing with ethical clearance number 96/II/2018/Komisi Bioetik. Exctraction process was done in Biological Laboratory of Pharmacy Faculty, Wahid Hasyim University. Antithrombocytopenia test was done in Pharmacology and Clinical Laboratory of Pharmacy Faculty, Wahid Hasyim University. The extraction methods are infundation and maceration. Forty-eight healthy Balb/C mice (calculation of the number of test animals using Federer's formula (Federer, 2011)) aged 6-

8 weeks weighing 20-25 g, acclimated for 7 days, and then were divided into 8 groups consisting of control group CMC Na 0.5 mL/20 gBW/day, control group aquadest 0,5mL/20gBW/day, 3 groups of peanut shell ethanol extract (0.019, 0.038, and 0.076) g/20 gBW/day, and 3 groups of peanut shell infusa (0.026, 0.052; 0.104) g/20gBW/day. All mice were counted of the platelets number and then given 26 UI/20gBB subcutaneous heparin inductions (human dose conversion 5,000 UI every 12 hours) (Bakta, 2006). Twenty four hours later, the number of platelets was recalculated to determine the occurrence of thrombocytopenia. Furthermore, the subjects were treated according to the doses treatment orally. Twenty four hours after treatments, the number of platelets was counted to determine the effectiveness of peanut shell infusa and extract to elevate blood platelet levels (Sundaryono, 2011; Kartika, 2016). Blood platelet levels were determined using the Rees Ecker method (Gandasoebrata, 2004).

Dose of peanut shell ethanol extract was referring to empirical usage of boiling 10 grams of peanut shell in 1000 mL of water (Dyah, 2016). The percentage of peanut shell weights on peanuts and yield of extract were 24.5% and 29.82%, respectively. The conversion factor from human to mouse was 0.0026 and pharmacokinetics factor is 10, then the dose of peanut shell ethanol extract for the mice was 0.0026 x 10 x 10 g x 24.5% x 29.82% = 0.019 g/20gBW. The dose is defined as the lowest dose. Three rank doses use a multiple of two (Kementerian Lingkungan Hidup, 2015) to obtain a dose sequence of 0.019; 0.038; 0.076 g/20gBW.

The same base was used as a reference dose of peanut shell infusion. Ten grams of peanut shell multiplied by the dose conversion factor in the mice of 0,0026 for weight 20 gram where the dose was determined as the lowest dose to be given. Three rank doses use a multiple of two so that the dose sequence is 0,026g/20gBW; 0.052g/20gBB; and 0.104g/20gBW.

Materials and Tools

The peanut use in this study was obtained from the Pledokan Village, Dusun Resowinangun RT.01 RW.04 Semarang Regency, Sumowono Sub-District. The solvent extractions were ethanol (Brataco, 70%) and aquadest (Brataco). The ingredients for therapeutic effectiveness test of platelet count were heparin (Fahrenheit, Invicolt 5000 IU/mL) as thrombocytopenia inductor, Rees Ecker solution (Indo Reagen) as dilution solution to calculate platelet number, Aquadest (Brataco) and CMC-Na (Brataco) as thrombocytopenia control.

The tools used to make the peanut shell infusion as follows electric scales (Ohaus Pioneer), oven (Pyramid and Sense), wooden spoon, electric stove (Maspion), infusa pot, thermometer (Sellery), spoon, flannel, funnel, and glassware (Iwaki Pyrex). The tools used to test the effectiveness of platelet count enhancers were test animal scales (Acis), mouse cages, peroral sonde for mice, disposable syringes (Terumo), erythrocyte pipes (Assistant), glass decks (ISO Lab), hemocytometers (Neubauer), scalpels (Lotus), microscopes (Yazumi), and camera (Iphone 5s).

The Extraction Process

Five kilograms of peanut seeds were cleaned and roasted at a temperature of 40ºC. Dried peanut was characterized by changing the color of the epidermis and easily destroyed when kneaded. Peanut seeds then immediately sifted so that the epidermis of peanuts regardless of the seeds. Water content of peanut shell was measured by means of moisture balance. Peanut shell with water content <10% reduced size by blender.

