talenta PUBLISHER

Indonesian Journal of Pharmaceutical and Clinical Research

IMMUNOMODULATORY EFFECTS OF *Phaleria macrocarpa* LEAF EXTRACT IN NORMAL AND CYCLOPHOSPHAMIDES INDUCED IN WISTAR RATS

Andy Febriady, Urip Harahap*, Yuandani

Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia

Abstract. Cyclophosphamide is an antineoplastic drug belonging to the alkylating agents commonly used in treating cancer. However, the use of cyclophosphamide causes a decrease in the body's immune system by reducing lymphocyte proliferation. The curent study was conducted to evaluate the immunomodulatory effect of ethyl acetate extracts of the leaf of the *P. macrocarpa* (EEADMD) and the ethanol extracts of the leaf of the *P. macrocarpa* (EEDMD) in normal and cyclophosphamide induced rats. This study used rats classified into two major groups: normal rats and rats induced by cyclophosphamide which were then given *P. macrocarpa* leaf extract until day 14. On day 4, the test animals were infected with 1% *Staphylococcus aureus* suspension in. Induced with cyclophosphamide 70 mg/kg BW was carried out on the 8th and 13th days, and then the immunomodulatory activity was tested using total leukocyte analysis, leukocyte differential, and delayed type hypersensitivity response. The results showed that EEDMD 400 mg/kg BW in normal rats and EEDMD 100 mg/kg BW in cyclophosphamide induced rats could increase total leukocytes and leukocyte differential with a significant difference to the negative group ($p < 0.05$). The results of the delayed hypersensitivity response test of EEADMD and EEDMD at a dose of 100 mg/kg BW with cyclophosphamide induction and normal rats could give an increase in rat paw volume with a significant difference to the negative group ($p < 0.05$). This study shows that EEADMD and EEDMD have an immunomodulatory effect on increasing total and differential leukocytes and leukocyte differential and delayed type hypersensitivity response.

Keywords: *Phaleria macrocarpa, immunomodulator, leukocytes, delayed type hypersensitivity*

Abstrak. Siklofosfamid sebagai suatu obat antineoplastik golongan *alkylating agent* yang biasa dipergunakan dalam menangani kanker. Namun, penggunaan siklofosfamid menyebabkan penurunan sistem imun tubuh, dengan menurunkan proliferasi limfosit. Ekstrak etil asetat daun mahkota dewa (EEADMD) dan ekstrak etanol daun mahkota dewa (EEDMD) mempunyai kandungan flavonoid dan saponin yang mempunyai efek imunomodulator dengan cara bekerja kepada limfokin (interferon- γ) yang diciptakan oleh sel T maka bisa memberi rangsangan sel-sel fagosit dan meningkatkan produksi sitokin seperti interleukin dan interferon. Tujuannya dari kegiatan meneliti ini yaitu supaya memahami aktivitas ekstrak etil asetat dan etanol dari daun *P. macrocarpa* terhadap hewan uji yang diinduksikan dengan antigen. Penelitian ini mempergunakan tikus yang diklasifikasikan jadi dua kelompok besar, yakni tikus normal tanpa induksi siklofosfamid dan tikus yang diinduksi siklofosfamid yang kemudian diberikan ekstrak daun mahkota dewa sampai hari ke 14, pada hari ke 4 hewan uji diinfeksi dengan suspensi *Staphylococcus aureus* 1% pada kelompok yang diinduksi dengan siklofosfamid 70 mg/kg bb dilakukan dalam hari ke 8 dan 13 selanjutnya diuji aktivitas imunomodulator dengan metode analisis total leukosit, diferensial leukosit dan hipersensitivitas tipe lambat. Hasil penelitian diperoleh data bahwa EEDMD 400 mg/kg BB tanpa induksi siklofosfamid dan EEDMD 100 mg/kg bb dengan induksi siklofosfamid dapat memberi peningkatan total leukosit serta

diferensial leukosit dengan perbedaan yang bersignifikan kepada kelompok negatif ($p < 0,05$). Hasil pengujian respon hipersensitivitas tipe lambat EEADMD serta EEDMD dosis 100 mg/kg BB dengan induksi siklofosfamid dan tanpa induksi siklofosfamid bisa memberi peningkatan volume kaki tikus dengan perbedaan yang signifikan terhadap kelompok negatif ($p < 0,05$). Penelitian ini menunjukkan bahwa EEADMD dan EEDMD menunjukkan efek imunomodulator terhadap peningkatan total leukosit dan diferensial leukosit dan respon hipersensitivitas tipe lambat.

