

# Immunostimulatory Activities of Pegagan Embun (*Hydrocotyle sibthorpioides* Lam.) in White Male Mice

Afriwardi<sup>1,\*</sup>, Yufri Aldi<sup>2</sup>, Dwisari Dillasamola<sup>2</sup>, Yolanda Alfiyah Larakhansa<sup>2</sup>, Elsa Badriyya<sup>2</sup>

Afriwardi<sup>1,\*</sup>, Yufri Aldi<sup>2</sup>, Dwisari Dillasamola<sup>2</sup>, Yolanda Alfiyah Larakhansa<sup>2</sup>, Elsa Badriyya<sup>2</sup>

<sup>1</sup>Faculty of Medicine, Andalas University, INDONESIA.

<sup>2</sup>Faculty of Pharmacy, Andalas University, INDONESIA.

## Correspondence

Afriwardi

Faculty of Medicine, Andalas University, INDONESIA.

E-mail: afriwardi@med.unand.ac.id

## History

- Submission Date: 09-11-2020;
- Review completed: 27-12-2020;
- Accepted Date: 04-01-2021.

DOI : 10.5530/pj.2021.13.47

## Article Available online

<http://www.phcogj.com/v13/i2>

## Copyright

© 2021 Phcogj.Com. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

## ABSTRACT

**Introduction:** Pegagan embun (*Hydrocotyle sibthorpioides* L.) has many pharmacological activities, such as improving the immune system. **Aim:** This research aims to study the immunomodulatory effect of pegagan embun herbs ethanol extract (*Hydrocotyle sibthorpioides* L.) determined by phagocytic activity and capacity of macrophages, total and percentage of leukocytes. **Methods:** 25 male white mice were divided into 5 equal groups. Negative control group were given Na CMC 0.5%, the extract treated group were given pegagan embun ethanol extract at doses of 10, 50, 200 mg/kgbw, and the positive control group was given Stimuno 50 mg/kgbw orally for 7 days. On the 8<sup>th</sup> day, the total and percentage of leukocytes were counted through blood sample taken intravenously. The mice were then induced with *Staphylococcus aureus* suspension. After one hour, the peritoneal fluids was taken to determine the macrophages activity and capacity. The macrophages phagocytic activity and capacity, total and percentage of leukocytes were analyzed by One-Way Anova followed by Duncan Multiple Range Test ( $p < 0.05$ ). **Results:** The results show significant increase of concentration ( $p < 0.05$ ) towards macrophages phagocytic activity and capacity, and total leukocytes count. Percentage of leukocytes show that lymphocytes increase significantly ( $p < 0.05$ ), meanwhile neutrophils segments decrease significantly ( $p < 0.05$ ). **Conclusion:** It can be concluded that ethanol extract of pegagan embun herb at doses of 10, 50, 200 mg/kgbw shows immunostimulatory activity.

**Key words:** *Hydrocotyle sibthorpioides* Lam., *Staphylococcus aureus*, Phagocytosis, Macrophage, Leukocytes.

## INTRODUCTION

The immune system protects human from foreign substances and pathogens such as viruses, bacteria, parasites, and fungi. There are two types of immune response such as specific and non-specific immune response. Non-specific immune response works rapidly to protect human from microorganisms while specific immune response shows specific response towards specific microbes.<sup>1,2</sup>

Non-specific immune response protect the body by phagocytosing antigen such as bacteria without regarding its difference from other foreign substances. The most important phagocytic cells in this process is the macrophages. Macrophages come from adult monocytes in the tissues. The two main functions of macrophages are destroying antigens and presenting them to lymphocytes T as macrophages acts as Antigen Presenting Cells (APC).<sup>3</sup>

An immunostimulant can increase body defense mechanism. Generally, an immunostimulant is defined as a compound that can increase body defense mechanism specifically and non-specifically through cellular or humoral response.<sup>3</sup> Certain plants contains compound that show immunostimulatory activity. One of those plants is known as pegagan embun (*Hydrocotyle sibthorpioides* Lam.) herbs that is widely used in Chinese traditional medicines to treat immune

and liver related disease.<sup>4</sup> Pegagan embun has several medicinal properties such as anti-swelling, anti-inflammatory, diuretics, antibiotics, antipiretics, detoxificans, and expectorants. Farong Yu *et al.* reported that *Hydrocotyle sibthorpioides* extract shows excellent anti-tumour activities and helps repairing mice's immunologic functions.<sup>6</sup>

Based on the above explanations, a research to determine the immunomodulatory activity of *Hydrocotyle sibthorpioides* extract was conducted. The determined parameters were macrophages phagocytic activity and capacity, total leukocytes count, and percentages of leukocytes in white male mice induced by *Staphylococcus aureus*.

## MATERIALS AND METHODS

### Time and place

This study was carried out for 4 months at Research Laboratory and Immunology and Serology Laboratorium of Faculty of Pharmacy of Andalas University.

### Apparatus

The apparatus used in this study were measuring cylinder (Pyrex), erlenmeyer (Pyrex), gavage needle (Terumo), rotary evaporator (Ika), filter paper (Whatman), analytical balance (Ohaus), microscope (Olympus), TLC plate (Merck), centrifuge, WBC pipette (Assistant), mice cage, and surgical scissors.