The extraction by the maceration method was made by weighing 1.210 g of peanut shell powder, inserted in the container plus 70% ethanol as much as 1:10 (1.210 g of peanut shell : 12.100 mL of 70% ethanol). The initial step 75% of the solvent (9.075 mL) was used for the maceration stage for 3 days, 25% of the solvent (3.025 mL) was used for the remaceration stage

for 2 days. The maceration container is conditioned to be protected from light in order to avoid decomposition of the compound content while being shaken repeatedly. The maserate concentrate was thickened using a rotary evaporator at 40ºC.

The extraction with infusa method was performed by weighing 1.04, 2.08, and 4.16 grams of peanut shell infused in each 20mL of aquadest for 15 min which begins to be calculated when the temperature reaches 90ºC while stirring, then the solution was filtered using a clean flannel cloth and placed in flask.

Calculation of Platelet Count

The calculation of platelet number was using the direct method with Rees Ecker solution, where the solution serves as a coloring agent in platelets. The sodium citrate contained in the Ress Ecker solution use as a preventive coagulation and maintaining red blood cells; formalin as a blood fixation; whereas blue dye on platelets is obtained from briliant crecyl blue. Blood was taken through a lateral vein in the tail of a mouse using an erythrocyte pipette. The first blood drop on the tail of the mouse was discarded and the next droplet taken with an erythrocyte pipette until the "0.5" mark. Then the Rees Ecker solution was sucked up to the "101" mark, then the dilution occurs 200 times. The blood suspension and Ress Ecker solution were shaken until homogeneous, then the mixture was dropped on the Neubauer count chamber measuring 1 × 1 mm^3 and observed under a 40 × magnification microscope. Platelet count data obtained from the number of platelets seen in the counting booth then multiplied 2000. Platelets under a microscope looks shiny light blue small size is around erythrocytes, dispersed or clustered (Gandasoebrata, 2004).

Data Analysis

The quantitative data obtained was the number of Balb/C mice platelets before and after treatment. The platelet count data in all treatment groups fulfilled the normality requirement, then tested using Dependent T-test to see if there was any significant difference between platelet number before and after treatment of each group. The increase in platelet number between the groups should be meet to the normality and homogeneity requirements, then the difference were tested with 2 ways Anova and continued with Tukey test. Data analysis used 95% of confidence level.

RESULTS AND DISCUSSION

Balb/c mice were used in this study because they have a responsive and easy-to-observe for its immune system (Wahidah, 2010). The age of Balb/c mice used was 6-8 weeks, describing the condition of adult age in humans. Balb/c mice used are male because the immune system is not affected by reproductive hormones (Inayah, 2008).

Heparin can cause a state of thrombocytopenia in the form of heparin induced thrombocytopenia (HIT). The mechanism of heparin in causing thrombocytopenia was in the increasing of antithrombin activity and the formation of antibodies against platelets forms the heparin-platelet factor complex 4 so that thrombocyte is easily destroyed by the macrophage system in the liver and spleen, more than 50% (Bakta, 2006). Mice are said to be thrombocytopenia when platelet number after heparin induction are significantly lower than platelet number before heparin induction.

The platelet count before heparin induction in this study was calculated on the first day to see that the platelet number of mice were within the normal range according to the Minister of Environment and Forestry of the Republic of Indonesia, which 960,000 – 1,600,000 /µL of blood.

The average comparison of platelet number before and after 24 hours of heparin induction in all treatment groups was presented in Figure 1. This research can be concluded that Balb/c mice have thrombocytopenia after 24 hours of heparin induction, where platelet number after treatment is lower and significantly different when compared with platelet number before treatment ($p < 0.05$). This is consistent with the Bakta's research on 2006 that thrombocytopenia due to heparin may occur within 24 hours to 5 days after heparin administration.

