Pharmacogn J. 2023; 15(2): 375-383
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Complete Blood Profile after administration of Hydrocotyle sibthorpioides Lam. extract in capsule form

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History

- Submission Date: 06-01-2023;
- Review completed: 24-02-2023;
- Accepted Date: 27-02-2023.

DOI : 10.5530/pj.2023.15.58

Article Available online

http://www.phcogj.com/v15/i6

ABSTRACT

Pegagan embun (*Hydrocotyle sibthorpioides* Lam.) has been studied as an immunostimulant, increasing macrophage cell activity and phagocytosis capacity. Based on that circumstance, the study aims to prove the immunostimulating effect by measuring the activity of *Hydrocotyle sibthorpioides* Lam. extract in the capsule for the complete blood profile of healthy volunteers. The number of volunteers used was twenty people, and the volunteers were divided into two groups. The first group was given a placebo capsule without *Hydrocotyle sibthorpioides* Lam. extract. The second group was given a capsule with *Hydrocotyle sibthorpioides* Lam. extract with a dose of 67 mg, which was taken once a day for three days. Blood sampling was obtained before and after taking the capsule preparation. Observation of the complete blood profile was conducted by investigating changes in blood parameters such as haemoglobin levels, number of erythrocytes, number of leukocytes, hematocrit values, number of thrombocytes and the percentage of leukocyte types (banded neutrophils, segmented neutrophils, eosinophils, basophils, monocytes, and lymphocytes). The second group given *Hydrocotyle sibthorpioides* Lam. extract showed a significant effect on the increase in haemoglobin levels, number of thrombocytes and hematocrit values ($p<0.05$). The percentage of leukocyte type values showed that lymphocytes increased significantly ($p<0.05$). In contrast, the segmented neutrophil increased but did not show a significant difference with the percentage of banded neutrophils, eosinophils, basophils and monocytes ($p>0.05$). There was a non-significant result in all parameters for the first group, which was administrated with a placebo capsule without *Hydrocotyle sibthorpioides* Lam. extract.

Key words: *Hydrocotile sibthorpioides* Lam., Immunostimulants, Haematology, Haemoglobin, Erythrocytes, Leukocytes, Hematocrit, Basophils, Eosinophils, Banded neutrophil, Segmented neutrophil, Monocytes, Lymphocyte, Thrombocytes.

INTRODUCTION

Immunostimulants can increase the body's defence against viral infections by enhancing the immune system and non-specifically cellular and humoral defence mechanisms. Immunostimulating compounds can be obtained from plants. The mechanism used by the body as protection against infectious diseases is the immune system. The immune system plays a role in the blood and has cells known as neutrophils, eosinophils, basophils, monocytes, and lymphocytes.[1] The specific and non-specific immune systems work together to fight infection. The immune response consists of various cells and dissolved molecules secreted by these cells. The primary cells involved in immune reactions are lymphocytes (B cells, T cells, and NK cells), phagocytes (neutrophils, eosinophils, monocytes, and macrophages), accessory cells (basophils, mast cells, and platelets).[2]

Immunostimulating compounds can be obtained from plants. One of the plants used by the Indonesian to boost the immune system is Pegagan embun (*Hydrocotyle sibthorpioides* Lam.). It has been used as a traditional medicine in fresh and dry ingredients. Research conducted by Yu *et al.* reported that *Hydrocotyle sibthorpioides* extract produced excellent antitumor effects and demonstrated the ability to influence the immunological function of mice.[3]

Hydrocotyle sibthorpioides Lam. has been studied as an immunostimulant, increasing the activity and phagocytosis capacity of macrophage cells, having an anti-inflammatory effect when used topically,[4] reducing TNF α levels, increasing the activity of NK cells and CD8 cells from male white mice exposed to antigen H5N1 virus.[5] Furthermore, the ethanol extract of *Hydrocotyle sibthorpioides* Lam. has also been shown to have a haematopoietic effect on anaemic mice.[6] In addition, the safety of *Hydrocotyle sibthorpioides* Lam. has been studied by observing the toxicity tests of LD50,[7] SGOT and SGPT,[12] Creatinin Clearance, as well as observing the histology of liver tissue and kidneys of mice using the extract of *Hydrocotyle sibthorpioides* Lam..[7]

The percentage value of leukocytes in the pre-clinical test showed that *Hydrocotyle sibthorpioides* Lam. extract at doses of 10, 50, and 200 mg/kg had a significant effect on the percentage of segmented neutrophils and lymphocytes ($p<0.05$).[4] The percentage of segmented neutrophils decreased after administration of *Hydrocotyle sibthorpioides* Lam. extract. Therefore, it can be assumed that macrophages play an essential role in phagocytosis. It proved by increasing chemotactic factors that increase the ability of phagocytosis.[10]

Through pre-clinical and toxicity tests carried out previously, it can be concluded that *Hydrocotyle sibthorpioides* Lam. can be used as an immune system-enhanced drug. Furthermore, it is necessary to conduct a clinical trial. Based on the description above, the study aims to assess the effect of *Hydrocotyle sibthorpioides* Lam. extract capsules to

Cite this article: Afriwardi, Fauzan A, Aldi Y, Umar S. Complete Blood Profile after administration of Hydrocotyle sibthorpioides Lam. extract in capsule form. Pharmacogn J. 2023;15(2): 375-383.

determine the drug's effect on the complete blood profile by examining its levels in healthy human blood.

MATERIALS AND METHODS

Tool

The tools used in this study were glassware (Pyrex), Erlenmeyer (Pyrex), capsule filler, blender (Panasonic), porcelain crucible, drying cupboard, mortar and pestle, rotary evaporator (Ika), microscope (Olympus), rough balance (Ohaus), analytical balance (Vibra AJ), Cell Dyn-Ruby Hematology analyser (Abbott), UV-Vis spectrophotometry.