**Cite this article:** Afriwardi, Aldi Y, Dillasamola D, Larakhansa YA, Badriyya E. Immunostimulatory Activities of Pegagan Embun (*Hydrocotyle sibthorpioides* Lam.) in White Male Mice. Pharmacogn J. 2021;13(2): 368-75.

## Materials

The materials used in this study were *pegagan embun* (*Hydrocotyle sibthorpioides* Lam.), stimuno (Dexa Medica, Batch No. : SLBZ4289), quercetin (Sigma-Aldrich, CAS Number: 117-39-5), aquadest (Andeska Laboratory), carbomethylcellulose (Na CMC), Giemsa stain 0,5% (Merck), ethanol 70% (Andeska Laboratory), *Staphylococcus aureus* (Microbiology Laboratory of Faculty of Pharmacy of Andalas University), nutrient agar (Merck), nutrient broth (Merck), physiologic NaCl (Andeska Laboratory), Turk solution (Sagara Husada Mandiri), and white male mice (Wistar).

## Extract preparation

1 kg of dried *pegagan embun* were finely grinded (Mesh 20) and macerated with ethanol 70%. An amount of fine dried powder and solvent were added into a mecerator with a ratio of 1:10. The mixture was stirred occasionally for 6 hours, left for 18 hours, and then filtered. The above processes were repeated two times with the same ratio of dried powder and solvent. The macerates were collected and concentrated by rotary evaporator until a crude extract was obtained.<sup>7</sup> The extract was tested before use. The tested parameters were organoleptic, phytochemical screening, and TLC profile.

## Animal model

The animal used in this research was 25 naive white male mice, 2-3 months old that weigh 20-30 g. The test animal were grouped into 5 groups consisted of 5 animals each. The negative control group was given Na CMC 5% suspension, group 2,3, and 4 were given *pegagan embun's* extracts with doses of 10, 50, and 200 mg/kgbw while positive control group was given stimuno with doses of 50 mg/kgbw. The extract was given orally for 7 days.

## Bacteria culture

*Staphylococcus aureus* R. (SA) was cultured into slanted nutrient agar (NA) and then inoculated into a new NA using the inoculating needle. Then the bacteria was incubated for 24 hours at 37°C. *Staphylococcus aureus* R. was then moved into the nutrient broth (NB) and incubated at 37°C for 24 hours. It was then centrifuged at 2500 rpm for 25 minutes until a pellet was formed and suspended with physiological NaCl.<sup>8</sup>

## Total leucocyte count

Fresh blood was taken using WBC pipette until the number 0.5 and turk solution was taken until the number 11 then shook for 3 minutes. The first 2 drops of the solution in the pipette were removed. Then, 1 drop of the solution was dropped on the hemocytometer. Leave for 2 minutes for the leukocytes to be sedimented. The white blood cells were counted at the 4 corner of the counting chamber.<sup>9</sup>

$$\text{Total leukocyte count} = \text{Total of leukocytes} \times \frac{20}{0,4}$$

## Percentage of leukocytes

On the 8<sup>th</sup> day, blood smear was made on the object glass and left to dry. Methanol was dropped on the dried blood smear and left to dry for 5 minutes. Giemsa stain was added and left to dry for 20 minutes. The blood smear was then rinsed with distilled water and left to dry. Immersion oil was added and examined the smear under the microscope. Count the number of eosinophils, segmented neutrophils, banded neutrophils, lymphocytes, and monocytes at 1000x magnification<sup>9</sup>.

## Macrophages phagocytic activity and capacity test

The mice were acclimatized for 7 days then administered with *Hydrocotyle sibthorpioides* extract suspension for 7 days. On the 8<sup>th</sup> day, blood sample was collected and the animals were administered

with 0.5 ml *Staphylococcus aureus* R. suspension intraperitoneally. After 1 hour, the animals were sacrificed and peritoneal fluid sample was collected with a micropipette. The peritoneal fluid was smeared on the object glass, fixated with absolute methanol for 5 minutes, stained with Giemsa, left for 20 minutes, rinsed with distilled water, and left to dry. Immersion oil was added before examining the smear under the microscope with 1000x magnification. Macrophages phagocytic activity was determined based on the percentage of phagocyte that carried out phagocytosis out of 100 phagocytes. Macrophages phagocytic capacity was determined based on the number of phagocytosed *Staphylococcus aureus* R by 50 active phagocytes.<sup>10,11</sup>

$$\% \text{ Macrophages activity} = \frac{\text{number of active macrophages}}{\text{total macrophages}} \times 100\%$$

## Data analysis

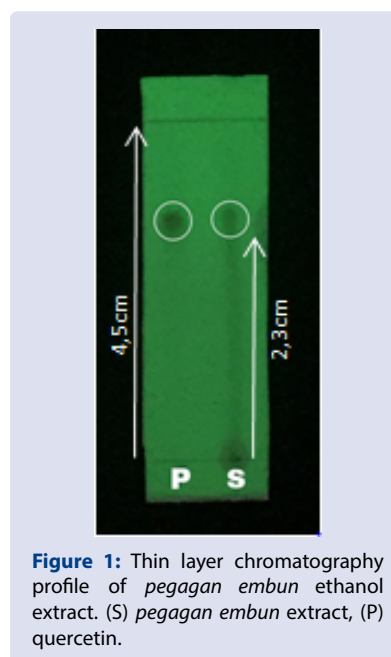
The macrophages phagocytic activity and capacity, total leukocyte count, and percentage of leukocytes were analyzed statistically by one-way ANOVA and continued with Duncan's Multiple Range Test.