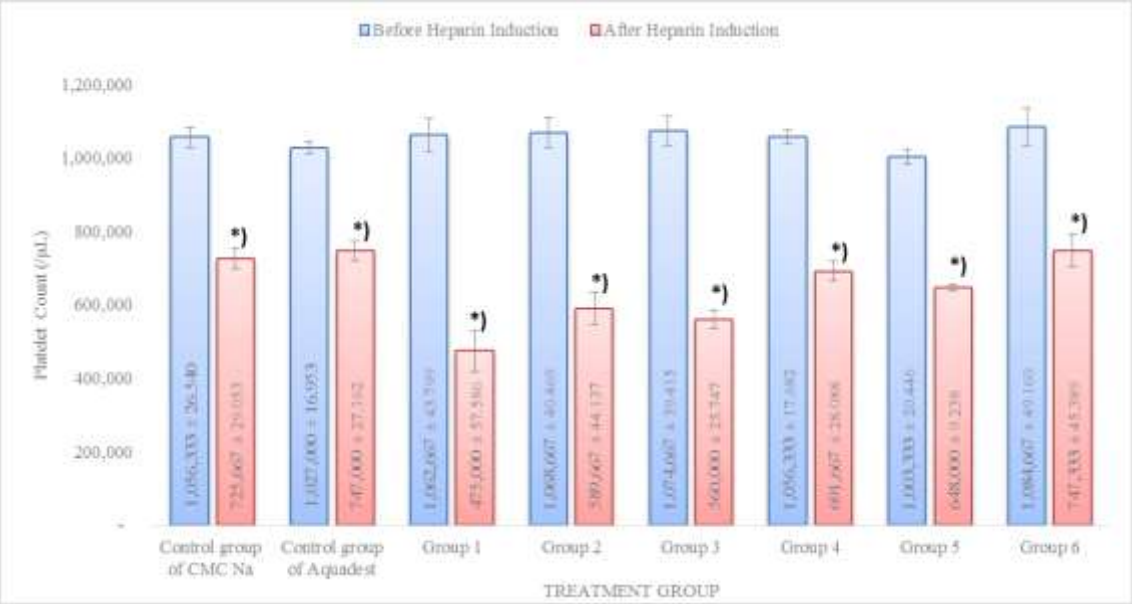

Figure 1. The average of platelet counts before and after 24 hours of heparin induction

Information :
Control Group of CMC Na : Treatment group of CMC Na with a dose of 0.5 mL/20gBW/day
Control Group of Aquadest : Treatment group of aquadest with a dose of 0,5mL/20gBW/day
Group 1 : Treatment group of peanut shell ethanol extract with a dose of 0.019 g/20gBW/day
Group 2 : Treatment group of peanut shell ethanol extract with a dose of 0.038 g/20gBW/day
Group 3 : Treatment group of peanut shell ethanol extract with a dose of 0.076 g/20gBW/day
Group 4 : Treatment group of peanut shell infusa with a dose of 0.026 g/20gBW/day
Group 5 : Treatment group of peanut shell infusa with a dose of 0.052 g/20gBW/day
Group 6 : Treatment group of peanut shell infusa with a dose of 0.104 g/20gBW/day
*) : Dependent t-test resulted platelet count after treatment is lower and significantly different when compared with platelet count before treatment ($p < 0.05$)

Platelet number before and after treatment can be seen in Fig. 2. CMC Na was used as a control of thrombocytopenia from ethanol extract treatment of peanut shell. CMC Na as a carrier substance because ethanol extract of peanut shell can not dissolve with aquadest. While the aquadest control group was used as a control group of thrombocytopenia from peanut shell infusa treatment. Both control groups are simultaneously for the method validation process, that as an extract carrier or as a solvent solution should not have the effect of increasing platelets. The results of data analysis showed that both CMC Na and aquadest did not have activity to increase platelet count (p. 0.082 and 0.056).

