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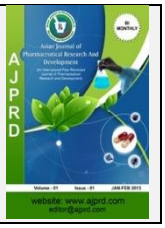

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Review Article

Imunomodulatory Effect of Red Tip Leaf's (Syzygiummyrtifolium Walp.) Ethanol Extract on Male Rat

Rony Andreas Harka Sinulingga, Poppy Anjelisa Z Hasibuan*, Yuandani, Rosidah, Aminah Dalimunthe

Fakultas Farmasi, Universitas Sumatera Utara, Indonesia

A B S T R A C T

The pattern of modern life demands that everything must be done very fast and instant. The quality of the consumed food, air pollution, lack of exercise and stress can cause the body's endurance to decline continuously. The Red Tip (*Syzigium myrtifolium* Walp.) has been researched to have a flavonoid compound that has benefits as an anti-inflammatory and have potentially as an immunomodulator. The purpose of this study is to prove the effect of immunomodulator on the activity of phagocytosis, the total number and the differential of leukosit, the antibody titer value, and delayed type hypersensitivity response. Group treatment to test the clearance of carbon divided into 7 groups, each consisting of 5 male mice including the group of CMC-Na 0.5 %, Imboost ® dose 22.5 mg/ kgBB, the red tip leaf's ethanol extract with dose of 50, 100, 200, 400, and 800 mg/kg orraly distributed for 7 days, and on the 8th day the carbon suspension was given using i.v. The blood intake were done in certain times and then the absorbance was measured using UV-Vis spectrophotometer. The liver and spleen of the rats were taken and weighed. The treatment group for antibody titer was divided into 7 groups with each consisting of 5 male mice using the same treatment as above except positive control that has been used levamisole on 25 mg/kgBB dose that has been distributed orraly for 14 days. The non spesific immune respond utilizing carbon cleanses method by measuring the carbon elemination speed and total amount and leukocyte differential. The specific immune response testing using antibody titers was done based on glutination which was formed and the delayed type hypersensitivity testing based on the mice swollen foot. The research outcome shows that the distribution of the red tip leaf's ethanol extract with dose of 100, 200, 400, and 800 mg/kgBB is increasing the phagocytosis activity significantly compared to CMC-Na 0.5% ($p < 0.05$). The of the red tip leaf's ethanol extract is increasing the total of leukocytes and neutrophils of the segment. The distribution of the red tip leaf's ethanol extract with dose of 50, 100, 200, 400, and 800 mg/kgBB is increasing the formation of rat's immune cell antibodies significantly compared to CMC-Na 0,5% ($p < 0,05$).This research prove that the red tip leaf's ethanol extract has an effect of imunostimulator againts the phagocytosis activity, the total sum and leukocytes differential along with antibody titer value and the delayed type hypersensitivity.

Key Word: Imunomodulator, *Syzigium myrtifolium* Walp, and Leukosit

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*Address for Correspondence:
Poppy Anjelisa Z Hasibuan, Fakultas Farmasi, Universitas Sumatera Utara, Indonesia

INTRODUCTION

Modern lifestyle demands that everything is done quickly and instantaneously. The quality of the food, air pollution, lack of exercise, and stress can cause the immune system to continue to decline. This condition causes pathogenic microbes such as viruses, bacteria, parasites, fungi to quickly enter and attack the body resulting in various infectious and degenerative diseases, which can even cause premature aging [1-5]. umans have a complete defense system against pathogenic organisms. However, the emergence of disease manifestations is not only influenced by pathogenic organisms. However, it is also influenced by a weak body defense system, with the weakening of the body's immunity, even light exposure will cause a dreadful disease, especially in the event of a deadly infection [6-8].

The immune system is all the mechanisms that the body uses to maintain the body's integrity as protection against the dangers posed by various substances in the environment [9]. Two types of immune responses occur when an antigen

invasion occurs, namely the nonspecific immune response or innate immunity and the specific immune response or acquired immunity. The nonspecific immune response is generally innate immunity in the sense that a response to a foreign substance can occur even though the body has not been previously exposed to it. One of the body's efforts to defend itself against antigens' entry, for example, bacterial antigens, is to nonspecifically destroy the bacteria involved with the phagocytosis process [10]. Apart from phagocytosis, another manifestation of a nonspecific immune response is an inflammatory reaction. Immune system cells are scattered throughout the body, but when an infection occurs in one place, the immune cells and their products will be concentrated on the infection site. This reaction can occur due to the release of specific mediators by several types of cells, such as histamine released by basophils and mastocytes. Chronic inflammation involves the role of white blood cells, especially mononuclear cells, including monocytes, macrophages, and lymphocytes [11]. Inflammation is the body's normal protective response to tissue injury caused by physical trauma, harmful chemicals, and microbiological agents. A specific immune response occurs when a particular antigen invasion occurs, to which the body has been exposed before [12].

A substance that can correct an imbalance in the immune system is called an immunomodulator [13]. This material plays a role in protecting the body from incoming foreign objects to not disturb the body's functions.

