THE EFFECT OF BAJAKAH (*SPATHOLOBUS LITTORALIS HASSK*) STEM EXTRACT ON CALCULATION OF LEUKOCYTE IN MICE (*MUS MUSCULUS*)

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ABSTRACT

Background: Oxidative stress plays a role in the obesity mechanism, thus leads to premature aging. Bajakah tampala is a plant that has a great potential to be developed as a medicinal, flavonoids, and tannins. High antioxidant capacity in Bajakah stem may effectively lessen oxidative stress and reduce fat mass and body weight accordinfly. This study aimed to examine the effect of bajakah stem extract (*Spatholobus littoralis hassk*) on leukocyte level in mice (*Mus musculus*)
Subjects and Methods: This was a randomized controlled trial was conducted at YATSI school of health sciences laboratorium, from June to August 2021. A sample of 30 mice aged 8-12 weeks, weighted 20-30 grams, and exposed to *Salmonella typhimurium* was selected for this study randomly. Sampele was divided into 5 groups and received: (1) aquadest (control). (2) bajakah stem extract dose 25 mb/kgBW, (3) bajakah stem extract dose 50 mg/kgBW, (4) bajakah stem extract dose 100 mg/kgBW. And (5) 30 mg/kgBW modulator drugs. The dependent variable was Bajakah stem extract. The independent variable was leukocytes. The data were analyzed by ANOVA test.
Results: Bajakah stem extract dose 100 mg/kgBW reduced leukocytes in mice exposed to *Salmonella typhimurium* ($p<0.001$).
Conclusion: Bajakah stem extract dose 100 mg/kgBW reduced leukocytes in mice exposed to *Salmonella tyhimurium*.

Keywords: spatholobus littoralis hassk, leukocytes, immunity

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BACKGROUND

The immune system is a system of body defense mechanisms against a disease. The immune system protects the body from attacks by various organisms that can cause disease. In order to avoid the source of the disease, the immune system needs to be stabilized and improved. Good nutritional intake such as consuming foods rich in vitamins and minerals are ways to increase immunity.

Indonesia as one of the mega diversity countries has abundant and diverse natural wealth. Exploration of natural resources, especially plants that have the potential for medical ingredients, and continues to grow. The use of natural ingredients as herbal medicines tends to be more in demand by the public because it is safe or has low side effects. One of the natural resources that can be used for health, especially to increase immunity at this time is the tampala bajakah (Spatholobus littoralis). The potential of bajakah tampala is not widely known by the public. In Indonesia,

https://doi.org/10.26911/ICPHmedicine.FP.08.2021.03

precisely in the Central Kalimantan region, bajakah is known as a traditional medicine by drinking boiled water from the stems.

The results of the phytochemical screening test showed that the main content of bajakah was saponins, flavonoids, and tannins (Saputera & Ayuchecaria, 2018). Saponin compounds trigger the immune system and antibodies work more efficiently, reduce the frequency of fever, flu, and antimicrobials (Faradisa, 2008; Noorlaili et al., 2019). Flavonoids induce an increase in interleukin-2 which plays a role in T cell proliferation and stimulates phagocytic cells (Nugroho, 2012). Tannins stimulate phagocytic cells, are antitumor, and antibacterial (Yusuf, 2019). Currently, research on the potential of piracy is still limited only as a cancer medication and wound healer.

Based on the exposure of some of the research results above, Bajakah stems contain chemical compounds that have effectiveness on immunity, so they have high potential to be developed as herbal medical ingredients to increase immunity. Consuming a drink derived from the trunk of Bajakah, which is rich in secondary metabolism such as saponins, flavonoids, and tannins, can increase immunity. Literature studies show this compound functions as an immunostimulant. Therefore, further studies are needed to prove the benefits of bajakah on the immune system seen in the percentage of leukocytes.

SUBJECTS AND METHOD

1. Study Design

The study used an experimental design with a randomized complete design (RCD), which consisted of 5 treatments (dose) and 8 replications. The research was carried out from February to July 2021 at the Pharmacology Laboratory of STIKes Yatsi Tangerang.

2. Population and sample: populasi

The target population in this study were male white mice. Samples were taken using simple random sampling method. The sample size was calculated using the Federer formula. The overall sample size used in this study was 30 mice. The mice used were males with healthy conditions, aged between 8-12 weeks, and weighing around 20-30 g.