## RESULTS AND DISCUSSIONS

The herb that was used in this study is *pegagan embun*. The herb was identified as *Hydrocotyle sibthorpioides* Lam, comes from a family of Araliaceae Lam. by Anda Herbarium, Department of Biology of Faculty of Mathematics and Natural Sciences of Andalas University.

116,126 g of extract was obtained from 1 kg of dried *pegagan embun* with an extraction yield of 11,61%. The extraction yield was in accordance with specification stated by the Herbal Pharmacopeia (2008). The organoleptic test results show that *pegagan embun* extract has thick blackish green appearance, specific odour, and bitter taste. Phytochemical screening tests show that the ethanol extract contains alkaloid, flavonoid, phenolic, and saponin compounds.

Thin layer chromatography (TLC) profile is a qualitative test to identify the marker compound (quercetin) in *pegagan embun* extract. The stationary phase used was TLC Silica gel 60 F<sub>254</sub> Aluminum TLC plate. The mobile phase used were n-hexane and ethyl acetate with a ratio of 6:4. The TLC profile was examined under UV light with a wavelength of 254 nm. The retention factor (Rf) of the extract and pure quercetin was 0,51. This show that the extract contains quercetin because it has the same Rf as pure quercetin, which can be seen in Figure 1.

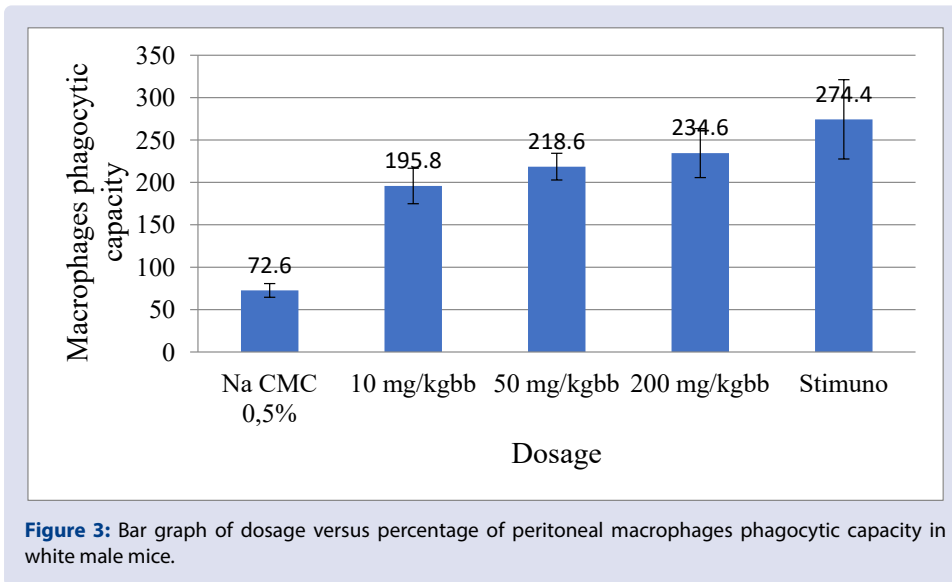
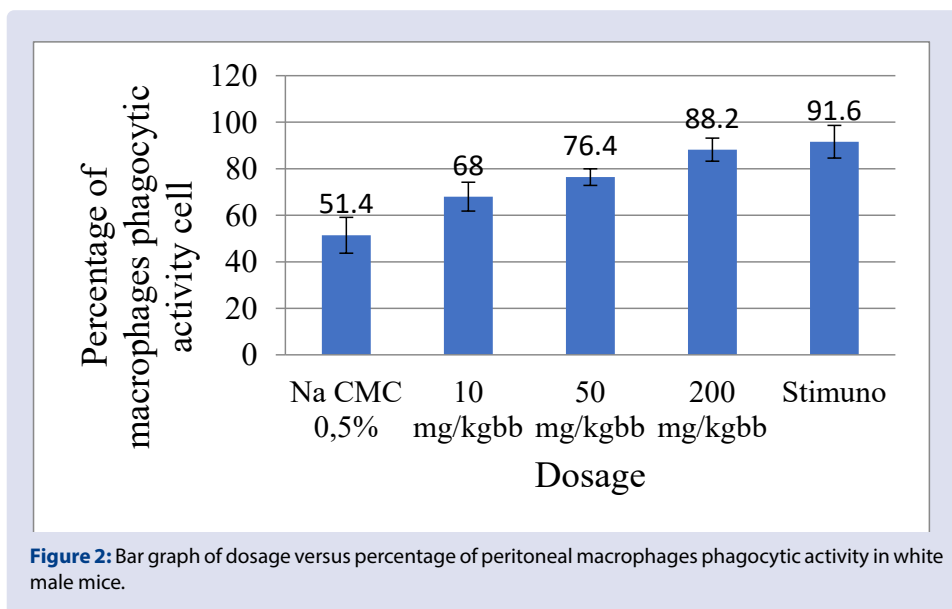


**Figure 1:** Thin layer chromatography profile of *pegagan embun* ethanol extract. (S) *pegagan embun* extract, (P) quercetin.