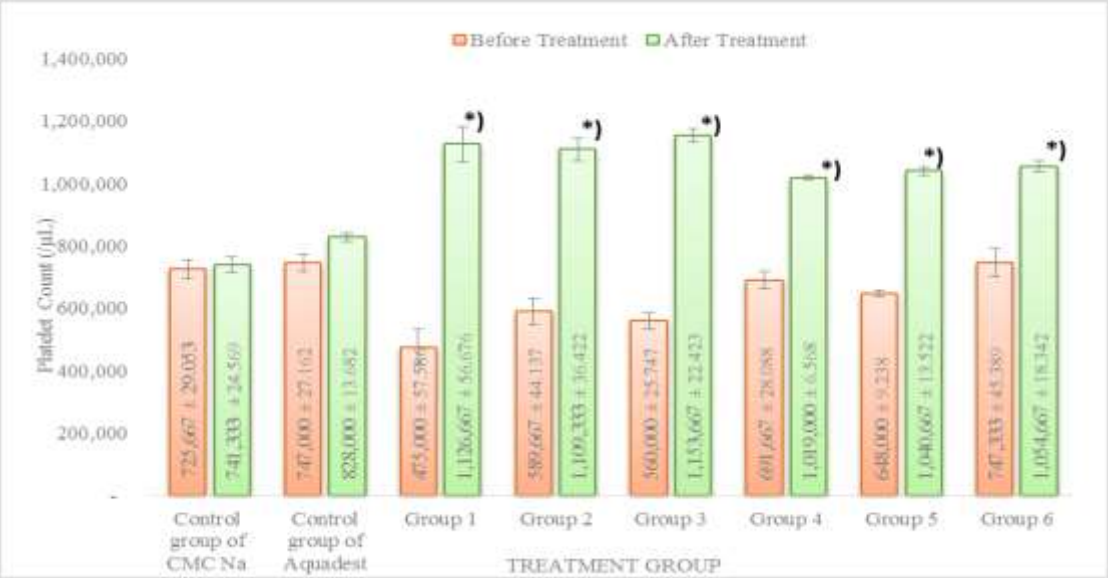

Figure 2. The average of platelet counts before and after 24 hours of treatment

Information :
Control Group of CMC Na : Treatment group of CMC Na with a dose of 0.5 mL/20gBW/day
Control Group of Aquadest : Treatment group of aquadest with a dose of 0,5mL/20gBW/day
Group 1 : Treatment group of peanut shell ethanol extract with a dose of 0.019 g/20gBW/day
Group 2 : Treatment group of peanut shell ethanol extract with a dose of 0.038 g/20gBW/day
Group 3 : Treatment group of peanut shell ethanol extract with a dose of 0.076 g/20gBW/day
Group 4 : Treatment group of peanut shell infusa with a dose of 0.026 g/20gBW/day
Group 5 : Treatment group of peanut shell infusa with a dose of 0.052 g/20gBW/day
Group 6 : Treatment group of peanut shell infusa with a dose of 0.104 g/20gBW/day
***)** : Dependent t-test resulted platelet count after treatment is higher and significantly different when compared with platelet count before treatment ($p < 0.05$)

The results of dependent t-test showed that ethanol extracts of peanut shell (doses of 1-3) and peanut shell infusion (doses of 4-6) were able to increase platelet count after 24 hours of treatment significantly. The increase in platelet number in this study was likely to occur from flavonoid compounds contained in peanut shells. Sundaryono (2011) was stated that the administration of total flavonoid compounds from *Jatropa multifida* Linn stem orally with a dose of 0.028 mg/KgBW on *Mus musculus* male Swiss Webster was able to raise the platelet number by 543,000/mm^3, while at a dose of 0.056 mg/KgBW can increase the number of platelets by 813.000/mm^3.

The increase of platelet number in the three groups of peanut shell ethanol extracts, and the three groups of peanut shell infusa (figure 3) were then analyzed using Two Way Anova to determine if there were significant differences between treatment groups. The result of the analysis shows the difference between groups.