3. Study Variables

The independent variable in this study was the provision of bajakah stem extract, the dependent variable was the number of leukocytes in male mice

4. Operational Definition of Variables

Giving the extract of bajakah stem obtained by maceration of powder with ethanol solvent. Administered orally via a gastric probe, the dose obtained is based on the usual human dose converted to mice. Mice were injected orally with the treatment in the form of extract of the root of the pirates dissolved in 100 ml of 2% Na-CMC according to the dose as determined. The extract was administered from the 1-30th day after the adaptation period (2 weeks) orally using a gastric probe every 08.00 WIB. The dose used was 0 or given aquadest (K1/control), 25 mg/kg (K2), 50

https://doi.org/10.26911/ICPHmedicine.FP.08.2021.03

mg/kg (K3), and 100 mg/kg BW (K4) and 30 mg/kg immuno-modulator brand X (K5).

Determination of the number of leukocytes is the number of leukocytes as a result of blood examination of mice on the 34th day, the leukocyte count was observed. Counting leukocytes using a haemocytometer with the solution used is Turk. Calculations using a microscope magnification 10X.

Salmonella typhimurium infection was carried out in day 31, mice infected with *salmonella typhimurium* bacteria. *Salmonella typhimurium* infection was carried out by intraperitoneal injection of 0.5 ml of inoculum.

5. Study Intrument

The instruments used are observation sheets and haemocytometer.

6. Data analysis

Data was processed using ANOVA. The difference in ANOVA results was followed by DMNRT at 1% level.

7. Research Ethics

This research has passed the ethical test by the Research Ethics Committee of the Yatsi Tangerang School of Health with the number: 072/LPPM-STIKES YATSI/VI/2021 on June 2, 2021.

RESULTS

Data on the number of leukocytes are shown in table 1, the average number of leukocytes in K1 is 3,400 mm, K2 is 4,633.33 mm, K3 is 9,200 mm, K4 is 12,525 mm and K5 is 9516.67 mm (table 1).

Table 1. Leukocyte count

Mencit	Jumlah leukosit (mm)	Mean
K1		
M1	3700	
M2	3500	3400.00
M3	3000	
K2		
M1	5450	
M2	4550	4633.33
M3	3900	
K3		
M1	9075	
M2	8850	9200.00
M3	9675	
K4		
M1	13050	
M2	13350	12525.00
M3	11175	
K5		
M1	9300	
M2	9800	9516.67
M3	9450	

https://doi.org/10.26911/ICPHmedicine.FP.08.2021.03

Tabel 2. Normality test

Parameter	Statistic	df	p
Leukosit	0.89	15	0.09

Table 2 shows that the leukocyte parameter data is normally distributed. It Shown by a significant value that is equal to 0.091 (p <0.010). Thus, the data analysis used on the leukocyte parameter is the ANOVA test and it could be stated that the results of the ANOVA test there are differences in treatment with the control group (P < 0.010), then the analysis will continue using the Duncan Multiple New Range Test (DMNRT).

Tabel 3. ANOVA Test Effect of Giving *Bajakah* Stem Extract on Leukocyte Count of Male Mice

Parameter	F count	p	α = 1 %
Leukosit	89.455	<0.001	0.1

Table 3 shows that the significant value of the leukocyte parameter obtained (<0.001) is smaller than the alpha value (α = 0.010). This shows that the administration of pirated extract and brand X immunomodulator to mice that have been infected with salmonella typhimurium bacteria has a significant effect on leukocytes of male mice compared to the control group. Therefore, the data on the number of leukost were further tested by DMNRT.

Table 4. DMNRT Test Effect of Giving *Bajakah* Stem Extract on Leukocyte Count of Male Mice

Dosis Perlakuan	Rata-rata Leukosit (mm)
K1	3,400.00 [a]
K2	4,633.33 [a]
K3	9,200.00 [b]
K4	12,525.00 [c]
K5	9,516.67 [b]

Note: K1 = 0 mg/kg. K2 = 25mg/kg, K3 = 50 mg/kg, K4 = 100 mg/kg, K5 = 30 mg/kg immunomodulator brand X. Notations (a, b and c) in the same column indicate significant differences in the 1% DMNRT test.