Immunostimulant is a substance that acts as an immune enhancer or enhancer that can be obtained by using herbal immunostimulants. One of the herbs used is the red shoot plant (Syzygium myrtifolium Walp.) Which has been studied as an immunomodulator with high antioxidant activity [14]? Previous research stated that the flavonoid compounds in green leaves of red shoot plants (Syzygium myrtifolium Walp.), namely dimethyl cardamonin (DMC), have activities as hepatoprotectors, cytoprotective, anti-inflammatory, antiviral, antihyperglycemic, and antiapoptosic effects [14]. Phytochemical tests have been reported that the red shoot leaves contain cardenolide flavonoids, tannins, saponins, and glycosides [14].

The use of red shoot leaves shows a positive effect because of its wide use, especially in treating infectious and inflammatory diseases, so that examination as an immunomodulator is necessary. The immune system activity test can be carried out by various methods, namely by looking at the phagocytosis activity using the carbon clearance method, slow type hypersensitivity response, and antibody titer hemagglutination test. Phagocytic activity test uses the carbon clearance method to determine the nonspecific immune system's image by measuring the activity of phagocytic cells that phagocyte pathogenic organism that enter the body and counting the total number of leukocytes and the differential of leukocytes. The antibody titer hemagglutination test method is a picture of the specific immune system by looking at hemagglutination visually.

MATERIAL AND METHOD

Materials

laboratory glassware, aluminum foil, electric balance (Vibra), rotary evaporator (Heidolph), blender (National), a set of distillation equipment for determination of moisture, round bottom flask (pyrex), test tube (pyrex), funnel, filter paper , spot plate, tube clamp, spatula, vaporizer plate, mortar and stamfer, animal balance, 1 ml syringe (Terumo), oral sonde, surgical kit, velocity 18R refrigerated centrifuge (Dynamic), microtube, microtitration plate, micropipette (Socorex) , UV-Visible (Shimadzu) plestimometer and spectrophotometer.

Animals

The animal used was 70 male rats weighing 150-200 grams. 35 rats were used for the carbon clearance test and the remaining 35 rats for the antibody titer test and the slow-type hypersensitivity test. Before treatment, experimental animals were conditioned for two weeks in a suitable cage to adapt to their environment and homogenize their food.

Extraction

A total of 500 grams of red shoot leaf Simplicia powder is put into a closed vessel, 75 parts of 96% ethanol solvent are added until all of the powder is immersed, then the vessel is closed and left for 5 days protected from light while occasionally stirring. Then it is filtered, and the waste is rinsed again with 25 parts of 96% ethanol solvent, put in a vessel and stored in a place protected from sunlight for 2 days, then pour it down. (Ministry of Health, 1979). All macerate is combined and concentrated with a rotary evaporator's help at a temperature not more than 40oC until a thick extract is obtained.

Carbon clearance test

The immunomodulatory effect test of the ethanol extract of red shoot leaves was determined using the carbon cleaning method by measuring the absorbance using a UV-Visible spectrophotometer (Wagner, 1993). A total of 35 rats were divided into 7 treatment groups:

Group I: 0.5% CMC-Na suspension

Group II: Imboost® suspension at a dose of 22.5 mg / kgBW

Group III: EEPM suspension at a dose of 50 mg / KgBW

Group IV: EEPM suspension at a dose of 100 mg / KgBW

Group V: EEPM suspension at a dose of 200mg / KgBW

Group VI: EEPM suspension at a dose of 400 mg / KgBW

Group VII: EEPM suspension at a dose of 800 mg / KgBW

Each group was given an oral suspension once a day for 7 consecutive days. On the 8th day the ends of the rats were cut off. Taken and put into a tube containing Na-citrate, then 25µl of blood is taken and 4 ml of 1% acetic acid is added and then vortexed to lyse red blood cells, this first blood is used as a blank (0 minutes), then 0.1 ml of carbon suspension is injected intravenously through a vein in the tail, and at 5, 10, 15, and 20 minutes after the injection of carbon, blood is drawn, collected in a tube containing Na-citrate, then 25µl of blood is drawn. -Each 4 ml of 1% acetic acid is added and then vortexed to lyse red blood cells, then the absorbance is measured using a UV-Visible spectrophotometer at a wavelength of 640.5 nm. After 12 hours of blood collection, the mice were sacrificed, the liver and lymph organs of the

rats were taken and weighed, then the liver and lymph nodes were recorded (Aldi, et al., 2013).

Constant carbon elimination rate (K), phagocytosis index (α), and stimulation index were calculated.

Antibody Titer Test and Hypersensitivity Test slow type

The immunomodulatory effect of the ethanol extract of red shoot leaves was determined using an antibody titer test by looking at agglutination. The dosage is determined based on the orientation data that has been done previously.