Kata kunci: *mahkota dewa, imunomodulator, leukosit, hipersensitivitas tipe lambat*

Received 09 August 2022 | Revised 24 October 2022 | Accepted 05 December 2022

1. Introduction

Infectious diseases are defined by the presence of various pathogens in the human body. These pathogens can be eradicated from the body by immune cells such as macrophages and neutrophils. Low immune conditions trigger the development of pathogens and eventually cause clinical symptoms. Every human being has an immune system. This system supports repairing human deoxyribonucleic acid (DNA) and preventing infections caused by bacteria, fungi, viruses, and other organisms[1].

.Leukocytes are part of the body's defense system. The advantage of Leukocytes is that most of these cells are specifically transported to areas of severe inflammation to provide a quick and strong defense against possible infection. Various efforts to discover new drugs have been developed, especially from natural ingredients in Indonesia, which are rich in natural resources and have great potential for finding new drugs to cure various diseases. Mahkota dewa (*Phaleria macrocarpa*) has multiple pharmacological activities, *P. macrocarpa* contains flavonoids and saponins, which can increase immunomodulation by increasing the effectiveness of lymphokine proliferation produced by T cells to stimulate phagocytosis cells to increase phagocytosis and leukocytes. The present study was conducted to evaluate immunomodulatory effects of *P. macrocarpa* on rats induced cyclophosphamide [2], [3].

2. Methods

2.1. Materials

The materials used in this study were the leaves of the god crown, PBS, ethanol, ethyl acetate, and n-hexane, *Staphylococcus aureus*. *P. macrocarpa* obtained in Medan, Indonesia. Samples identified in Medan.

2.2. Extraction

The extract was prepared through a multilevel maceration process, in which the simplicia powder was mixed with n-hexane, ethyl acetate, and 96% ethanol in a specific ratio. The simplicia powder was macerated three times with ethyl acetate solvent. Afterward, the powder was macerated with 96% ethanol solvent three times. The maceration container was filled with 500 grams of dry simplicia powder. The added 5 L of solvent was soaked for the first 6 hours

while stirring occasionally, then allowed to stand for 18 hours. The result is further separated by pouring and filtering. The extraction process was carried out three times with the same type and amount of solvent. All maceration collected was then evaporated through a rotary evaporator until it produced a thick extract [4], [5].

2.3. Phytochemical Screening

The simplicia and extracts, ethyl acetate, and ethanol of P. macrocarpa leaves were tested for the content of secondary metabolites contained therein, namely for alkaloids, flavonoids, saponins, tannins, glycosides, steroids, and triterpenoids [6], [7].

2.4. Delayed Type Hypersensitivity

Test animals were classified into two large groups: cyclophosphamide-induced and non-cyclophosphamide groups. The division of the group consists of the following:

Group I: received 1% CMC-Na suspension (negative control)

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

Group III : received EEAMD preparations at a rate of 100 mg/kg BW

Group IV : received EEAMD preparations at a rate of 200 mg/kg BW

Group V : received EEAMD preparations at a rate of 400 mg/kg BW

Group VI : received EEMD preparations at a rate of 100 mg/kg BW

Group VII : received EEMD preparations at a rate of 200 mg/kg BW

Group VIII : received EEMD preparations at a rate of 400 mg/kg BW

The treatment was started on day 0 and conducted once a day for 14 days. On the fourth day, each group was injected intraperitoneally with 0.1 ml of *S. aureus* bacterial suspension in PBS, an antigen.In the cyclophosphamide-induced group, cyclophosphamide suspension was given on the 8th and 13th days. Delayed type hypersensitivity response test was carried out on the 14th day. Measuring the volume of the rat's paw on the right side, which had previously been marked with a volume measurement limit using a marker, the volume of the rat's paw was measured as the initial volume (V_0). An intraplantar injection of 0.1 ml of *S. aureus* bacterial suspension was carried out again in the sole of his right foot. Rat leg volume was measured on day 15 (after 24 hours) with a digital plethysmometer. Measurements were carried out by immersing the rat's paw in a tube containing triton, and an increase in the scale on the plethysmometer was seen as a specific time volume (Vt) of the rat's foot. The swelling volume