Materials

The materials were *Hydrocotyle sibthorpioides* Lam., aquadest, ethanol 70%, ethanol 96%, rutin, silica gel 60 $F_{254,}$ Mayer's reagent, Dragendorf's reagent, HCl, metal Mg, $FeCl_3$, $HgCl_2$, $AlCl_3$, sodium acetate, aquadest (Andeska Laboratory), carboxymethyl cellulose (Na CMC), Giemsa stain 0.5% (Merck), Aerosil, Amylum Manihot, Magnesium stearate, lactose (Brataco).

Making *Hydrocotyle sibthorpioides* Lam. extract

The first process begins with preparing *Hydrocotyle sibthorpioides* Lam. extract by collecting 5000 g fresh *Hydrocotyle sibthorpioides* Lam. plants, then cleaning it from dirt by washing it with running water, and then airing it to dry simplicia. The dried simplicia was crushed using a blender and sieved using sieve no. 48. This refinement aims to widen the surface of the simplicia so that the process of withdrawing the active substance is more effective and efficient. Also, the solvent can penetrate easily so that the withdrawal of the active substance is complete. The extraction process carried out by the maceration method. It chosen because of the advantages of using a large number of samples, simple implementation, does not require special treatment and does not go through a heating process so that the compounds contained in the sample are not easily decomposed. Sample maceration conducted using 70% ethanol solvent.[9] Extraction was conducted by the maceration method by soaking one part of finely powdered simplicia plus ten parts of 70% ethanol solvent into a dark-coloured. The mixture was stirred occasionally for six hours, set aside for eighteen hours, and then filtered. Repeat this process twice with the same ratio of powder and solvent. All maceration was collected and then evaporated with a rotary evaporator at 40^{0}C until a thick extract was obtained. The viscous extract was then made into a dry extract through freeze-drying.

The use of ethanol as a universal solvent is due to its ability to quickly dissolve polar, semipolar and nonpolar compounds.[5] Maceration was carried out for 24 hours with three repetitions using 70% ethanol (1:10). The powder is soaked for the first 6 hours while stirring occasionally and allowed to stand for 18 hours. Maceration was carried out with two repetitions and then filtered using filter paper. The maceration results were collected and then evaporated using a rotary evaporator to obtain a thick extract.[5]

Capsule formulation

A 500 mg capsule chosen (0# size) with an orange cap and yellow capsule body. Saccharum lactis (SL) is the placebo powder used to fill the capsule. It weighed 335 mg per capsule, and the water content was less than 10%. This capsule should disintegrate in less than thirty minutes.

The dosage used in making *Hydrocotyle sibthorpioides* Lam capsules is 10 mg/kg human body weight. So, the weight of the extract in 1 capsule is 67 mg. All ingredients were weighed according to the concentration in the formula. Put Amylum Manihot, aerosil, and dry extract of *Hydrocotyle sibthorpioides* Lam. into the mortar, grind until homogeneous, then add magnesium stearate and lactose. Grind until homogeneous, and put into capsule shell no.0#.

Extract standardisation

Hydrocotyle sibthorpioides Lam. extract was characterised specifically and non-specifically. Non-specific characterisation consists of water contains with a 5.56 % of yield: 2.25 of total ash value: and 0.07% insoluble acid ash. In contrast, the specific characterisation consisted of organoleptic tests, identity parameters, chemical content tests, thin layer chromatography, and determination of total flavonoid content.

Evaluation of *Hydrocotyle sibthorpioides* Lam. extract powder

The powder was readily formulated then subjected to an evaluation test before being put into the capsule to ensure the effectiveness of the powder. The evaluation tests were the flow rate test, compressibility test, and angle of repose test.

Evaluation of *Hydrocotyle sibthorpioides* Lam. capsules

The finished capsules are then subjected to evaluation tests to ascertain how effective the capsules are. The evaluation tests carried out were organoleptic, weight uniformity, and disintegration time tests.

Clinical trial test protocol

The study used 20 healthy volunteers, 20-21-year-old, who were divided into two groups, called the group that was given preparations without the active substance and the group that was given the active substance. Then blood samples were taken before and after administration of the test preparations, which were taken once a day for three days. The protocol began with an initial examination of the patient's blood profile (before administering the test preparation/H_0). The patient received a test preparation *Hydrocotyle sibthorpioides* Lam. capsule, consumed once after eating in a day and repeated for three days. At the end of the study, the healthy volunteer's blood profile was examined to see changes in the parameters tested (after administration of the test preparation/H_1).

Sample determination and examination

A health analyst carries out blood sampling, and the collection performed after the patient has fasted for 6-12 hours. Blood was taken as much as 3 ml through the median cubital vein in the elbow fold, first cleaned above the puncture site with 70% alcohol and allowed to dry, then a tourniquet was placed on the upper arm, the median cubital vein was stabbed at an angle of 45 degrees. Blood is aspirated to flow into the syringe; the tourniquet is released, the needle withdrawn while pressing the puncture hole with alcohol cotton, and the puncture site is closed with a plaster. The blood obtained is centrifuged to form blood plasma, which used to examine blood profiles (haemoglobin levels, erythrocyte counts, leukocyte counts, hematocrit values, platelet counts, and percentage values), basophils, eosinophils, segmented neutrophils, banded neutrophil, lymphocytes, and monocytes). The examination was carried out in a Sejawat laboratory using a haematology analyser machine.

Data analysis

The research data were analysed statistically using the *Simple Paired Test* analysis method with IBM SPSS Statistics 25 Version.

Ethics approval

Ethical approval was obtained from the Faculty of Medicine Ethics Committee, Universitas Andalas, with an ethical letter contract number: 1072/UN.16.2/KEP-FK/2022.