Based on the results of the DMNRT test (table 4) at = 0.01 it was found that the treatment given to K1 and K2 was not significantly different. also with the treatment of K3 and K5. However, the K4 treatment which was given the extract of the bajakah stem at a dose of 100 mg/kg was significantly different compared to the treatment K1 (control), K2, K3 and K5 at = 0.01. This indicates that the administration of the extract of the root of the bajakah in male mice infected with salmonella typhimurium was significantly different in the number of leukocytes between the control group and the K3, K4 and K5 treatments. The treatment dose that had the most significant effect was the treatment with a pirate extract dose of 100 mg/kg (K4)

https://doi.org/10.26911/ICPHmedicine.FP.08.2021.03

DISCUSSION

The number of leukocytes was observed using a microscope. The normal number of leukocytes in mice is between 6x103–15x103/mm3. The number of leukocytes was measured on the 4th day after being injected with salmonella typhimurium bacteria. An increase in the number of leukocytes can be caused by an infection. Leukocytes (white blood cells) blood components that function for the immune system, Increased production of white blood cells indicates the body's resistance against harmful foreign agents.

Based on the results of the measurement of the number of leukocytes in male mice that had been given extract of the rods and immunomodulator brand X for 30 days, after that they were exposed to 0.5 ml of salmonella typhimurium bacteria which was given once in each treatment. The K1 and K2 groups did not have a significant increase in leukocytes, the K3 and K5 groups had an increase in leukocytes but more in K4. This indicates the existence of an infectious process and a process of resistance to infection. Salmonella typhimurium is thought to be able to activate the immune system in mice, resulting in an immune process. The provision of bajakah stem extract is proven to be able to help the process of resistance from infection.

Salmonella typhimurium is a serotype that can be isolated from both animals and humans. This bacterium can cause various diseases such as in humans. It can cause gastroenteritis and food poisoning, in animals it causes infectious enterocolitis and septicemia. Salmonella typhimurium is an intracellular facultative that is easily phagocytosed but cannot be destroyed so it is difficult to destroy. These bacteria live intracellularly which causes complement antibiotics and granulocyte cells cannot reach the bacteria. The way that can be done to destroy *salmonella typhimurium* bacteria is to stimulate the function of macrophages until they are activated to destroy and eliminate these bacteria (Gamasinta, 2015).

Herbal products as immunostimulants can be used as a complementary therapy of choice in accelerating the healing process against a bacterial infection. Giving additional intake in the form of extracts bajakah stems which function as anti-inflammatory can minimize infections caused by *salmonella typhimurium* bacteria. According research results (Yusuf, 2019), tampala pirates were positive for phenolic, flavonoid, tannin and saponin tests. Supporting previous research, phytochemical screening that has been carried out on the extract of Bajakah tampala stems showed a positive content of phenolic compounds, tannins and saponins (Ayuchecaria et al., 2020). Phenolic compounds are compounds that can have antioxidant activity (Onkar et al., 2012) .The flavonoids in the rods have anti-cancer and anti-inflammatory properties. It is evident from research (Risya, 2020), which states that there is a decrease in bell-2 which describes the occurrence of the apoptosis process, so it has the potential for cancer cell apoptosis.

Ginger also contains compounds such as those found in bajakah stems, namely saponins, alkaloids and

https://doi.org/10.26911/ICPHmedicine.FP.08.2021.03

flavonoids which have anti-microbial and anti-fungal properties (Omoya & Akharaiyi, 2012). States that giving ethanol extract of rambai fruit peel can significantly affect the number of leukocytes, the content in the rind of rumbai fruit, namely alkaloids, saponins, flavonoids and phenolics. This means that the content in the trunk of the bajakah is the same as that in the rind of the rambai fruit (Meutia, 2020).

The content is also found in the leaves of the gods, the leaves of the gods contain alkaloids, saponins, flavonoids, essential oils and tannins (Rivai et al., 2012).Supported by research by (Marlinda et al., 2017), which states that leaves of the gods can restore the number of leukocytes in patients with leukemia. Therefore, the content of Bajakah stem extract with the highest dose (100 mg/kg) in this study was able to increase the number of leukocytes in mice, so that it could fight infection due to salmonella typhimurium bacteria.

ACKNOLEDGMENT

The author would like to thank LLDIKTI Region IV which has fully funded this research through the Research Grants of the Ministry of Education, Culture and Technology. In addition, the authors also thank STIKes YATSI Tangerang for facilitating and supporting the research.

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