of rat feet was determined based on the difference between the volume at a particular time (Vt) and the initial volume (V_0) [8].

2.5. Test for Measurement of Total Leukocyte Counts and Leukocyte Differentials

The treatment began on day 0 and was administered daily for 14 days. Each group was injected intraperitoneally with 0.1 ml of S. aureus bacterial suspension in PBS, an antigen, on day 4. The group that was given a dose of cyclophosphamide at a rate of 70 mg/kg body weight had the treatment administered on the 8th and 13th days. After the 14th day, 1 ml of blood was taken and put into a tube containing sodium citrate. An examination of total leukocytes and leukocyte cell differentiation was performed at the USU Hospital Laboratory in Medan, North Sumatra.

3. Results and Discussion

The results of the phytochemical screening of the ethanol and ethyl acetate extracts of *P. macrocarpa* leaves were obtained to gather data on the secondary metabolites contained in these plants. The results of the phytochemical screening can be seen in Table 1.

Table 1 Phytochemical Screening Examination Results

No	Examination of secondary metabolites	Ethyl acetate extract	Ethanol extract
1	Alkaloids	-	-
2	Flavonoids	+	+
3	Glycosides	+	+
4	Saponins	-	+
5	Tannins	+	+
6	Steroids/triterpenoids	+	+

The reaction between the extract and magnesium powder, and concentrated hydrochloric acid produced a red solution. Hence, the conclusion was that it contained flavonoids. Flavonoid compounds in *P. macrocarpa* leaves' ethyl acetate or ethanol extract were treated with magnesium powder and concentrated hydrochloric acid to produce a red solution (Depkes RI, 1995). The addition of FeCl3 to the extract gives a slight black-green color, indicating the presence of a class of tannin compounds [4] The ethanol extract of *P. macrocarpa* leaves, with the addition of hot distilled water and vigorous shaking, was tested to produce constant foam and then tried to accumulate HCl 2 N, which indicated the presence of saponins [5]. Checking for saponin compounds in the ethanol extract showed positive result because the saponin content was extracted in ethanol solvent and saponin compounds were not found in the ethyl acetate extract. The glycosides present in the ethyl acetate and ethanol extract of *P. macrocarpa* leaves were identified using the Molisch reagent and concentrated sulfuric acid, which resulted

in the formation of a purple ring [5]. The triterpenoid/steroid compound groups in the ethyl acetate and ethanol extracts of *P. macrocarpa* leaves were identified using the Liebermann-Burchard reagent, which produced a pink and purple pattern, indicating the presence of triterpenoid compounds.

Table 2 Effect of ethyl acetate and ethanol extracts of *P. macrocarpa* leaves on the total leukocyte count

Treatment group	Total Leukocytes 10^9/L (mean ±SD)	
	Without Cyclophosphamide Induction	Cyclophosphamide Induced
Negative control	6,83 ± 0,02[bc]	6,62 ± 2,13[bc]
EAADMD 100 mg/kg BW	12,71 ± 1,16[a]	8,78 ± 2,16
EAADMD 200 mg/kg BW	12,32 ± 2,47[a]	5,98 ± 0,35[bc]
EAADMD 400 mg/kg BW	13,77 ± 6,54[a]	10,33 ± 2,36[a]
EEDMD 100 mg/kg BW	7,62 ± 1,20[cb]	5,24 ± 1,62[bc]
EEDMD 200 mg/kg BW	12,39 ± 4,90[a]	11,04 ± 1,63[a]
EEDMD 400 mg/kg BW	12,16 ±3,23[a]	8,86 ± 1,40 [a]
Positive control	11,49 ± 0,04[a]	8,97 ± 3,06[a]
Normal	12,26 ± 0,12[a]	12,26 ± 0,12[a]

Notes :

The effect of ethyl acetate and ethanol extracts of *P. macrocarpa* leaves on the total leukocyte count: a. (p) <0.05, there is a significant difference with the negative control group; b. (p) <0.05, there is a significant difference with the positive control group; c. (p) <0.05, there is a significant difference with the normal control group.