Table 1: Capsules formula.

Ingredient	F1	F1
Hydrocotyle sibthorpioides Lam. extract	-	67 mg
Aerosil	2%	2%
Amylum Manihot	2%	2%
Magnesium stearate	1%	1%
Lactose	qs	qs

Table 2: Evaluation results of capsule powder.

Evaluation Type	Results	Condition
Flow rate	8.21 grams/second	4-10 grams/second
% Compressibility	14.3%	11-15%
Angle of repose	32.31^0	21-35^0

Table 3: Evaluation results of capsule.

Evaluation Type	Results	Condition
Organoleptic	Qualify	Clean, dry, no particles attached to the capsule shell
Weight uniformity	Qualify	<10%
Disintegration time	2 min 41 second	<15 minutes

Table 4: Average Haemoglobin levels in the placebo-filled capsule group Saccharum lactis (SL) and the capsule group containing *Hydrocotyle sibthorpioides* Lam. extract.

Group	Haemoglobin Level (gr/dL)	
	Pre	Post
Placebo	14.94±0.6	14.90±0.85
Hydrocotyle sibthorpioides Lam. extract.	14.92±0.7	15.32±0.8

Table 5: The results of the average percentage of leukocyte type values in the placebo capsule group containing *Saccharum lactis* (SL) and group of *Hydrocotyle sibthorpioides* Lam.

Percentage of Leukocyte Type Value (%)				
	Placebo (Pre)	Placebo (Post)	Hydrocotyle sibthorpioides Lam. (Pre)	Hydrocotyle sibthorpioides Lam. (Post)
Basophils	0	0	0.1± 0.31	0
Eosinophils	2.2± 0.7	1.6± 0.3	2.1± 0.99	1.5± 0.85
Banded N.	3.3± 0.95	3± 1.05	3.4± 1.17	3± 1.05
Segment N.	56.1± 4.5	59.6± 8.9	57.3± 6.89	58.2± 8.2
Lymphocytes	29.5± 2.06	29± 4	29.7± 5.85	34.2± 4.9
Monocytes	5.7± 1.05	5.8± 0.6	5± 0.8	6± 1.2

RESULTS

Haemoglobin levels

The results for haemoglobin levels showed in Table 4 and Figure 1. The group that was given *Hydrocotyle sibthorpioides* Lam. extract capsules showed a significant increase in haemoglobin levels ($p<0.05$).

The results of erythrocytes value showed an increase in the administration of *Hydrocotyle sibthorpioides* Lam. extract capsules in healthy volunteers, but there was no significant effect ($p>0.05$) (Table 7).

Furthermore, the results obtained on the number of leukocytes showed an increase in the administration of *Hydrocotyle sibthorpioides* Lam extract capsules in healthy volunteers (Figure 3). However, there was no significant effect ($p>0.05$) (Table 8).

The results for the haematocrit values showed in (Figure 4). The group that was given *Hydrocotyle sibthorpioides* Lam extract capsules showed a significant increase in the hematocrit value ($p<0.05$) (Table 9).

The results for the number of thrombocytes showed in Figure 5. The group given the extract of *Hydrocotyle sibthorpioides* Lam. capsules showed a significant effect in increasing the number of thrombocytes ($p<0.05$) (Table 11). After administration of Hydrocotyle sibthorpioides Lam. capsules, the average number of thrombocytes was 263,500/mm.

The results for the percentage of leukocyte cell types showed in Table 5 and Figure 6. The group that was given *Hydrocotyle sibthorpioides* Lam. extract capsules showed a significant increase in lymphocyte values $p<0.05$ (Table 11), the average lymphocyte value after administration of *Hydrocotyle sibthorpioides* Lam capsules by 34.2%.

Statistical analysis results

The results of statistical analysis on haemoglobin levels, number of erythrocytes, number of leukocytes, haematocrit values, number of thrombocytes and leukocyte count values on lymphocyte values. The research data were analysed statistically using the *Simple Paired Test* analysis method. Significant results with significance were taken at $p<0.05$.

DISCUSSION

The complete blood profile observed in blood parameters such as haemoglobin levels, number of erythrocytes, number of leukocytes, haematocrit values, number of thrombocytes and the percentage of leukocyte types, such as basophils, eosinophils, banded neutrophils, segmented neutrophils, monocytes, and lymphocytes in humans using *Hydrocotyle sibthorpioides* Lam. as a test substance. However, it assessed beforehand to determine the sample identity.

After the thick extract of *Hydrocotyle sibthorpioides* Lam. obtained, the extract standardised to test non-specific and specific parameters to obtain a safe and quality-assured extract. Non-specific parameters carried out included: water content, ash content, and acid-insoluble ash content. The drying shrinkage value provides a limit or minimum range for the compound lost in the drying process.[9]

Furthermore, examining the specific parameters of *Hydrocotyle sibthorpioides* Lam. extract was carried out to provide an objective identity and a specific name for the plant used. The organoleptic examination aims to provide a simple initial recognition of the *Hydrocotyle sibthorpioides* Lam. extract used, a viscous extract with a characteristic odour, dark brown colour and bitter taste. The chemical content test of the extract included a phytochemical test, in which the reaction between *Hydrocotyle sibthorpioides* Lam. extract and its specific reagents showed that the *Hydrocotyle sibthorpioides* Lam. extract positively contained flavonoids, phenolics, terpenoids, and saponins.

In the Thin Layer Chromatography (TLC) test, the eluent or mobile phase used was n-butanol:acetic acid:water (4:1:5). The test solution and sample solution were then daubed on the F254 silica gel plate at the lower limit outlined in pencil. The plate is 10 cm long and 3 cm wide with a top and bottom border of 1 cm each. Once visible, the silica plate was inserted into the eluent or mobile phase chamber. Previously, the camber was saturated using filter paper. The plate was removed and dried, then measured under UV light (λ 366 nm) using an AlCl3 stain spotter to produce spots.

