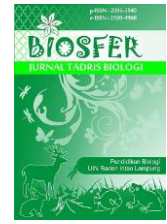




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The Potential of Billygoat Weed Leaf Extract (*Ageratum Conyzoides*) in Increasing Platelet Counts of Male Mice (*Mus Musculus*)

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ABSTRACT

Billygoat weed (*Ageratum conyzoides*) is mainly used to treat wounds and fever. People also do not understand the use of these leaves in the treatment to increase the platelet counts. The objectives of this research Objectives were to test whether the Billygoat Weed's leaves can increase platelet count of male mice and to find out what is the most effective dose of the Billygoat Weed on the platelet count of male mice. This research was done by employing the true experimental approach with the completely randomized design which consisted of 5 treatments, namely the negative control (plain water), the positive control (acetylsalicylic acid), 1.68 mg/kgBW, 2.52 mg/kgBW, and 3.36 mg/kgBW on five mice in each treatment. The increase in the platelet counts, based on the results of One-Way ANOVA with a p-value of 0,0004, the Billygoat Weed extract was able to increase the platelet counts. Through further testing, the smallest significant difference showed that a dose of 3.36 mg/kgBW was effectively able to increase the platelet counts of male mice with an average of 11140.80/mm³.

Potensi Ekstrak Daun Babandotan (*Ageratum Conyzoides*) Dalam Meningkatkan Jumlah Trombosit Pada Mencit Jantan (*Mus Musculus*)

ABSTRAK: *Ageratum conyzoides* hanya dimanfaatkan untuk mengobati luka dan demam, masyarakat juga belum paham dalam pemanfaatan daun babandotan dalam pengobatan kenaikan jumlah trombosit. Tujuan penelitian 1). Menguji apakah daun babandotan mampu meningkatkan jumlah trombosit pada mencit jantan dan 2). Mengetahui berapakah dosis yang paling efektif dari ekstrak daun babandotan pada jumlah trombosit mencit jantan. Menggunakan true experimental dengan rancangan penelitian acak lengkap (RAL), terdiri dari 5 perlakuan ; kontrol negatif (air biasa), kontrol positif (asam asetilsalisilat), 1,68 mg/gbb, 2,52 mg/gbb dan 3,36 mg/gbb dengan masing-masing perlakuan menggunakan 5 ekor mencit. Berdasarkan hasil peningkatan jumlah trombosit diuji menggunakan One Way ANOVA dengan pvalue 0,0004 maka ekstrak mempunyai pengaruh dalam meningkatkan jumlah trombosit dan dengan uji lanjutan beda nyata terkecil yang hasil

*menunjukkan adanya peningkatan jumlah trombosit pada mencit jantan (*Mus musculus*) yaitu dosis 3,36 mg/gbb yang mampu meningkatkan jumlah trombosit dengan efektif dengan rata-rata 11140,80/mm³.*

INTRODUCTION

Blood is vital for all humans because it has the function of sending substances and oxygen needed by the body tissues (Sugianto & Zundi, 2017). Blood is composed of two parts, namely blood cells and blood plasma (Firani, 2018; Syam et al., 2016). Blood cells are composed of the blood cell (erythrocytes), white blood cells (leukocytes), and blood fragments (platelets) (Yolanda & Kurnia, 2015; Yolanda & Prayama, 2016).

Platelet plays an important role in hemostasis (Anwar, 2018; Motulo et al., 2015; Ujiani et al., 2018; Wirawan, 2007). Platelets have a function in the mechanism of blood protection to stop bleeding (Hajiriah & Intan, 2019; Khasanah & Suyadi, 2014). Platelet abnormalities, namely the thrombocytopenia and thrombocytosis (Bahctiar, 2019; Masihor et al., 2013; Yuliani, 2013), might be caused by many factors. Thrombocytopenia is an abnormality that emerges when the platelet count is less than normal amounts which are 150,000/ μ l (Rasyada et al., 2014; Rofinda, 2012). Decreased platelet production in the bone marrow can be caused by a viral infection (Masihor et al., 2013; Utari et al., 2018; Zulkarnain et al., 2017), the maturation of the platelet precursor cells in the bone marrow, radiation, bone marrow aplasia, malignancy or cancer in bone marrow, as well as drugs that suppress the production of blood cells in the bone marrow.

The destruction of platelets in the blood can be caused by immune or non-immune processes (Lubis et al., 2015). Primary diseases that are often associated with ITP are autoimmune diseases, especially the antibody and phospholipid syndrome, and viral infections including Hepatitis C, Human Immunodeficiency Virus

or HIV (Wijaya, 2020), Cytomegalovirus, Epstein-Barr virus, Dengue virus, and certain drugs (Karsy et al., 2018).

research had been done on the effects of Billygoat Weed (*Ageratum conyzoides*) toward germination (Isda et al., 2013), larvae (Lumowa, 2011), and wet transport (Aini et al., 2014).



(a) (b)
Figure 1. (a) and (b) Billygoat weed (*Ageratum conyzoides*)

However, research on the effects of Billygoat Weed (*Ageratum conyzoides*) in increasing platelet counts has not been done. Thus, this research tested the potential of Billygoat Weed leaf extract in increasing the platelet counts on male mice with reduced platelet counts.

METHOD

This laboratory study was carried out to examine the effects of Billygoat Weed (*Ageratum conyzoides*) leaf extracts in increasing platelet counts of male mice (*Mus musculus*) with thrombocytopenia. The instruments used were the M.1600 digital binocular microscope, glass object, and 1 ml disposable syringe.