The results for macrophages phagocytic activity in animals given doses of 10, 50, 200 mg/kgbw of extract, and 50 mg/kgbw of stimuno were shown in Table 1 and Figure 2. Groups that were administered with *pegagan embun* extract show higher macrophages phagocytic activity compared to group that was administered with Na CMC 0,5%. One-way ANOVA test shows that *pegagan embun* extract influence macrophages phagocytic activity significantly ( $p < 0.05$ ). Duncan's multiple range test show that macrophages phagocytic activity increases with increasing

doses ( $p < 0.05$ ). Group that was administered 200 mg/kgbw *pegagan embun* extract show the same macrophages phagocytic activity as group administered with stimuno ( $p > 0.05$ ).

The results for macrophages phagocytic capacity were shown in Table 2 and Figure 3. Groups treated with *pegagan embun* extract at doses of 10, 50, and 200 mg/kgbw show significant increase in macrophages phagocytic capacity ( $p < 0.05$ ). Groups treated with extract show higher



**Table 1:** Percentage of peritoneal macrophages phagocytic activity in control groups and male white mice treated with *pegagan embun* (*Hydrocotyle sibthorpioides* Lam.) extract.

Percentage of macrophages phagocytic activity (%)	
Doses	Mean ± SD
Na CMC 0,5 %	51,40 ± 7,70 <sup>a</sup>
10 mg/kgbw	68,00 ± 6,20 <sup>b</sup>
50 mg/kgbw	76,40 ± 3,57 <sup>c</sup>
200 mg/kgbw	88,20 ± 4,96 <sup>d</sup>
Stimuno 50 mg/kgbw	91,60 ± 7,02 <sup>d</sup>

macrophages phagocytic capacity compared to group administered with Na CMC 0.05% ( $p < 0.05$ ). There was no significant difference in macrophages phagocytic capacity between groups given extract at doses of 10 mg/kgbw and 50 mg/kgbw. Based on the previous study, flavonoid has been proven to increase IL-2 and proliferation of lymphocytes.<sup>12</sup> Lymphocytes proliferation will influence CD4+ cell that activates Th1 cell. Activated Th1 cell will then influence Specific Macrophage Activating Factor (SMAF) wherein SMAF are multiple molecules, for example, IFN- $\gamma$ . IFN- $\gamma$  will activates macrophage and increase their phagocytic activity and capacity. This results in faster rate of phagocytosis of bacteria by macrophages.<sup>2</sup>

The results for total leukocyte count of five tested groups were shown in Table 3 and Figure 5. Groups treated with *pegagan embun* extract at doses of 10, 50, and 200 mg/kgbw show significant increase in total leukocyte count ( $p < 0.05$ ). Groups treated with extract show higher total leukocyte count compared to group administered with Na CMC 0.05% ( $p < 0.05$ ). Group treated with 200 mg/kgbw extract show the highest total leukocyte count compared to groups treated 10 and 50 mg/kgbw extract ( $p < 0.05$ ). There was no significant difference in total leukocyte count between groups given extract at doses of 10 mg/kgbw and 50 mg/kgbw ( $p > 0.05$ ). There was no significant difference in total leukocyte count between animals given extract at doses of 200 mg/kgbw and stimuno ( $p > 0.05$ ). Based on the previous study, increase in total leukocyte count indicates increase in immune response.<sup>13</sup>

The results for percentage of leukocytes were shown in Table 4. One-way ANOVA test shows that *pegagan embun* extract at doses of 10, 50, and 200 mg/kgbw influence the percentage of segmented neutrophils

and lymphocytes significantly ( $p < 0.05$ ) but show no significant difference towards percentages of banded neutrophils, eosinophils, and monocytes ( $p > 0.05$ ). There was no significant difference in percentages of segmented neutrophils in group treated with 10 mg/kgbw extract compared to group treated with doses of 50 mg/kgbw ( $p > 0.05$ ) and they show the same effect as 50 mg/kgbw stimuno. The percentage of segmented neutrophil decreases after the administration of *pegagan embun* extract. Hence, it can be assumed that macrophages played the most important role in phagocytosis or there was an increase in chemotaxis factor that increase the phagocytosis ability.<sup>2</sup>

*Pegagan embun* extract significantly increases the percentage of leukocytes as shown in Figure 6. Groups treated with extract at doses of 10 and 50 mg/kgbw show no significant difference in percentage of leukocytes compared to control group ( $p > 0.05$ ). Group given extract at doses of 200 mg/kgbw shows higher percentage of leukocytes compared to group given extract at doses of 50 mg/kgbw. There was no significant difference on the percentage of leukocytes in group treated with extract at doses of 200 mg/kgbw compared to group treated with stimuno at doses of 50 mg/kgbw. Increase in percentage of lymphocytes indicates that *pegagan embun* extract could stimulate specific immune response.<sup>2</sup>

This study show that *pegagan embun* (*Hydrocotyle sibthorpioides* Lam.) extract at doses of 10, 50, and 200 mg/kgbw show immunostimulant properties through increasing the activity and capacity of macrophages, total leukocyte count, percentage of lymphocytes, and decreasing the number of segmented neutrophils. Extract at doses of 50 and 200 mg/kgbw show similar immunostimulatory effect.