A total of 35 rats were divided into seven groups with the following divisions:

Group I: 0.5% (w / v) CMC Na suspension as negative control

Group II: levamisole suspension at a dose of 25 mg / kg BW

Group III: EEPM suspension dose 50 mg / kgBW

Group IV: EEPM suspension dose 100 mg / kgBW

Group V: EEPM suspension at a dose of 200 mg / kg BW

Group VI: 400mg / kgBW EEPM suspension

Group VII: EEPM suspension dose 800mg / kgBW

The treatment was started from day 0 and was given once a day for 14 days. Each experimental animal group was injected with 0.1 ml of Staphylococcus aureus bacterial suspension 108 cells/ml in PBS as antigen intraperitoneally on day 4. On day 14, each rat's blood sample was taken through a vein in the tail. The method is with modification, namely the end of the rat tail is sliced using a razor blade, then the blood that comes out is sucked using a 1 ml syringe. The blood sample is collected in a microtube (microtube), then centrifuged at 1900 rpm using a centrifuge at 4oC for 10 minutes, and took the serum. The hemagglutination technique determined the antibody titer value. 25 µl of serum was dropped into 96 holes microtitration plate well, added PBS and Staphylococcus aureus bacteria suspension with the same volume, and diluted twice (1: 2; 1: 4; 1: 8; 1:16; 1:32; 1: 64; 1: 128; 1: 256; 1: 512; 1: 1024; 1: 2048; 1: 4096) then incubated at 37oC for 1 hour and observed hemagglutination visually (Makare, et al., 2001; Puri and Bagchi, 1993). The antibody titer value is determined based on the last dilution in which the antibody is still detected by visually visible hemagglutination. The antibody titer value is then transformed by [2log (titer) +1] (Rahmi, 2011).

Delayed Hypersensitivity Test was performed on day 14. Measurement of the volume of the mouse's feet on the right side, which previously had a limit mark on the volume measurement using a marker, was measured as the initial volume (Vo). Inject 0.1 ml of Staphylococcus aureus suspension in the right foot again. The rats' feet' volume was measured on day 15 (after 24 hours) with a digital plestimometer. Measurements were made by dipping the rat's feet into a tube containing the triton, and the scale increase was seen on the plestinometer as the volume of time (Vt) of the mouse feet. The volume of rat foot swelling is determined based on the difference between the volume of a particular time (Vt) and the initial volume (Vo) (Ahirwal, 2015).

Analysis of Data

The research data were analyzed using the SPSS program. The homogeneity and normality of the research data were determined to determine the statistical analysis used. Data were analyzed using the one-way ANOVA test to determine the average difference between treatments. If there are differences, then use the Post Hoc Tuckey test to find out which variables have differences. Based on the significance value $p < 0.05$ is considered significant.

RESULT AND DISCUSSION

Phytochemical screening result

Phytochemical screening is carried out to determine the active compounds contained in simplicia and extracts. Phytochemical screening is the simplest, fastest, and most selective method that can be used to identify groups of active compounds and determine the presence of biologically active compounds distributed in plant tissue.

Examinations carried out on simplicia and ethanol extract of red shoot leaves include examinations for the class of flavonoids, alkaloids, steroids / triterpenoids, saponins, tannins and glycosides. The results of these examinations can be seen in Table 4.1 below.

RESULT

Tabel 1: Phytochemical result

NO	Compounds	Result	
		Simplisia	Extract
1.	Alkaloida	-	-
2.	Flavonoida	+	+
3.	Steroida / Triterpenoida	-	-
4.	Saponin	+	+
5.	Tanin	+	+
6.	Glycoside	+	+

Information : (-) : negative compound, (+) : positive compound

The tables above shows that both the simplicia and the ethanol extract of the red shoot leaves contain the same active compounds, namely flavonoids, saponins, tannins, and glycosides. Still, there are no alkaloid and steroid/triterpenoid compound groups.

Carbon clearance result

A carbon clearance test or carbon elimination rate was performed to determine the reticuloendothelial system (RES). This test is performed by measuring the elimination rate of carbon particles injected intravenously into the bloodstream to

measure the phagocytosis mechanism by phagocytic cells (Wagner, 1993). When

colloid carbon is injected intravenously, colloid carbon is stabilized by gelatin. It does not cause thrombosis in the lungs and carbon particles will be eliminated by immobilized macrophage cells in the liver lymph. Carbon clearance test measurements were carried out at the 5th, 10th, 15th, and 20th minutes after giving carbon using a UV-Visible spectrophotometer based on the absorbance measurement of carbon particles.Blood taken from the rats' tail end was measured using a UV-Vis spectrophotometer at a wavelength of 640.5 nm in each predetermined minute. The results of the carbon clearance test can be seen in Table 4.2

Tabel: 2 Carbon clearance method

No	Group	Minutes- (MEAN ± SD)				K (MEAN ± SD)
		5 minutes	10 minutes	15 minutes	20 minutes	
1	CMC Na 0,5%	0,1548 ± 0,002[b]	0,1426 ± 0,001[b]	0,1358 ± 0,002[b]	0,1211 ± 0,001[b]	0,0071 ± 0,000[b]
2	Imboost	0,1050 ± 0,009[a]	0,0893 ± 0,002[a]	0,0788 ± 0,004[a]	0,0616 ± 0,002[a]	0,0145 ± 0,001[a]
3	EEDPM 50 mg/kg bb	0,1490 ± 0,002[*b]	0,1370 ± 0,004[*b]	0,1259 ± 0,003[*b]	0,1141 ± 0,003[*b]	0,0078 ± 0,000[*b]
4	EEDPM 100 mg/kg bb	0,1412 ± 0,002[ab]	0,1302 ± 0,002[*b]	0,1119 ± 0,006[ab]	0,1013 ± 0,002[ab]	0,0096 ± 0,001[*#]
5	EEDPM 200 mg/kg bb	0,1374 ± 0,003[ab]	0,1258 ± 0,008[ab]	0,1058 ± 0,008[ab]	0,0912 ± 0,003[ab]	0,0119 ± 0,000[*#]
6	EEDPM 400 mg/kg bb	0,1232 ± 0,002[ab]	0,1133 ± 0,05[ab]	0,0927 ± 0,005[ab]	0,0808 ± 0,008[ab]	0,0123 ± 0,003[#a]
7	EEDPM 800 mg/kg bb	0,1180 ± 0,004[ab]	0,1033 ± 0,007[ab]	0,0870 ± 0,005[#a]	0,0735 ± 0,006[#a]	0,0138 ± 0,003[#a]