Bioactive compounds in *Hydrocotyle sibthorpioides* Lam. extract one of them is a flavonoid which can increase the proliferation of lymphocytes or T cells. In addition, flavonoids have been shown to inhibit the production of macrophage cytokines and reduce T-cell inflammation. Polyphenol molecules also have the potential to enhance

Table 6: **Analysis of haemoglobin data.**

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Means	std. Deviation	std. Error Means	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	preex - postex	-.4000	.3367	.1065	-.6408	-.1592	-3,757	9	005
Pair 2	Prektrl-postktrl	.0400	.5562	.1759	-.3579	.4379	.227	9	.825

Table 7: **Erythrocyte data analysis.**

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Means	std. Deviation	std. Error Means	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	preex - postex	-.11400	.41578	.13148	-.41143	.18343	-.867	9	.408
Pair 2	prektrl - postktrl	.05400	.26492	.08377	-.13551	.24351	.645	9	.535

Table 8: **Leukocyte data analysis.**

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Means	std. Deviation	std. Error Means	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	preex - postex	-310,000	445,845	140,989	-628,938	8,938	-2,199	9	055
Pair 2	prektrl - postktrl	40,000	596,657	188,680	-386,823	466,823	.212	9	.837

Table 9: **Analysis of hematocrit data.**

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Means	std. Deviation	std. Error Means	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	preex - postex	-1.4100	1.4791	.4677	-2.4681	-.3519	-3015	9	.015
Pair 2	prektrl - postktrl	.0900	.5322	.1683	-.2907	.4707	.535	9	.606

Table 10: **Thrombocytes data analysis.**

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Means	std. Deviation	std. Error Means	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	preex - postex	-12100,000	14533.104	4595771	-22496.356	-1703644	-2,633	9	.027
Pair 2	prektrl - postktrl	3100,000	29266781	9254969	-17836.194	24036194	.335	9	.745

Table 11: **Lymphocytes data analysis.**

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Means	std. Deviation	std. Error Means	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	preex - postex	-4.500	2.991	.946	-6.639	-2.361	-4.758	9	.001
Pair 2	prektrl - postktrl	2.900	6.367	2.014	-1.655	7.455	1.440	9	.184

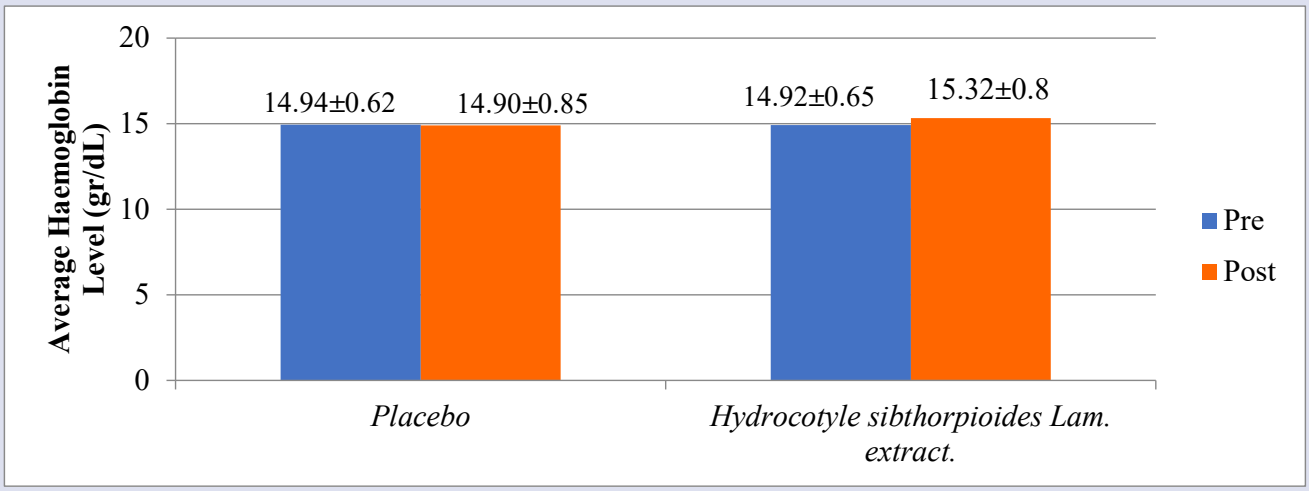

Figure 1: Graphic the average haemoglobin level of the placebo-filled capsule group *Saccharum lactis* (SL) and the capsule group of *Hydrocotyle sibthorpioides* Lam. Extract.