**Table 2: Mean of peritoneal macrophages phagocytic capacity in control groups and male white mice treated with *pegagan embun* (*Hydrocotyle sibthorpioides* Lam.) extract.**

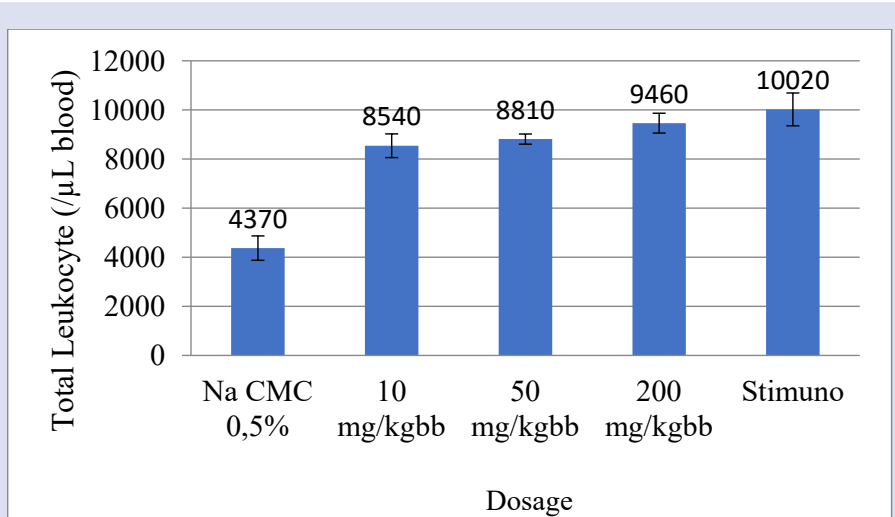
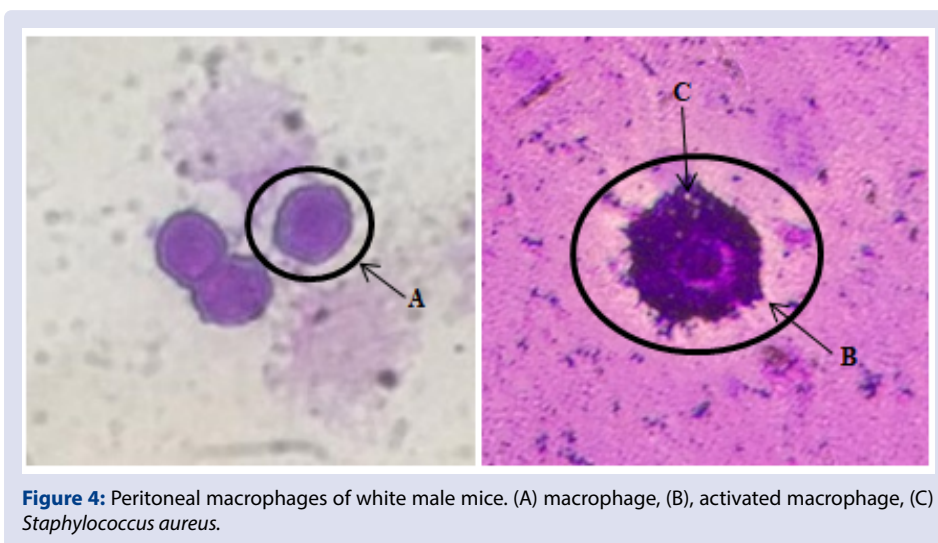
Mean of peritoneal macrophages phagocytic capacity	
Doses	Mean $\pm$ SD
Na CMC 0,5 %	72,60 $\pm$ 8,08 <sup>a</sup>
10 mg/kgbw	195,80 $\pm$ 20,93 <sup>b</sup>
50 mg/kgbw	218,60 $\pm$ 15,75 <sup>bc</sup>
200 mg/kgbw	234,60 $\pm$ 28,91 <sup>c</sup>
Stimuno	274,40 $\pm$ 46,77 <sup>d</sup>

**Table 3: Mean of total leukocyte count in control groups and male white mice treated with *pegagan embun* (*Hydrocotyle sibthorpioides* Lam.) extract.**

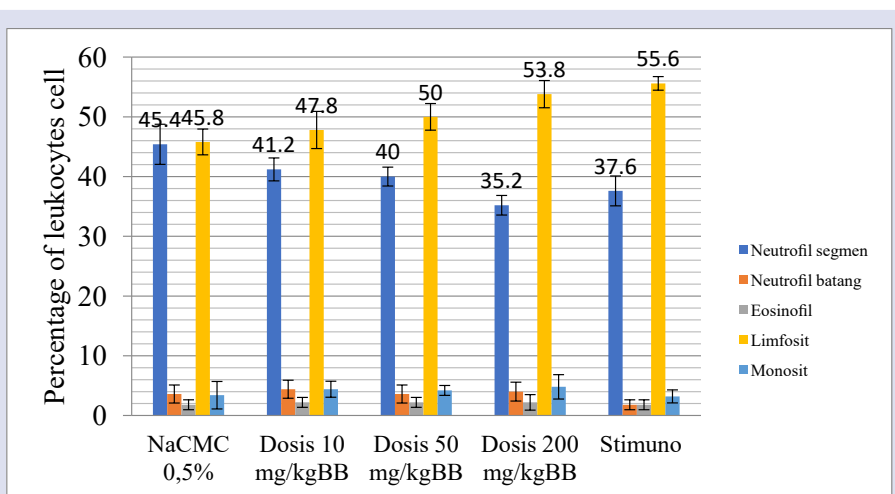
Total leukocyte ( $\mu$ L blood)	
Doses	Mean $\pm$ SD
Na CMC 0,5 %	4370 $\pm$ 496,99 <sup>a</sup>
10 mg/kgbw	8540 $\pm$ 482,70 <sup>b</sup>
50 mg/kgbw	8810 $\pm$ 207,36 <sup>b</sup>
200 mg/kgbw	9460 $\pm$ 403,73 <sup>c</sup>
Stimuno	10020 $\pm$ 668,58 <sup>c</sup>