Information : * No significant difference with group CMC Na 0,5% # No significant difference with group Imboost,
[a] Significant difference withgroup CMC Na 0,5%, [b] Significant difference with Imboost

Carbon clearance test data obtained from the 5 to 20 minutes were statistically processed using the Statistical Program Service Solution (SPSS). The data obtained were tested for normality using the Kolmogorov-Smirnov then continued with the One Way ANOVA parametric test. The analysis was continued with Post Hoc Tukey to see whether there was a difference in the effect of giving red shoot ethanol extract from the smallest dose to the most massive dose with the 0.5% CMC Na group (negative control) and Imboost suspension (positive control).

At the 5th minute, it was seen that there was no significant difference between the 0.5% CMC Na suspension group and the EEDPM 50 mg / kg bw suspension group (p> 0.05) but it was significantly different from the Imboost suspension group, EEDPM 100 mg / kg BW, 200 mg / kg BW, 400 mg / kg BW

and 800 mg / kg BW (p <0.05). Whereas for the Imboost suspension group there was a difference with the CMC Na 0.5% suspension, EEDPM 50 mg / kg, 100 mg / kg, 200 mg / kg, 400 mg / kg and 800 mg / kg (p < 0.05).

In the 10th minute the group giving 0.5% CMC Na suspension was not significantly different from the EEDPM suspension group 50 mg / kg BW and 100 mg / kg BW (p> 0.05) but significantly different from the Imboost suspension group, EEDPM 200 mg / kg BW, 400 mg / kg BW and 800 mg / kg BW (p <0.05). The Imboost suspension group had a significant difference with the CMC Na 0.5% suspension, 50 mg / kg BW EEDPM, 100 mg / kg BW EEDPM, 200 mg / kg BW, 400 mg / kg BW and 800 mg / kg BW (p. <0.05).

At the 15th minute there was no significant difference between the 0.5% CMC Na suspension group and the EEDPM 50 mg / kg bw suspension group (p> 0.05) but it was significantly different from the Imboost suspension group, EEDPM 100 mg / kg, 200 mg / kg, 400 mg / kg and 800 mg / kg of body weight (p <0.05). In the Imboost suspension group there was a difference with the CMC Na 0.5% suspension, EEDPM 50 mg / kg BW, EEDPM 100 mg / kg BW, 200 mg / kg BW, 400 mg / kg BW (p <0.05) but not has a difference with the group giving EEDPM suspension 800 mg / kg bw (p> 0.05).

In the 20th minute the 0.5% CMC Na suspension treatment group had differences with the Imboost suspension group, EEDPM 100 mg / kg, 200 mg / kg, 400 mg / kg and 800 mg / kg of body weight (p <0 , 05) but there was no difference with the group giving EEDPM suspension 50 mg / kg body weight (p> 0.05). In the group giving the Imboost suspension there was no difference with the group giving EEDPM suspension 800 mg / kg bw (p> 0.05) but it was different from the group giving 0.5% CMC Na suspension, EEDPM 50 mg / kg bw, EEDPM 100 mg / kg BW, 200 mg / kg BW, and 400 mg / kg BW (p <0.05).

From Table 4.3 above, it can also be seen that the value of the carbon elimination constant is calculated based on the net carbon value. In the 0.5% CMC Na group there was a significant difference with the Imboost group, the EEDPM dose of 400 mg / kg BW and the dose of 800 mg / kg BW (p <0.05) but did not differ from the EEDPM group at the dose of 50 mg / kg BW. 100 mg / kg body weight and 200 mg / kg body weight (p> 0.05). Whereas in the Imboost group there was a significant difference with the CMC Na 0.5% and EEDPM groups at a dose of 50 mg / kg bw (p <0.05) but there was no significant difference with the EEDPM group at a dose of 100 mg / kg, 200 mg / kg BW, 400 mg / kg BW and 800 mg / kg BW (p> 0.05).

The highest elimination constant value was in the group giving the Imboost suspension of 0.0145 and the smallest was in the 0.5% CMC Na suspension group, which was 0.0071. In the group giving the ethanol extract suspension of red shoots the most significant value was at a dose of 800 mg / kg bw. It can be seen from the dose given that the higher the dose given, the constant value will also increase. Fast or not phagocytic cells carry out the phagocytosis process can be seen from the size of

the constant value of carbon elimination, because the greater the value of carbon elimination is obtained, the faster the phagocytosis process will be (Aldi, 2013).