Table 2 shows that the rats that received EEADMD 100 mg/kg BW and EEDMD 200 mg/kg BW in the group of mice that did not receive cyclophosphamide had leukocyte counts that were significantly different from the negative control class. There were no significant differences between the normal and positive control groups.

The rats that received EEADMD 400 mg/kg BW and EEDMD 200 mg/kg BW that received cyclophosphamide had leukocyte counts that significantly differed from the negative control group. There were no significant differences between the normal and positive control groups.

Table 3 Effect of ethyl acetate and ethanol extracts of *P. macrocarpa* leaves in normal rats on differential leukocyte counts

Treatment Group	Leukocyte Differential (Mean ± SD)				
	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Negative control	1,31 ± 0,02[bc]	4,40 ± 0,11 [bc]	0,81 ± 0,01 [bc]	0,12 ± 0,01 [bc]	0,05 ± 0,02 [bc]
Positive control	3,12 ± 0,12[a]	7,34 ± 0,11[a]	3,91 ± 0,06[ac]	0,24 ± 0,02[ac]	0,12 ± 0,01[a]
EAADMD 100 mg/kg BW	3,73 ± 1,03[bc]	5,31 ± 1,01[ac]	1,22 ± 0,27[ac]	0,11 ± 0,03[ac]	0,12 ± 0,06[b]
EAADMD 200 mg/kg BW	2,41 ± 1,21 [abc]	5,90 ± 2,33[abc]	2,61 ± 0,90[abc]	0,12 ± 0,04[ac]	0,11 ± 0,05[b]
EAADMD 400 mg/kg BW	3,47 ± 1,65[b]	7,22 ± 4,58[b]	4,28 ± 1,95[b]	0,12 ± 0,01[ac]	0,15 ± 0,07[b]
EEDMD 100 mg/kg BW	1,42 ± 1,67[ac]	2,38 ± 1,07[abc]	2,36 ± 0,85[abc]	0,08 ± 0,05[ac]	0,07 ± 0,02
EEDMD 200 mg/kg BW	2,66 ± 2,06[ab]	4,38 ± 2,01[ac]	3,73 ± 1,95[ab]	0,28 ± 0,39[b]	0,11 ± 0,04[b]
EEDMD 400 mg/kg BW	3,33 ± 0,46[b]	6,34 ± 2,62[ab]	3,63 ± 0,51[ab]	0,23 ± 0,09[ab]	0,17 ± 0,04[ab]
Normal control	1,2 ± 0,12[a]	7,63 ± 0,29[a]	4,45 ± 0,09 [ab]	0,35 ± 0,05[ab]	0,18 ± 0,01 [a]

Notes :

Effect of ethyl acetate and ethanol extracts of *P. macrocarpa* leaves on leukocyte differential: a. (p) <0.05, there is a significant difference with the negative control group; b. (p) <0.05, there is a significant difference with the positive control group; c. (p)<0.05, there is a significant difference with the normal control group.