**Table 4: Percentage of leukocytes in control groups and male white mice treated with *pegagan embun* (*Hydrocotyle sibthorpioides* Lam.) extract.**

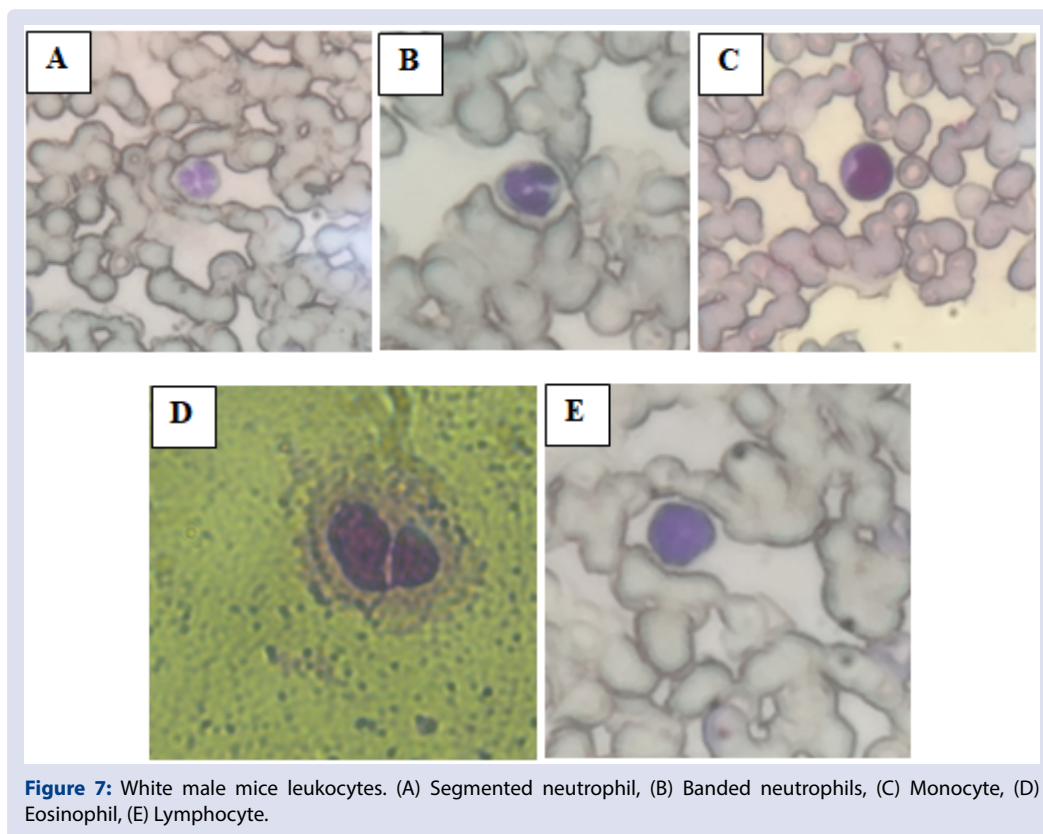
Doses	Percentage of leukocytes (%)				
	Segmented neutrophils	Banded neutrophils	Eosinophils	Lymphocytes	Monocytes
Na CMC 0,5 %	45,40 $\pm$ 3,36 <sup>d</sup>	3,60 $\pm$ 1,51	1,80 $\pm$ 0,83	45,80 $\pm$ 2,16 <sup>a</sup>	3,40 $\pm$ 2,30
10 mg/kgbw	41,20 $\pm$ 1,92 <sup>c</sup>	4,40 $\pm$ 1,51	2,20 $\pm$ 0,83	47,80 $\pm$ 3,11 <sup>ab</sup>	4,40 $\pm$ 1,34
50 mg/kgbw	40,00 $\pm$ 1,58 <sup>bc</sup>	3,60 $\pm$ 1,51	2,20 $\pm$ 0,83	50,00 $\pm$ 2,23 <sup>b</sup>	4,20 $\pm$ 0,83
200 mg/kgbw	35,20 $\pm$ 1,64 <sup>a</sup>	4,00 $\pm$ 1,58	2,20 $\pm$ 1,30	53,80 $\pm$ 2,28 <sup>c</sup>	4,80 $\pm$ 2,04
Stimuno	37,60 $\pm$ 2,50 <sup>ab</sup>	1,80 $\pm$ 0,83	1,80 $\pm$ 0,83	55,60 $\pm$ 1,14 <sup>c</sup>	3,20 $\pm$ 1,09



**Figure 5:** Bar graph of dosage versus total leukocyte count in white male mice.



**Figure 6:** Bar graph of dosage versus percentage of leukocytes in white male mice.



**Figure 7:** White male mice leukocytes. (A) Segmented neutrophil, (B) Banded neutrophils, (C) Monocyte, (D) Eosinophil, (E) Lymphocyte.

## ACKNOWLEDGMENT

Profound thanks towards Research Institutions and Community Service (LPPM) of Andalas University that has funded this research.

