

The Effect of Kayu Akway (*Drymis* sp) Extract on The Number of Leukocyte of The Male Mice (*Mus musculus* L)

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ABSTRACT: Kayu Akway (*Drymis* sp) is an endemic herb plant in West Papua. Akway plant is a plant traditionally used by Papua citizen for various benefits, such as increasing endurance and durability, vitality, controlling birth range, and also curing kudis. The objective of this research was to determine the effect of Kayu Akway extract on the number of leukocyte of male mice (*Mus musculus* L). The experimental method used in this research is randomized complete design with 3 blocks of treatments and 4 times repetition with given orally for two weeks. The block of treatments are control (K), dose of oral 1.3 ml/kg body weight (K1), and dose of oral 3.3 ml/kg body weight (K2). The data of leukocyte number is analyzed by Analysis of Variance (ANOVA) and then continued by Duncan Multiple Range Test at 95% confidence interval. The result showed that statistically insignificant number of leukocyte in K1 and K2 treatment when compared with control. In the contrary, the lowest leukocyte number was found in the control treatment that was untreated with oral extract. While increased the number of leukocyte was found in the K1 treatment and the highest leukocyte number was found in the K2 treatment. It concluded that seen a trend increase in the number of leukocyte on the second dose compared to control.

Keywords: *Drymis* sp extract, Leukocyte number, Male mice

INTRODUCTION

Haematological study both humans and in animal sciences is regarded to be an important index of the physiological state of the individual. Blood is one of the most important specimen studied during parasitic infections and diseases in mice as experimental models. The total leukocyte count (TLC) is one of an important diagnostic tool to assess the host immune status and resistance to disease or infection (Astavief, 1966; Garside and Behnke, 1989). The differential leukocyte count (DLC) is a significant parameter in the blood picture of an animal, especially during any kind of stress from disease, trauma, and infection. The leukocytes of most strains of mice mainly contain neutrophils, lymphocytes, monocytes and eosinophils, while basophils are almost absent (Hardy, 1941). Most of the routine haematological tests described by Dacie (Dacie, 1964) for humans and by Schalm (Schalm *et al.*, 1961) for larger animals are applicable to rats and mice, using electronic cell counters.

The balance between free radical and antioxidant in the body is one of that affects health. It is caused by a deficiency of the antioxidant inputs enough food consumption. One of an antioxidant source that come from outside the body can be obtained from plants. One of the anti oxidant is believed to be the potential akway (*Drymis* sp). It is based on studies (Santoso *et al.*, 2004), which he also informed that simplisia the bark of a plant Akway high alkaloid, saponin and tannin.

Akway plants (*Drymis* sp) is defined as of herbaceous plants from the family winterceae with high 1 to 4 m, part the edge of a leaf shaped and oblong leaves somewhat slippery (Anon, 2005). In Indonesia, these plants only in the regions Papua, especially in hilly areas Manokwari. Akway including rare plants growing in the Arfak Mountains natural heritage, the Regency of Manokwari at an altitude of 2500 m (Parubak, 2007). The classification of plants Akway in taxonomic kingdom plantae is a phylum of tracheophyta, subfilum tracheophytina, magnoliopsida

class, subclass magnoliidae, winterales the ordo, winteraceae family, subfamily winteroideae, the genus *drymis*, and species *drymis* sp (Heywood 1993 in Santoso *et al.*, 2004).

Akway used as traditional medicine herbs Sougb tribe in the District of Sururey Papua. This plant used to treat malaria and to increase endurance in do servile work, as well as for increase vitality of the body (Paliling, 2004). So far not yet never reported the influence of treatment extract kayu Akway (*Drymis* sp) to the number of leukocytes in physiology of the body normal. Therefore to discover the potential kayu Akway as one of the traditional herbs done study on the effect of extract kayu Akway (*Drymis* sp) to the number of leukocytes using male mice (*Mus musculus* L) as animal models.

The general goal of this study is to interpret the status of the blood profile during treatment extract kayu Akway (*Drymis* sp) in male mice (*Mus musculus* L) in comparison to control treatment and then look at the normal physiological conditions. It has already been proved that the blood picture may undergo huge changes during the life time (Khan and Zafar, 2005). The blood profile can undergo drastic changes with certain conditions such as, stress, infections and intoxications. The objective of this research was to determine the effect of kayu Akway (*Drymis* sp) extract on the number of leukocyte of male mice (*Mus musculus* L).

MATERIALS AND METHODS

Plant material

Kayu Akway (*Drymis* sp) were collected from the traditional market in the Regency of Manokwari, West Papua. The semidry material (5 g) was dried in air under shade, and crushed to get a fine powder, then it was boiled in a soxhalate apparatus with 50 mL of aquadest for 15 minute in 90°C temperature. After that the crude extract was obtained after removal of the solvent, the extract poured in a beaker glass and closed an aluminum foil paper than stored in low temperature.

Animal models

Twelve adults male mice (*Mus musculus* L) weighting (18-25 g) were obtained from the Genetics Laboratory, Animal Husbandry Faculty, University of Papua. They were kept in plastic cages with free access to water and food, with a 12 h dark and 12 h light cycle.