Phagocytosis Index

The phagocytosis index is obtained based on the constant value of the carbon clearance test. It is measured as an assessment of the phagocytic ability or activity of phagocytic cells against antigens where macrophage phagocytosis is one of the most widely used parameters to evaluate the health or immune function. The results of the phagocytosis index can be seen in Table 4.3

Tabel 3: Phagocytosis index result

No	Group	phagocytosis index
1	CMC Na 0,5%	6,7912 ± 0,83[b]
2	Imboost	9,3559 ± 0,63[a]
3	EEDPM 50 mg/kg bb	6,9234 ± 0,99[*b]
4	EEDPM 100 mg/kg bb	7,7076 ± 0,99[*#]
5	EEDPM 200 mg/kg bb	8,4723 ± 0,33[*#]
6	EEDPM 400 mg/kg bb	8,5327 ± 1,23[*#]
7	EEDPM 800 mg/kg bb	8,8849 ± 0,71[*#]

Information : * No significant difference with group CMC Na 0,5% # No significant difference with group Imboost
[a] Significant difference withgroup CMC Na 0,5% [b] Significant difference with Imboost

The results obtained from the phagocytosis index showed that the group giving the 0.5% CMC Na suspension did not have a significant difference with the group giving the EEDPM suspension at a dose of 50 mg/kg BW ($p > 0.05$), but it was different from the group giving the Imboost suspension ($p < 0.05$). In the treatment group given the Imboost suspension, it was not different from the EEDPM suspension group of 100 mg / kg, 200 mg / kg, 400 mg / kg and 800 mg / kg of body weight ($p > 0.05$) but was different from the treatment group given. 0.5% CMC Na suspension and 50 mg / kg BW EEDPM suspension.

Red shoot plants contain chemical compounds that are beneficial to health. The secondary metabolite compounds contained in the red shoot leaf extract are alkaloids, triterpenoids, steroids, saponins, phenolics, and flavonoids. The content contained in green leaves of red shoots (Syzygium myrtifolium Walp.) Is a flavonoid compound, namely dimethyl cardamonin (DMC) which has activity as a hepatoprotector, cytoprotective, anti-inflammatory, antiviral, antihyperglycemic, and antiapoptosic effect (Memon, 2014).

Based on the results of research from (Amal, 2013) that red shoots contain α-pinene (32.32%), β-pinene (12.44%), trans-caryophyllene (11.19%), 1, 3, 6-octatriene (8.41%). , delta-3-carene (5.55%), α-caryophyllene (4.36%), and α-limonene (3.42%), which have high antioxidant activity. Flavonoids found in red shoots increase phagocytic activity and have immunomodulatory activity (Diska, 2017). Macrophages function to destroy bacteria / foreign objects that enter the body. Macrophages play a role in phagocytosis of pathogens and stimulate lymphocytes and immune cells to respond to pathogens. An increase in phagocytosis will accompany the increased performance of macrophages.

Stimulation index

The stimulation index value was obtained by comparing the treatment group's phagocytosis index value with the phagocytosis index value of the control group. The results of the stimulation index value of each treatment can be seen in Table 4.4

Table 4: Stimulation Index

No	Group	Stimulation index (MEAN ± SD)
1	Imboost	1,3979 ± 0,25
2	EEDPM 50 mg/kg bb	1,0215 ± 0,12[#]
3	EEDPM 100 mg/kg bb	1,1447 ± 0,20[#]
4	EEDPM 200 mg/kg bb	1,2565 ± 0,12[#]
5	EEDPM 400 mg/kg bb	1,2727 ± 0,28[#]
6	EEDPM 800 mg/kg bb	1,3268 ± 0,24[#]

Information: # Significant difference with Imboost

From the table above, it is known that the treatment group with Imboost suspension with an average stimulation index value of 1.3979 was not different from the treatment group giving EEDPM suspension at a dose of 50 mg / kg BW with an average stimulation index value of 1.0215, 100. mg / kg BW with an average stimulation index value of 1.1447, 200 mg / kg BW with an average stimulation index value of 1.2565, 400 mg / kg BW with an average stimulation index value of 1.2727 and 800 mg / kg bw with an average stimulation index value of 1.3268.

Total of Leukocytes

Measurement of total leukocytes was carried out to see whether there was an increase in the number of leucocytes and their components after administration of the ethanol extract of red shoot leaves as a parameter to show the immunomodulatory activity of the ethanol extract of red shoots of leaves. Leukocytes play a role in cellular and humoral defense against foreign substances that enter the body. The results of the total leukocyte measurement can be seen in Table 4.5.