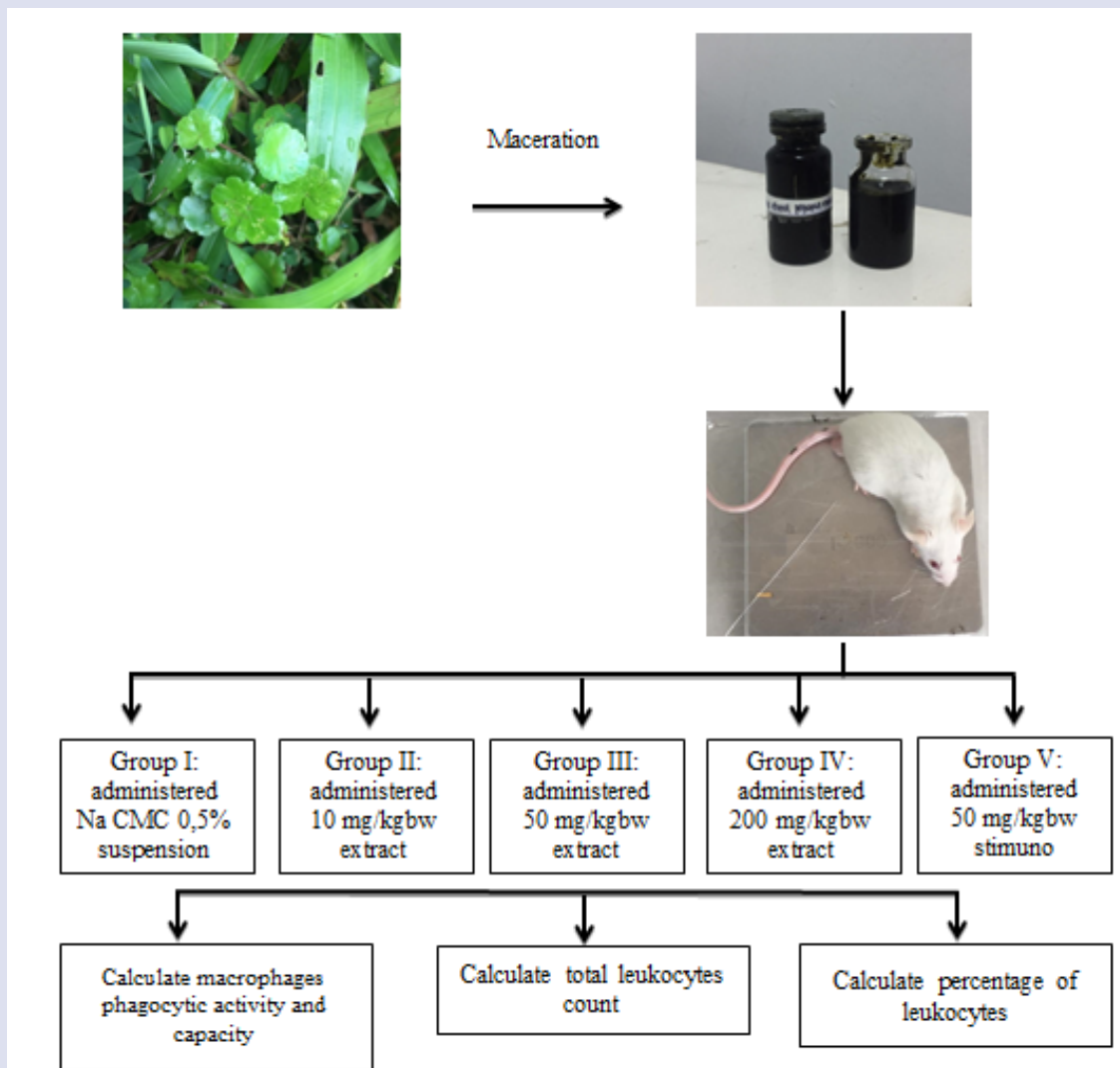
## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

## REFERENCES

1. Hasdianah, Dewi P, Peristiowati, Imam S. S. *Imunologi: Diagnosis Dan Teknik Biologi Molekuler*. Yogyakarta: Nuha Medika; 2014.
2. Burtis CA, Ashwood ER, Bruns DE. *Tietz textbook of clinical chemistry and molecular diagnostics-e-book*. Elsevier Health Sciences; 2012.
3. Hansen PJ. *Laboratory procedures use of Hemacytometer*. Florida: University of Florida; 2000.
4. Huang Q, Huang R, Zhang S. Protective effect of genistein isolated from *Hydrocotyle sibthorpioides* on hepatic injury and fibrosis induced by chronic alcohol in rats. *Toxicol Lett*. 2013;217(2):102-10.
5. Badrunasar A, Santoso H. *Tumbuhan Liar Berkhasiat Obat*. (Rachman E, Siarudin, eds.). Jawa Barat: Forda Press; 2016.
6. Yu F, Yu F, Mcguire PM, Li R, Wang R. Effects of *Hydrocotyle sibthorpioides* extract on transplanted tumors and immune function in mice. 2007;14:166-71.
7. Republic of Indonesia Ministry of Health. *Indonesian Herbal Pharmacopoeia*, ed 1. Jakarta; 2008.
8. Aldi Y, Megaraswita, D Dillasamola, Effect of *Elephantopus Scaber* Linn. leaf extract on mouse immune system. *Trop J Pharm Res*. 2019;18(10):2045-50.
9. Aldi Y, E Husni, R Yesika, Activity of Kincung Flowers (*Etilingera Elatior* (Jack) R.M.Sm.) on Total Leukocytes and Percentage of Leukocytes in Allergic Male White Mice. *Pharmacogn J*. 2020;12(1):44-51.
10. Aldi Y, Dillasamola D, Florina T, Friardi, Activity and Capacity Test of Macrophage Peritoneal Cell and Number Leukocyte of Ethanol Extract Purple Sweet Potato Peel *Ipomoea Batatas* (L.) Lam. *RJPBCS*. 2016;7(5):178-86.
11. Dillasamola D, Aldi Y, Kolobinti M. The Effect of Coriander Ethanol Extract (*Coriandrum sativum* L.) Against Phagocytosis Activity and Capacity of The Macrophage Cells and The Percentage of Leukocyte Cells in White Male Mice. *Pharmacogn J*. 2019;11(6):1290-8.
12. Hasnaeni, Wisdawati, Usman S. Pengaruh Metode Ekstraksi Terhadap Rendemen dan Kadar Fenolik Ekstrak Tanaman Kayu Beta-Beta (*Lunasia amara* Blanco). *J Farm Galen*. 2019;5(2):175-82.
13. Yan J, Jie W, XY, DZ. Influence of Flavonoid of *Astragalus Membranaceus*'s Stem and Leaf on the Function of Cell Mediate d Immunity in Mice. *CJIM*. 2001;7(2):117-20.