Experimental Design

The experimental method used in this research is randomized complete design with 3 blocks of treatments and 4 times repetition with given orally for two weeks. Mice were divided into three groups, each group consisting of 4 animals. Mice of group-I (K) as a control received food and distilled water ad libitum, group-II (K1) received orally a daily dose of kayu Akway (*Drymis* sp) at 1.3 ml/Kg b. wt. for two weeks, group-III (K3) received orally a daily dose of kayu Akway (*Drymis* sp) at 3.3 ml/Kg b. wt. for two weeks.

Hematological tests

Blood sample (1 ml) was obtained after two weeks of orally from each of the animals. Blood samples were collected by orbital sinus puncture from different experimental groups use microhematocrit blood tube into the corner of the eye then poured in EDTA rinsed vials (Hoff, 2000) for analyzing hematological parameters including total leukocyte count (TLC) were estimated by using the hemocytometer methods.

Statistical analysis

Data were analyzed by using SPSS version 16. The results were expressed as the mean \pm SD. The data of leukocyte number is analyzed by Analysis of Variance (ANOVA). The significance

of the mean difference between the control group and each of treatment groups was determined by Duncan Multiple Range Test at 95% confidence interval. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The kayu Akway (*Drymis* sp) extract resulted in statistically insignificant number of leukocyte in group-II (K1) and group-III (K2) treatment when compared with control (K). It concluded that seen a trend increase in the number of leukocyte on the second dose compared to control (Table 1).

Table 1. Total leukocyte count (TLC) (/mm³) of *Mus musculus* Luring treatment with kayu Akway (*Drymis* sp) extract

Batch	Mean ± SD
Control (K)	6750 ± 957.43
Treatment 1 (K1)	8100 ± 2289.11
Treatment 2 (K2)	8600 ± 432.05

In the contrary, the lowest leukocyte number was found in the control treatment that was untreated with orally extract. While increased the number of leukocyte was found in the K1 treatment and the highest leukocyte number was found in the K2 treatment. Leukocyte is a defense system to foreign objects which could result in inflammation and infection in the body. According to Arrington (1972) of normal leukocytes mice ranged between 6000 to 12600/mm³. Observations showed that the number of leukocytes of treatment mice is at the normal range.

This has resulted in the high defense of the mice body to foreign matter who enters into the body which can damage tissue. The role of antioxidant is by means of binding free radicals and molecules that very reactive. The high antioxidant consumption could increase system body for immunity foreign matter or antigen.

An orally kayu Akway extract treatment no significant in percentage of lymphocytes compared with the control group. This is because the experiments were not long time. It looks a tendency of instability the percentage of lymphocytes in the extracts kayu Akway group treatment. This is an increase in the percentage of lymphocytes in the group – II (K1) and a decrease in the percentage of lymphocytes in the group - III (K2) (Table 2). According to Arrington (1972) the percentage of lymphocytes in the normal mice ranging from 55 to 85 %. The observation shows that the percentage of lymphocytes mice because extract kayu Akway treatment be in the range of normal.

Table 2. Percentage lymphocytes (%) of *Mus musculus* L during treatment with kayu Akway (*Drymis* sp) extract

Batch	Mean ± SD
Control (K)	56.25 ± 2.50
Treatment 1 (K1)	57.75 ± 3.30
Treatment 2 (K2)	55.75 ± 1.89

Lymphocytes, the immunocompetent cells, are responsible for the immune response of the

host. The present increase in the percentage of lymphocyte, Lymphocytosis, reflects the host's immune response to overcome parasitic stress, after the phagocytic neutrophils failed in checking the invading parasites.

Lymphocytes is agranulosit leukocytes which has the size and form of which vary. In general, lymphocytes enter the circulatory system through the lymphatic vessels. Lymphocyte cells have the ability to conduct a recirculation in blood circulation so that the number of lymphocytes cells that enter and exit or leave blood circulation is relatively constant (Meyer *et al.*, 1975).

The ability of the recirculation lymphocytes very important especially in the process of the mechanism of the distribution of lymphoid cells. It is associated with the immune system response is accumulation a number of lymphocytes in the location of antigens in tissues and can relocated to another place in the network to perform an immune response (Jain, 1993).

In immune response, lymphocyte consisting of B and T cells which are the controllers in the immune system. B cells that differentiate into plasma cells will produce antibodies, whereas the T-cells can release a range of materials that have biological effects that are called limfokin (Medicastore, 2009).

The role of a lymphocyte very important in the activity of the production of humoral antibodies and the formation of cellular defense, as well as responsibility for the antibody diversity. The increase in cell lymphocytes can usually be found on the condition of stress, both physical and emotional, chronic infection, and chronic inflammation. While decrease the number of lymphocytes can be found in the incident a viral infection (decreasing temporary and will rise again the number of lymphocyte) (Meyer *et al.*, 1975).

CONCLUSIONS

To extract a kayu Akway in the dosage produce count of leukocyte and the percentage of lymphocytes mice in the range of normal. Invisible differences in the number of mice lymphocytes to extract kayu Akway treatment in the dosage to control. Observe this trend for increased the number of leukocytes and reducing the percentage of mice lymphocytes to extract kayu Akway are on the dosage compared to controls.

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