Table 5: Total Leukocytes

No	Kelompok	Total Leukocytes (MEAN ± SD)
1	CMC Na 0,5%	6697 ± 338,96[b]
2	Imboost	8500 ± 188,05[a]
3	EEDPM 50 mg/kg bb	6808 ± 256,23[*b]
4	EEDPM 100 mg/kg bb	6946 ± 543,52[*b]
5	EEDPM 200 mg/kg bb	7437 ± 541,39[*#]
6	EEDPM 400 mg/kg bb	7617 ± 520,05[*#]
7	EEDPM 800 mg/kg bb	8255 ± 347,85[#a]

Information : * No significant difference with group CMC Na 0,5% # No significant difference with group Imboost

[a] Significant difference withgroup CMC Na 0,5% [b] Significant difference with Imboost

The results shown in Table 4.6 show that the total number of leucocytes in the 0.5% CMC Na suspension group was smaller than the other groups. From the data processing, statistically, there was no significant difference between the 0.5% CMC Na group and the EEDPM group at a dose of 50 mg / kg, 100 mg / kg, 200 mg / kg and 400 mg / kg (p>0 , 05) whereas with the Imboost group and the EEDPM group the dose of 800 mg / kg BW had a significant difference (p <0.05). In the Imboost suspension group there was no difference with the group giving EEDPM suspension at a dose of 200 mg / kg, 400 mg / kg and a dose of 800 mg / kg bw (p> 0.05) and there was a significant difference with the group giving CMC Na suspension. 0.5%, EEDPM at a dose of 50 mg / kg and a dose of 100 mg / kg.

Leukocyte differential

Leukocyte differential measurements were carried out on leukocyte components including eosinophils, basophils, stem neutrophils, segment neutrophils, lymphocytes and monocytes. The differential number of leukocytes can be seen in Table 4.6.

Table: 6 Leukocyte differential

No	Group	Leukocyte differential (MEAN ± SD)					
		Eosinofil	Basofil	N. Batang	N. Segmen	Limfosit	Monosit
1	CMC Na 0,5%	4,3 ± 2,08[#]	0 ± 0	4,0 ± 2,00[#]	56,67 ± 5,51[#]	31,00 ± 6,25[b]	4,0 ± 1,00[b]
2	Imboost	3,0 ± 1,00[*]	0 ± 0	2,0 ± 0,00[*]	43,67 ± 4,73[*]	42,33 ± 2,52[a]	9,0 ± 2,00[a]
3	EEDPM 50 mg/kg bb	3,7 ± 1,53[*#]	0 ± 0	4,0 ± 1,00[*#]	51,67 ± 3,06[*#]	34,67 ± 3,06[*#]	6,0 ± 2,00[*#]
4	EEDPM 100 mg/kg bb	4,3 ± 2,08[*#]	0 ± 0	4,0 ± 1,00[*#]	50,33 ± 6,81[*#]	34,67 ± 5,86[*#]	6,7 ± 1,53[*#]
5	EEDPM 200 mg/kg bb	3,7 ± 1,53[*#]	0 ± 0	4,7 ± 1,53[*#]	47,00 ± 5,29[*#]	37,33 ± 2,52[*#]	7,3 ± 1,53[*#]
6	EEDPM 400 mg/kg bb	3,3 ± 2,08[*#]	0 ± 0	5,0 ± 1,00[*#]	47,33 ± 4,73[*#]	36,33 ± 3,06[*#]	8,0 ± 1,00[*#]
7	EEDPM 800 mg/kg bb	2,7 ± 1,53[#]	0 ± 0	3,7 ± 1,53[*#]	47,33 ± 1,53[#a]	43,67 ± 2,08[#a]	8,7 ± 1,53[#a]

Information: * No significant difference with group CMC Na 0,5%, # No significant difference with group Imboost

[a] Significant difference withgroup CMC Na 0,5%, [b] Significant difference with Imboost

From the results obtained on the differential measurement of leukocytes on eosinophil parameters, there was no significant difference between the 0.5% CMC Na group and the Imboost group, the EEDPM dose of 50 mg / kg, 100 mg / kg, 200 mg / kg, and 400. mg / kg BW and 800 mg / kg BW (p> 0.05). Likewise, the number of stem neutrophils was measured, where there was no significant difference between each treatment group (p> 0.05).

The measurement results on segment neutrophils were not significantly different between the 0.5% CMC Na group with Imboost, EEDPM at a dose of 50 mg / kg, 100 mg / kg, 200 mg / kg, and 400 mg / kg (p> 0.05) but significantly different from the EEDPM group at the dose of 800 mg / kg body weight (p <0.05). In the Imboost suspension group there was no significant difference with the CMC Na 0.5%, EEDPM doses of 50 mg / kg, 100 mg / kg, 200 mg / kg, 400 mg / kg and 800 mg / kg of body weight. (p> 0.05).

In the measurement of lymphocytes and monocytes, it was seen that in the CMC Na 0.5% group there was no difference with the EEDPM group at the dose of 50 mg / kg, 100 mg / kg, 200 mg / kg, and 400 mg / kg (p> 0, 05) whereas with the Imboost and EEDPM groups the dose of 800 mg / kg body weight was a significant difference (p <0.05). The Imboost group had no difference with the EEDPM group at the dose of 50 mg / kg, 100 mg / kg, 200 mg / kg, 400 mg / kg and 800 mg / kg (p> 0.05), but had differences. which was significant in the 0.5% CMC Na group (p <0.05).

Antibody Titer Test

The antibody titer assessment is a test of the humoral immune response that involves the formation of antibodies. The increase in antibody titer value occurs due to an increase in T cell activation, which stimulates B cells to form antibodies and increase B cell activation in the formation of

antibodies (Roit, 2002). The antibody titer value measurement used the hemagglutination method, which was carried out on day 14 after giving treatment to each group.