## GRAPHICAL ABSTRACT



## SUMMARY

*Pegagan embun* (*Hydrocotyle sibthorpioides* L.) is known for its immunostimulatory effect. The aim of this research is to study the effect of *pegagan embun* ethanol extract on macrophages phagocytic activity and capacity, total leukocyte count, and percentage of leukocytes. 25 male white mice were divided into 5 equal groups. Negative control group were given Na CMC 0.5%, the extract treated groups were given ethanol extract of *pegagan embun* herbs at doses of 10, 50, 200 mg/kgbw, and the positive control group was given Stimuno 50 mg/kgbw orally for 7 days. On the 8<sup>th</sup> day, the total and percentage of leukocytes were counted through the blood sample taken intravenously. The mice were then induced with *Staphylococcus aureus* suspension. The results shows significant increase of concentration ( $p < 0.05$ ) towards macrophages phagocytic activity and capacity, and total leukocytes count. Percentage of leukocytes show that lymphocytes increases significantly ( $p < 0.05$ ), meanwhile neutrophils segments decreases significantly ( $p < 0.05$ ). It can be concluded that ethanol extract of *pegagan embun* herb at doses of 10, 50, 200 mg/kgbw shows immunostimulatory activity through increasing macrophages phagocytic activity and capacity, increasing the number of lymphocytes, and decreasing the number of segmented neutrophils. *Pegagan embun* extract at doses of 200 mg/kgbw show similar immunostimulatory effect as 50 mg/kgbw stimuno.

## ABOUT AUTHORS



**Dr. dr. Afriwardi, Sp.KO.:** Born in Padang Kandis at 21 April 1967. Completed his education in Medicine Faculty in 1994 at Universitas Andalas, and his Specialist in Sports Medicine in Universitas Indonesia (2001) also the Doctoral Program in Biomedical Science at Universitas Andalas (2010). Currently, he is a lecturer in the Physiology Section of the Faculty of Medicine Universitas Andalas and has been a Dean at the Faculty of Dentistry of Universitas Andalas. His research interest in physiology and biomolecular research.



**Prof. Dr. Yufri Aldi, M.Si, Apt :** Currently as a lecturer functional positions at the Faculty of Pharmacy, Andalas University. Graduated from Faculty of Pharmacy Andalas University in 1989, then Master Program at Faculty of Pharmacy Andalas University in 1994 and Doctoral Program in Departement Biomedical, Faculty of Medicine, Andalas University in 2013. The research and expertise are in Farmaco-Immunology. Currently working as head of the Department Pharmacy Doctoral Program, Faculty of Pharmacy, Andalas University.



**Dwisari Dillasamola, M.Farm, Apt :** Currently as a lecturer functional positions at the Faculty of Pharmacy, Andalas University. Graduated from Faculty of Pharmacy Andalas University in 2004, then Master Program at Faculty of Pharmacy Andalas University in 2011. The research and expertise are in Farmaco-Immunology. Currently working as lecturer of Farmaco-Immunology and Clinical-pharmacy of Faculty of Pharmacy, Andalas University.



**Yolanda Alfiyah Larakhansa :** Currently as a student at Faculty of Pharmacy, Andalas University that had studied the effect of of *pegagan embun* (*Hydrocotyle sibthorpioides* Lam.) ethanol extract on macrophages phagocytic activity and capacity, and percentage of leucocytes in white male mice.



**Elsa Badriyya, M. Si. Apt.:** Currently a lecturer at the Faculty of Pharmacy, Andalas University. Graduated from the School of Pharmacy, Bandung Institute of Technology in 2016, then the Master's Program in 2017 at the Bandung Institute of Technology, School of Pharmacy. Currently working as a lecturer in Pharmacology and Clinical Pharmacy at the Faculty of Pharmacy, Andalas University.

**Cite this article:** Afriwardi, Aldi Y, Dillasamola D, Larakhansa YA, Badriyya E. Immunostimulatory Activities of Pegagan Embun (*Hydrocotyle sibthorpioides* Lam.) in White Male Mice. *Pharmacog J.* 2021;13(2): 368-75.