The mice were kept for about 4 days in storage with 12-hour lighting. The dosage of Billygoat Weed leaf extract in this research was adapted based on research on Betadine leaves (*Jatropha multifida* L.) conducted by (Sundaryono et al., 2016). On

that research, the doses given to the mice were 0.056 g/kgBW, 0.084 g/kgBW and 0.112g/kgBW. By converting the average body weight of mice (30 grams), the Billygoat Weed leaf extract given to the mice was 1.68 mg/kgBW, 2.52 mg/kgBW, and 3.36 mg/kgBW.

The blood was drawn from the tails of mice (*Mus musculus*). The platelet counts were determined by performing blood smears. The platelets were counted using a microscope within 24 hours after the treatments.

When extracting the blood from the mice's tails, the first drop of blood was removed and the next drop of blood was extracted with a syringe. The extracted drops of blood were placed at the upper end of glass objects to get good smear areas. The blood was then left alone to dry. After the blood had dried up, methanol drops were applied evenly and then once again left it to dry. Next, a small amount of Giemsa stain was applied to the dried-up blood on glass objects and then left them until the dye seeped into the blood. Lastly, the dyed glass objects were rinsed in distilled water.

Furthermore, to find the location of platelets, a microscope with an initial magnification of 4x10 was used and to see the platelets, a microscope with a magnification of 10x0.25 was used

The research data must be investigated for its normality. If the data is normally distributed, the One-way ANOVA test could be performed. Significant results can be said to be obtained or in other words, H_0 is rejected and H_1 is accepted if the result of the One-way ANOVA is less than 0.05. A further test needs to be done using the Least Significant Difference test (LSD) at the 0.05 significance level to determine which treatment is more influential to increase mice's platelet counts. (Agus, 2011).

RESULTS AND DISCUSSION

The platelet counts were measured three days after the (P0), (P1), (P2), (P3), and (P4) treatments. The results can be seen in Table 1.

Table 1. Platelet Counts Measurement Three Days after the Treatments

Treatments	Repetition	Averages up to $\pm 10^3 / \text{mm}$		
		H0	H2	H3
P(0)		9799,40 \pm 362,551	8490,40 \pm 1178,919	7966,80 \pm 597,252
P(1)		8884,80 \pm 1050,190	2224,60 \pm 665,269	7737,80 \pm 650,901
P(2)	5	8299,60 \pm 1315,173	3762,40 \pm 1432,230	7424,00 \pm 2085, 591
P(3)		7983,20 \pm 868,923	2273,80 \pm 1184,253	9577,40 \pm 1206,261
P(4)		7983,20 \pm 1657,947	3909,40 \pm 1138,475	11140,80 \pm 2220,261

Based on Table 1, within two applications of acetylsalicylic acid (P1) on H0 and H2, the platelet counts were reduced. The decreased platelet counts result in bleeding that cannot be stopped because the blood is difficult to clot. Thrombocytopenia is a condition where platelet counts drop below the normal levels. It might be caused by the failure of the bone marrow to produce platelets.

The application of acetylsalicylic acid to *Mus musculus* (P1) orally for 2 days resulted in a decreased platelet counts from 8884.80 (H0) to 2224.60/ mm^3 (H2). On the third day (H3), the platelet count increased to normal levels. This was due to the application of acetylsalicylic acid which was given orally for 2 days. On the third day, the application of acetylsalicylic acid was stopped so that the mice could recover.

On the P4 treatment group, after the Billygoat Weed leaf extract had been administered, the platelet counts increased from 3909.40 on the second day to 11140.80/ mm^3 on the third day (H3). The Billygoat Weed leaf extract was able to increase the platelet counts of male mice with thrombocytopenia to normal conditions (P0) on the third day. Thus, the Billygoat Weed leaf extract has the potential to increase the platelet counts of male mice.

Flavonoid can inhibit platelet aggregation (Irawati, 2015; Shalehah et al., 2015), because it inhibits the release of Arachidonic acid by impinging cells so that the metabolism pathway of Cyclooxygenase is inhibited. When the cyclooxygenase is inhibited, the thromboxane A2 cannot be formed (Putri et al., 2014). Thromboxane A2 is a type of thromboxane that is produced by activated platelets during hemostasis and has prothrombotic properties. It stimulates new platelet activation and increases platelet aggregation. Platelets and platelet destruction in thrombus clotting will be inhibited so the bleeding time will increase. Aspirin blocks the production of thromboxane A2 by acetylating residues near the active site of cyclooxygenase which is an enzyme that produces precursor endoperoxide cyclic thromboxane A2 (Memah, 2014).

Instead of using chemical drugs, medicinal plants could be used. One of the plants is *Ipomoea batatas* L. or known as sweet potato. It can increase the number of platelets by 224% because it contains polyphenol compounds, flavonoids (Hidayati & Harjono, 2017; Wati et al., 2014), and tannins (Nurhudiman et al., 2018; Rabbaniyah, 2015).

Phytochemical and pharmacological tests show that Billygoat Weed (*Ageratum conyzoides*) contains several chemical compounds, namely alkaloids, flavonoids, polyphenols, and tannins (Hikmah et al., 2018).

CONCLUSIONS AND SUGGESTIONS

Based on the research result, it can be concluded that the Billygoat Weed (*Ageratum conyzoides*) leaf extract can increase the platelet counts with a dose of 3.36 mg/kgBW. Other studies on Billygoat Weed (*Ageratum conyzoides*) need to be done in increasing the platelet counts of male mice (*Mus musculus*) with dengue fever with the right media and dosage. It is hoped that this research can be used as a reference for further research.

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