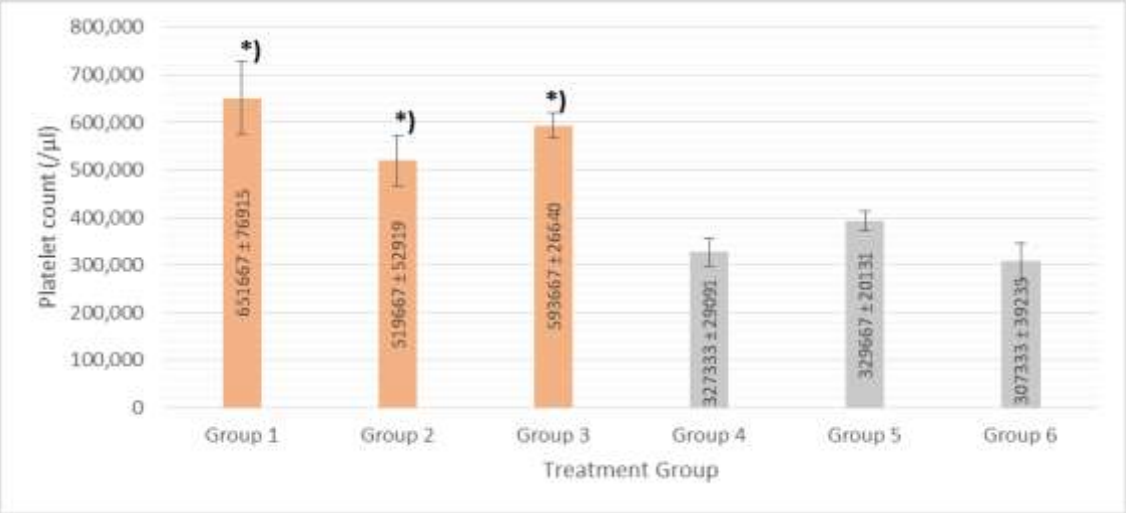

Figure 3. The increase of platelet counts after 24 hours of treatment group

Information :

Group 1	: Treatment group of peanut shell ethanol extract with a dose of 0.019 g/20gBW/day
Group 2	: Treatment group of peanut shell ethanol extract with a dose of 0.038 g/20gBW/day
Group 3	: Treatment group of peanut shell ethanol extract with a dose of 0.076 g/20gBW/day
Group 4	: Treatment group of peanut shell infusa with a dose of 0.026 g/20gBW/day
Group 5	: Treatment group of peanut shell infusa with a dose of 0.052 g/20gBW/day
Group 6	: Treatment group of peanut shell infusa with a dose of 0.104 g/20gBW/day
***)**	**:** LSD test resulted that increase of platelet counts of the peanut shell ethanol extract treatment group was significantly higher ($p < 0.05$) than the peanut shell infusa treatment group

The data analysis followed by LSD (Least Signifficant Difference) to determine which group had a significant difference ($p < 0.05$). The result of LSD test showed that the ability to increase the platelet number in the peanut shell ethanol extract treatment group was significantly better ($p < 0.05$) than the peanut shell infusa treatment group, which mean that peanut shell ethanol extract had better anti thrombocytopenia activity than peanut shell infusa. It was estimated that the cold extraction process using ethanol was able to dissolve active compounds that allegedly to increasing platelets properties better than heat extraction process that may damage the active compound. The increasing of dose for both ethanol extract and infusa of peanut shell do not result in increasing of platelet number.

The safety of peanut shell extract and infusa has been tested on Balb/C mice resulted single dose of peanut shell infusa with a dose of 0.026 g/20 gBW until 0.208 g/20 gBW did not cause death in mice (Fithria dkk, 2018[a]), and peanut shell extract had LD_{50} of 0,129 g/20g BW which was practically non toxic (Fithria dkk, 2018[b]).

CONCLUSIONS

Peanut shell ethanol extract was able to increase the platelet number significantly better than peanut shell infusa as follows of 651667±76915, 519667±52919, and 593667±26640/µl.

RECOMMENDATIONS

This study should be continued by determining the optimal dose as antithrombocytopenia from peanut shell ethanol extract, as well as its safety test on animal test.

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