The antibody titer value is determined based on the last dilution, where the antibody is still detectable by visually visible hemagglutination. The antibody titer value is then transformed by [2 logs (titer) + 1] (Hargono, 2000). The results of the antibody titer test can be seen in Table 4.7.

Tabel: 7 Antibody titer test

No	Group	Titer Antibody Value (MEAN ± SD)
1	CMC Na 0,5%	2,40 ± 0,92[b]
2	Levamisol	4,61 ± 0,60[a]
3	EEDPM 50 mg/kg bb	2,61 ± 0,92[*#]
4	EEDPM 100 mg/kg bb	3,41 ± 0,60[*#]
5	EEDPM 200 mg/kg bb	3,61 ± 0,92[*#]
6	EEDPM 400 mg/kg bb	4,01 ± 0,60[*#]
7	EEDPM 800 mg/kg bb	4,61 ± 0,60[#a]

Information : * No significant difference with group CMC Na 0,5% # No significant difference with group Levamisole
[a] Significant difference withgroup CMC Na 0,5%, [b] Significant difference with Levamisole

From the table above, it can be seen that there was no significant difference between the 0.5% CMC Na group and the EEDPM group at a dose of 50 mg / kg, 100 mg / kg, 200 mg / kg and 400 mg / kg (p>0 , 05) but there was a difference between the CMC Na 0.5% group with the Levamisol group and the EEDPM group at a dose of 800 mg / kg BW (p <0.05). In the Levamisol group there was a significant difference with the CMC Na 0.5% group (p <0.05) but not different from the EEDPM group at the dose of 50 mg / kg, 100 mg / kg, 200 mg / kg, 400 mg / kg body weight and 800 mg / kg body weight (p> 0.05).

Slow Type Hypersensitivity Test

The immunomodulatory effect of the ethanol extract of red shoot leaves can be seen by performing a slow-type hypersensitivity test which is a test of the immunomodulatory effect related to specific immune responses, namely by measuring the volume of swelling of the feet of the test animals. The slow type hypersensitivity response is a cellular immune response that involves the activation of Th cells, releasing pro-inflammatory cytokines and increasing the activity of macrophages characterized by swelling of the animal's legs (Roit, 2002).

Measurement of the volume of animal leg swelling using a digital Pletismometer. The volume of rats 'feet was measured on day 14 after treatment of each group as initial volume (V0), after which Staphylococcus aureus bacteria were injected as antigen into the soles of the rats' feet. On the 15th day, the foot volume was again measured as a defined time volume (Vt) after being left for 24 hours. The volume of rat foot swelling was determined based on the difference between the volume of a particular time (Vt) and the initial volume (V0). The results of measuring the volume of rat feet can be seen in Table 4.8

Table : 8 Leg swelling

No	Group	ΔV Leg Swelling (MEAN ± SD)
1	CMC Na 0,5%	0,50 ± 0,07[b]
2	Levamisol	1,60 ± 0,27[a]
3	EEDPM 50 mg/kg bb	0,73 ± 0,07[*b]
4	EEDPM 100 mg/kg bb	0,96 ± 0,10[*b]
5	EEDPM 200 mg/kg bb	1,16 ± 0,18[#a]
6	EEDPM 400 mg/kg bb	1,36 ± 0,29[#a]
7	EEDPM 800 mg/kg bb	1,45 ± 0,14[#a]

Information :* No significant difference with group CMC Na 0,5% # No significant difference with group Levamisole
[a] Significant difference withgroup CMC Na 0,5% [b] Significant difference with Levamisole

The results of the measurement of the swelling volume of the rats' feet that were injected with the Staphylococcus aureus bacteria which were seen in Table 4.9 showed that in the 0.5% CMC Na group there was no significant difference with the EEDPM treatment group at a dose of 50 mg / kg bw and 100 mg / kg bw (p> 0.05). However, there was a significant difference with the group giving Levamisol, EEDPM at a dose of 200 mg / kg BW, 400 mg / kg BW and 800 mg / kg BW (p <0.005). The Levamisol group was significantly different from the 0.5% CMC Na, 50 mg / kg BW and 100 mg / kg BW EEDPM (p <0.05), whereas the EEDPM group had a dose of 200 mg / kg BW, 400 mg / kg. kg BW and 800 mg / kg BW did not have a significant difference (p> 0.05).

CONCLUSIONS

The ethanol extract of red shoot leaves at doses of 50, 100, 200, 400, and 800 mg / kg BW increased the phagocytosis activity of macrophage cells in male mice injected with carbon suspension significantly compared to negative control CMC-Na 0.5% (p <0.05). EEDPM at a dose of 800 mg / kg bw showed a

significant phagocytic effect with Imboost positive control (p> 0.05). Ethanol extract of Pucuk Merah leaves increased the total number of leukocytes and the differential of leukocyte cells in male rats' neutropyl segment. Increasing the dose of red shoot ethanol extract increased the non-specific immune response in male rats in termsasing carbothe n elimination constant, phagocytosis index and stimulation index. The ethanol extract of red shoot leaves at doses of 50, 100, 200, 400 and 800 mg / kg bw can increase the antibody titer in male rats induced by Staphylococcus aureus bacteria compared to 0.5% CMC-Na (p <0.05). EEDPM dose of 800 mg / kg bw did not have significantly differ the Levamisol group (p> 0.05). EEDPM at doses of 200, 400 and 800 mg / kg bw can increase the swelling of the feet of male rats induced by Staphylococcus aureus bacteria compared to CMC-Na 0.5% (p <0.05). Increasing the dose of red shoot leaf ethanol extract, increasing the specific immune response in male mice saw an increased amount of agglutination and an increase in swelling of the rats' feet with increasing dose.