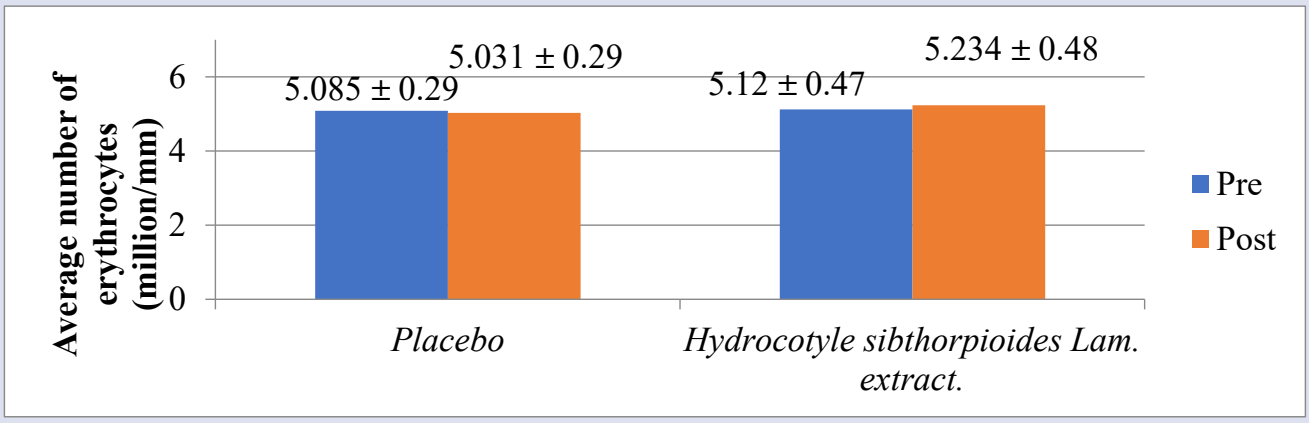

Figure 2: Graphic average amount erythrocytes the placebo capsule group containing *Saccharum lactis* (SL) and the capsule group of *Hydrocotyle sibthorpioides* Lam. Extract.

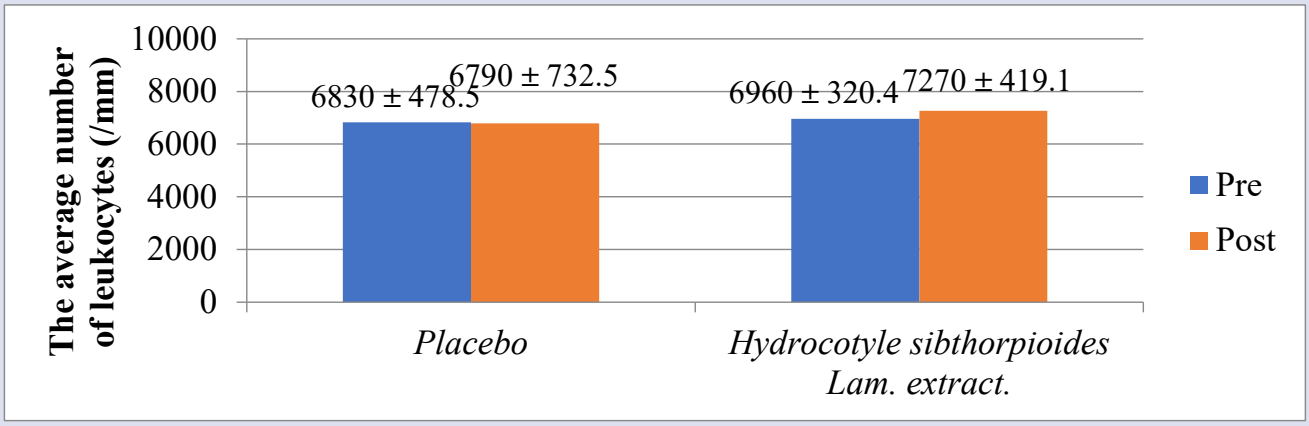

Figure 3: Graphic of the average leukocyte of the placebo capsule containing group *Saccharum lactis* (SL) and the capsule group of *Hydrocotyle sibthorpioides* Lam. Extract.

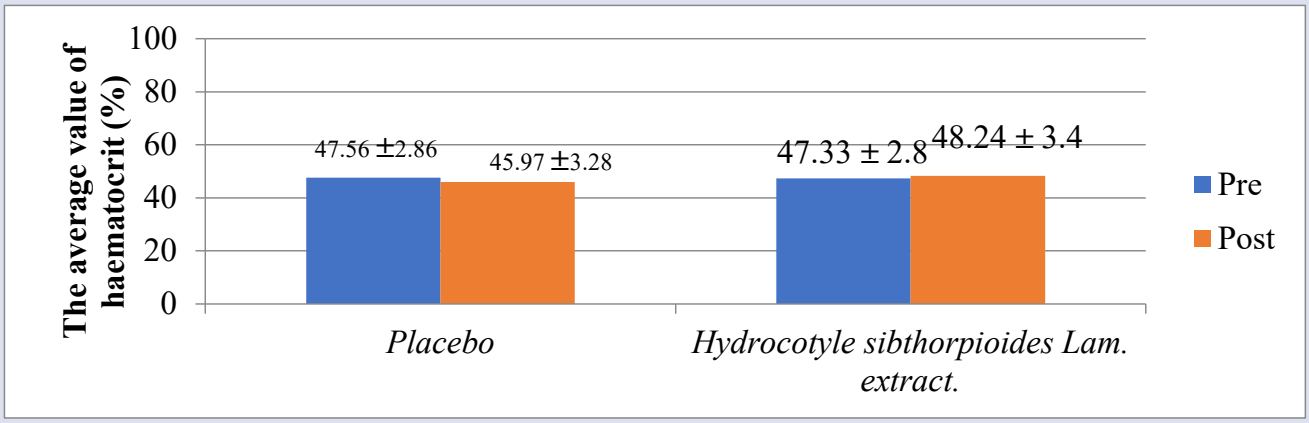

Figure 4: Graphic average result values of the placebo capsule containing haematocrit *Saccharum lactis* (SL) and the capsule group of *Hydrocotyle sibthorpioides* Lam. Extract.