REFERENCES

1. Nurfadhilah D, Yuandani Y, Hasibuan PA. Immunomodulatory Effects of Cermai Leaves (Phyllanthus acidus (L.) Skeels) Ethanol Extract on Normal Male Rats and Cyclophosphamide Induction. Open Access Macedonian Journal of Medical Sciences. 2022 Apr 8;10(A):782-7.
2. Rosidah R, Yuandani Y, Widjaja SS, Lubis MF, Satria D. The Immunomodulatory Activities of Saurauia vulcani Korth Leaves towards RAW 264.7 cell.
3. Yuandani Y, Jantan I, Laila L, Marianne M, Wira Septama A, Lintang N, Almadani P, A'ini S. Immunomodulatory Effects of Combined Ethanol Extracts of Curcuma mangga and Picria fel-terrae on Cellular-and Humoral-Mediated Immunity in Wistar Rats and Mice. Evidence-based Complementary & Alternative Medicine (eCAM). 2022 Sep 20.
4. Emori Y, Sasaki H, Hayashi Y, Nomoto K. Effect of Z-100, an immunomodulator extracted from human type tubercle bacilli, on the pulmonary metastases of Lewis lung carcinoma in attempt to regulate suppressor T cells and suppressor factor, IL-4. Biotherapy. 1996 Dec;9(4):249-55.
5. Ganguly S, Prasad A. Role of plant extracts and cow urine distillate as immunomodulator in comparison to Levamisole-A Review. Journal of Immunology and Immunopathology. 2010;12(2):91-4.
6. Majtan J. Honey: an immunomodulator in wound healing. Wound Repair and Regeneration. 2014 Mar;22(2):187-92.
7. Kalsum N, Sulaeman A, Setiawan B, Wibawan IW. Preliminary studies of the immunomodulator effect of the propolis Trigona spp. extract in a mouse model. IOSR Journal of Agriculture and Veterinary Science. 2017;10:75-80.
8. Shim JY, Kim MH, Kim HD, Ahn JY, Yun YS, Song JY. Protective action of the immunomodulator ginsan against carbon tetrachloride-induced liver injury via control of oxidative stress and the inflammatory response. Toxicology and applied pharmacology. 2010 Feb 1;242(3):318-25.
9. Sabdoningrum EK, Hidanah S, Ansori AN, Fadholly A. Immunomodulator y a nd Antioxidant Activities o f Phyllanthus niruri L. Extract a gainst t he Laying Hens Infected b y Escherichia c oli.
10. Gomes A, Datta P, Sarkar A, Dasgupta SC, Gomes A. Black tea (Camellia sinensis) extract as an immunomodulator against immunocompetent and immunodeficient experimental rodents. Oriental Pharmacy and Experimental Medicine. 2014 Mar;14(1):37-45.
11. Syahputra RA, Harahap U, Dalimunthe A, Nasution MP, Satria D. The Role of Flavonoids as a Cardioprotective Strategy against Doxorubicin-Induced Cardiotoxicity: A Review. Molecules. 2022 Feb 15;27(4):1320.
12. Syahputra RA, Harahap U, Dalimunthe A, Pandapotan M, Satria D. Protective effect of Vernonia amygdalina Delile against doxorubicin-induced cardiotoxicity. Heliyon. 2021 Jul 1;7(7):e07434.
13. Adrian A, Syahputra RA, Lie S, Nugraha SE. Amelioration of Cisplatin-induced Liver Injury by Extract Ethanol of Pometia pinnata. Open Access Macedonian Journal of Medical Sciences. 2021 Aug 27;9(A):665-8.
14. Chauhan PS, Satti NK, Suri KA, Amina M, Bani S. Stimulatory effects of Cuminum cyminum and flavonoid glycoside on Cyclosporine-A and restraint stress induced immune-suppression in Swiss albino mice. Chemico-biological interactions. 2010 Apr 15; 185(1):66-72.
15. Mahana NA, Abou Eldahab M, Abd El Aziz AT, Attia AE, Ramadan RS. Immunomodulatory Activity Of Moringa Oleifera And Curcuma Longa Extracts In Cyclophosphamide-Immunosuppressed Male Rats. Egyptian Journal of Zoology. 2018 Jun 1;69(69):89-106.
16. Auwal MS, Saka S, Mairiga IA, Sanda KA, Shuaibu A, Ibrahim A. Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of Acacia nilotica (Thorn mimosa). InVeterinary research forum: an international quarterly journal 2014(Vol. 5, No. 2, p. 95). Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.