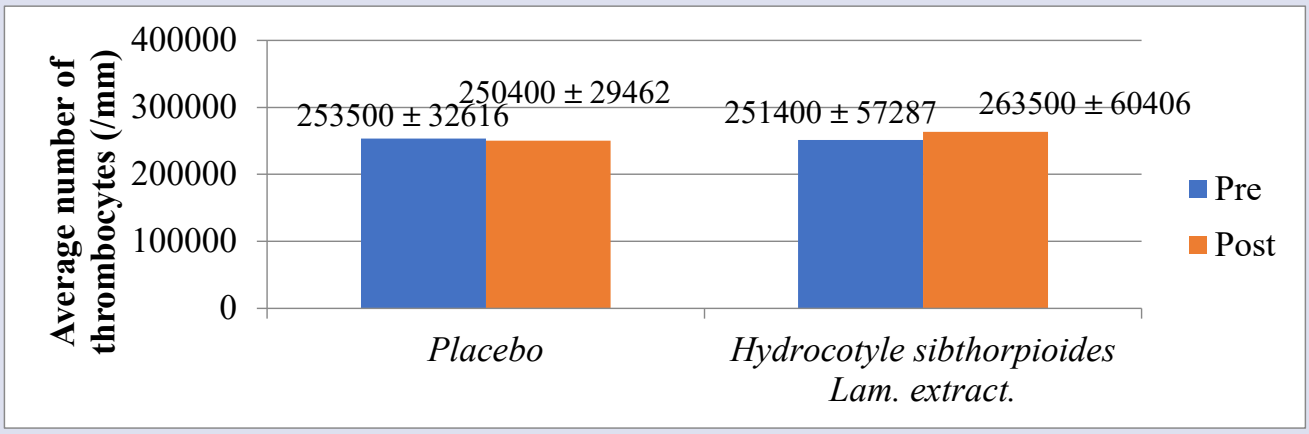

Figure 5: Graphic of average thrombocytes in the placebo capsule group containing *Saccharum lactis* (SL) and the capsule group of *Hydrocotyle sibthorpioides* Lam. Extract.

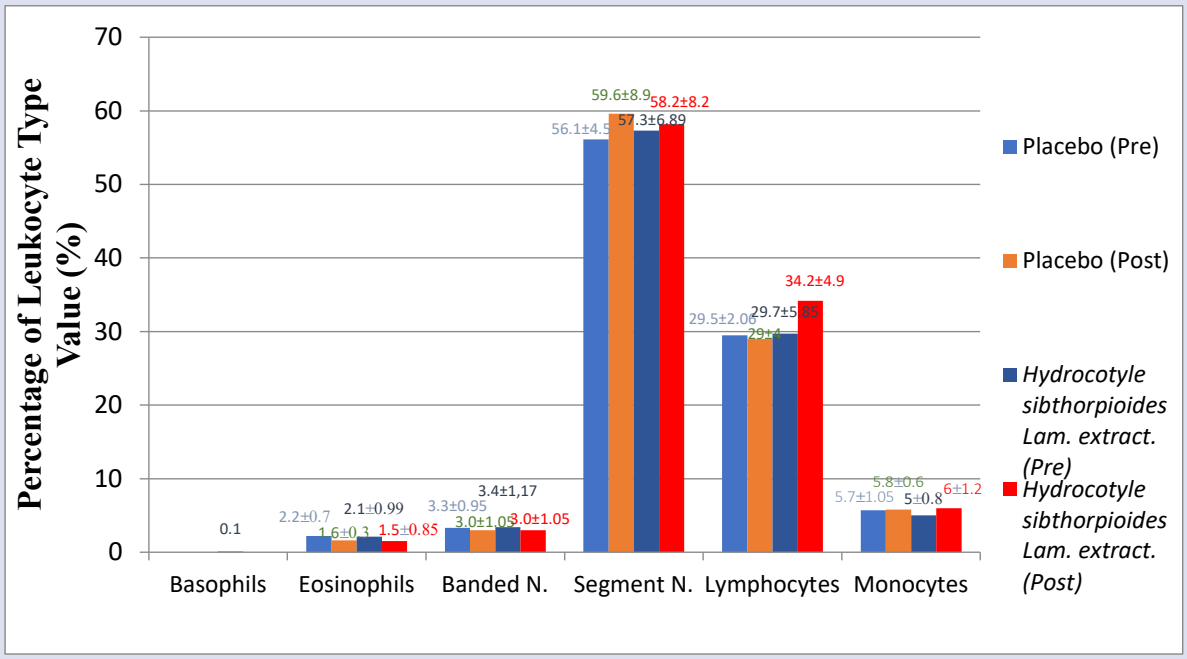

Figure 6: Graphic average percentage of leukocyte type values in the placebo capsule containing group *Saccharum lactis* (SL) and the capsule group of *Hydrocotyle sibthorpioides* Lam. Extract.

or inhibit innate and adaptive immune responses depending on their molecular structure. Flavonoids potentially aid in preventing and treating inflammatory diseases.[6] The flavonoid group can also act as an immunomodulator that can boost the immune system to ward off attacks by viruses, bacteria or other microbes.[7]

There were 11 parameters observed in this study. They are haemoglobin level, number of leukocytes, number of erythrocytes, haematocrit value, number of thrombocytes and leukocyte count values (basophils, eosinophils, banded neutrophils, segmented neutrophils, monocytes and lymphocytes).

The average results of haemoglobin levels before and after placebo administration were 14.94 and 14.90 gr/dL. In contrast, the results of the average haemoglobin level in the administration of *Hydrocotyle sibthorpioides* Lam. capsules before and after were 14.92 and 15.32 gr/dL. Based on these results, the group that was given *Hydrocotyle sibthorpioides* Lam. extract. showed a significant increase in haemoglobin levels with $p<0.05$. There was no significant difference in the group given placebo capsules containing *Saccharum lactis* (SL) with a $p>0.05$ of 0.825. Based on previous studies, administration of *Hydrocotyle sibthorpioides* Lam. extract to the factor of variation in dose and duration of administration of the test preparation had a significant effect on the number of erythrocytes, haemoglobin levels, and hematocrit values ($p<0.05$). Increasing the dose and duration of administration of *Hydrocotyle sibthorpioides* Lam. extract can increase hematopoietic parameters.[9] Overall, the results of the examination of volunteer levels were in the normal range of haemoglobin levels 14-18 gr/dL.[11]

The average results for the number of erythrocytes before and after placebo administration were 5,085 and 5,031 million/mm. At the same time, the results for the number of erythrocytes in the administration of *Hydrocotyle sibthorpioides* Lam. capsules before and after were 5,12 and 5,234 million/mm. In addition, the number of erythrocytes increased after administration of *Hydrocotyle sibthorpioides* Lam. capsules. However, there was no significant effect on the number of erythrocytes before and after the volunteers consumed capsules containing *Hydrocotyle sibthorpioides* Lam. extract ($p>0.05$). The formation of red blood cells is influenced by cells' functional ability to transport oxygen to the tissues. Erythrocyte cell production regulated by erythropoietin, a glycoprotein hormone in the blood in hypoxic conditions and then acts on the bone marrow to increase red blood cell formation.[6] The process of developing red blood cells from stem cells takes about seven days, and this process is called the process of erythropoiesis. Increasing the administration duration of Hydrocotyle sibthorpioides Lam. extract can increase the number of erythrocytes.[9] Overall, the examination results of volunteer levels were in the normal range for the number of erythrocytes 4,5-6,5 million/mm.[11]

The average results for the number of leukocytes before and after placebo administration were 6830 and 6790 /mm. In contrast, the results of the average number of leukocytes in the administration of *Hydrocotyle sibthorpioides* Lam. capsules before and after were 6960 and 7270 /mm. Overall the results of the examination of volunteer levels were in the normal range for the number of leukocytes at 5-10 hundred/mm.[11] The results showed that the number of erythrocytes increased after administration of *Hydrocotyle sibthorpioides* Lam. capsules. However, there was no significant effect on the average number of leukocytes before and after the volunteers consumed capsules containing *Hydrocotyle sibthorpioides* Lam. extract ($p>0.05$). *Hydrocotyle sibthorpioides* Lam. extract contains flavonoid compounds, where flavonoids have the potential as immunomodulators that can increase the number of leukocytes through increasing IL-2 and lymphocyte proliferation. Lymphocyte proliferation will affect CD_4 and then activate TH_1 cells, increasing the number of leukocytes.[12] Increasing the administration duration of Hydrocotyle sibthorpioides Lam. extract can significantly increase the number of leukocytes.[9]

Furthermore, the results of the average hematocrit value before and after administration of placebo capsules obtained values of 47.56% and 45.97%. While the average results of giving *Hydrocotyle sibthorpioides* Lam. capsules before and after the volunteers consumed the capsules were 47.33% and 48.24%. The results for the hematocrit value are shown in (Figure 4) The group that was given the *Hydrocotyle sibthorpioides* Lam. extract showed a significant increase in the haematocrit value ($p<0.05$). There was no significant difference in the group given the placebo capsule containing *Saccharum lactis* (SL). Previous studies stated that administration of *Hydrocotyle sibthorpioides* Lam. extract at the same dose had a significant effect on the number of erythrocytes, haemoglobin levels, reticulocyte values, and haematocrit values ($p<0.05$). Overall, the examination results of volunteer levels were in the normal range of 37-54% haematocrit values. Calculating the haematocrit value aims to be able to help diagnose anaemia. The haematocrit value in adult men is 42-54% and in women 37-47%.[11]

In the number of thrombocytes parameter, the average results before and after administration of placebo capsules were 253500/mm and 250400/mm. In addition, the average results of giving *Hydrocotyle sibthorpioides* Lam. capsules before and after the volunteers consumed the capsules were 251400/mm and 263500/mm. The results for the number of thrombocytes are shown in (Figure 5). The group that was given a capsule of *Hydrocotyle sibthorpioides* Lam. extract showed a significant increase in the number of thrombocytes ($p<0.05$). Overall, the examination results of volunteer levels were in the normal range for the number of thrombocytes, at 150-450 thousand/mm.[11]

Calculating the percentage of leukocyte cell types in volunteers after administration of *Hydrocotyle sibthorpioides* Lam. extract showed that the highest percentage was neutrophils, the second highest was lymphocytes, and the least was basophil cells. According to the literature, the most significant percentage of leukocyte cell types is 50-70% neutrophil cells, and the least are basophils, no more than 2%.[11] The mean results of segment neutrophil values before and after administering placebo capsules were 56.1% and 59.6%. While the average results of giving *Hydrocotyle sibthorpioides* Lam extract capsules before and after the volunteers consumed the capsules were 57.3% and 58.2%. In the results, the average lymphocyte values before and after administration of placebo capsules were 29.5% and 29%. While the average results of giving *Hydrocotyle sibthorpioides* Lam extract capsules before and after the volunteers consumed the capsules were 29.7% and 34.2%. Furthermore, a minor percentage of leukocyte cell types, namely basophil cells, with the average results before and after the placebo were 0% and 0%. While the average results of giving *Hydrocotyle sibthorpioides* Lam. extract capsules before and after the volunteers consumed the capsules were 0.1% and 0%. These results are below 2%, which means they are in the normal range for basophil cell values.[11]

The percentage of leukocyte type values that increased significantly ($p<0.05$), namely lymphocytes, with an average value of 34.2%, while neutrophil segments increased, but did not show a significant difference also banded neutrophils and monocytes ($p>0.05$). There was no difference in the percentage of segmented neutrophils in the capsule treatment group with *Hydrocotyle sibthorpioides* Lam. extract capsules compared to the placebo capsule group containing *Saccharum lactis* (SL). In the body, lymphocytes are the second most abundant type of leukocyte after neutrophils (20-40% of total leukocytes). The number of lymphocytes in children is relatively more than in adults, and these lymphocytes will increase if there is a viral infection.[11] The increase in lymphocytes is closely related to the increase in leukocytes. Lymphocyte proliferation will affect CD_4 and then activate TH_1 cells, increasing

the number of leukocytes.[12] Therefore, it assumed that macrophages play an essential role in phagocytosis or that there is an increase in chemotactic factors that increase the ability of phagocytosis.[4]

CONCLUSION

The study proved that *Hydrocotyle sibthorpioides* Lam. extract in capsule form at a dose of 67 mg could increase hematopoietic activity in the form of increased haemoglobin levels, haematocrit values and platelet cells in humans. *Hydrocotyle sibthorpioides* Lam. in capsule forms at these doses affected phagocytic cells by increasing lymphocyte cells. The average percentage of leukocyte type values showed significantly increased lymphocytes ($p<0.05$).

ACKNOWLEDGEMENT

The authors would like to thank the Rector Universitas Andalas through the Institute for Research and Service Society or "*Lembaga Penelitian dan Pengabdian Kepada Masyarakat*" (LPPM), which facilitated and funded the research in the scheme of "*Penelitian Dasar Unggulan Klaster Riset Publikasi Guru Besar Universitas Andalas*" (PDU KRP1GB UNAND), Batch I, the Year 2022, with Contract Number. T/18/UN.16.17/PT.01.03/KO-PDU-KRP1GB-Unand/2022 sign on 12 April 2022.

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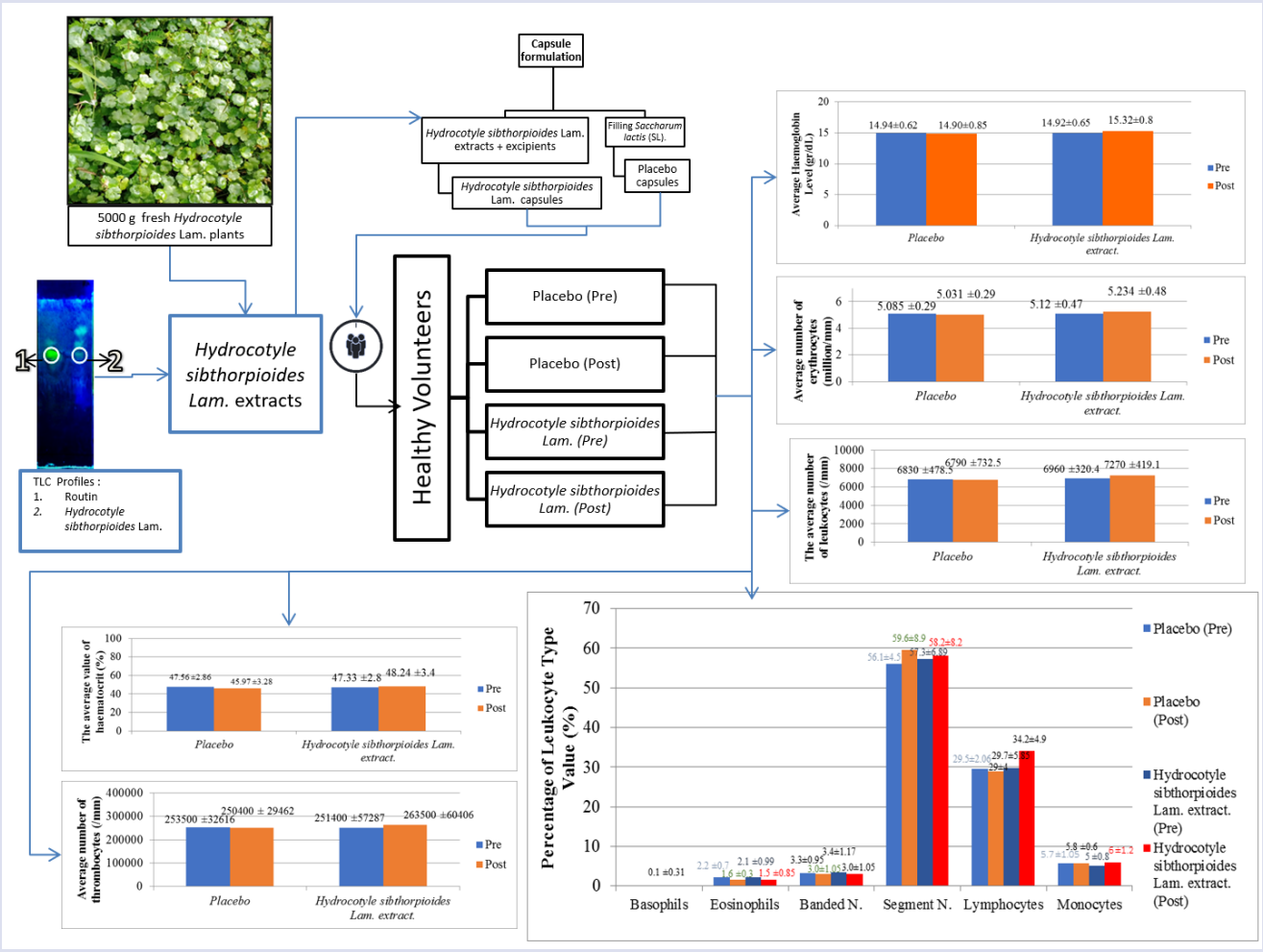
GRAPHICAL ABSTRACT



Cite this article: Afriwardi, Fauzan A, Aldi Y, Umar S. Complete Blood Profile after administration of Hydrocotyle sibthorpioides Lam. extract in capsule form. Pharmacogn J. 2023;15(2): 375-383.