There are five parameters that represent white blood cell differention, namely neutrophills, lymphocytes, monocytes, eosinophills and basophills. Table 3 shows that at EEADMD 200 mg/kg BW and EEDMD 100 mg/kg BW groups differed significantly from the negative control. It means that EEADMD 200 mg/kg BW and EEDMD 100 mg/kg BW were able to increase Neutriphills in normal rats and imunosupressed rats. The effects of ethyl acetate and ethanol extracts shows that EEADMD 100 mg/kg BW and EEDMD 100 mg/kg BW groups differed significantly from the negative control. It means that EEADMD 100 mg/kg BW and EEDMD 100 mg/kg BW were able to increase lymphocyte in normal rats and imunosupressed rats. Monocytes parameters shows that EEADMD 100 mg/kg BW and EEDMD 100 mg/kg BW groups differed significantly from the negative control. It means that EEADMD 100 mg/kg BW and EEDMD 100 mg/kg BW were able to increase monocyte in normal rats and imunosupressed rats.Eosinophils parameter shows that EEADMD 100 mg/kg BW and EEDMD 100 mg/kg BW groups differed significantly from the negative control. It means that EEADMD 100 mg/kg BW and EEDMD 100 mg/kg BW were able to increase eosinophils in normal rats and imunosupressed rats.Basophils parameter shows that EEDMD 400 mg/kg BW group differed significantly from the negative control. It means EEDMD 400 mg/kg BW were able to

increase basophills in normal rats and imunosupressed rats.

Table 4 Effect of cyclophosphamide-induced ethyl acetate and ethanol extracts of *P. macrocarpa* leaves on differential leukocyte counts

Treatment Group	Leukocyte Differential 10^9/L (Mean ± SD)				
	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Negative control	1,31± 0,18[bc]	2,40 ± 1,75 [bc]	0,18 ± 0,16 [bc]	0,02 ± 0,05 [bc]	0,07 ± 0,01 [bc]
Positive control	3,12 ± 0,34[a]	7,33 ± 1,84 [a]	3,91 ± 0,75 [a]	0,34 ± 0,16 [a]	0,17 ± 0,01 [a]
EAADMD 100 mg/kg BW	5,07 ± 1,67[abc]	6,31 ± 0,41[b]	1,45 ± 0,09[ac]	0,10 ± 0,04[abc]	0,19 ± 0,09b
EAADMD 200 mg/kg BW	2,41 ± 0,57	5,88 ± 0,47[b]	2,61 ± 0,03[abc]	0,12 ± 0,03[abc]	0,11 ± 0,20[a]
EAADMD 400 mg/kg BW	3,47 ± 0,60[b]	10,88 ± 1,2[abc]	3,95 ± 0,77[b]	0,12 ± 0,06[abc]	0,15 ± 0,03[b]
EEDMD 100 mg/kg BW	1,42 ± 0,60[ac]	2,38 ± 0,81[ac]	1,36 ± 0,19[ac]	0,14 ± 0,03[abc]	0,07 ± 0,16[ac]
EEDMD 200 mg/kg BW	3,66 ± 0,30[b]	5,72 ± 1,53[ab]	3,06 ± 0,72[b]	0,25 ± 0,07[abc]	0,13 ± 0,01[b]
EEDMD 400 mg/kg BW	3,33 ± 0,08[b]	7,68 ± 1,30[b]	2,63 ± 0,04[abc]	0,33 ± 0,06[bc]	0,16 ± 0,08[b]
Normal control	3,22 ± 0,12[a]	7,63 ± 0,29 [a]	4,45 ± 0,09 [a]	0,35 ± 0,05 [a]	0,18 ± 0,01 [a]

Notes :

Effect of ethyl acetate and ethanol extracts of *P. macrocarpa* leaves on leukocyte differential: a. (p) <0.05, there is a significant difference with the negative control group; b. (p) <0.05, there is a significant difference with the positive control group; c. (p)<0.05, there is a significant difference with the normal control group.

Table 4.4 shows that EEADMD 100 and EEDMD 100 groups differed significantly from the negative control. It means that EEADMD 100 and EEDMD 100 were able to increase neutrophills in cyclophosphamide-induced rats and imunosupressed rats. Effect of cyclophosphamide-induced ethyl acetate and ethanol extracts shows that EEADMD 400 and EEDMD 100 groups differed significantly from the negative control. It means that EEADMD 400 and EEDMD 100 were able to increase lymphocyte in cyclophosphamide-induced rats and imunosupressed rats. Monocytes parameter shows that EEADMD 100 and EEDMD 100 groups differed significantly from the negative control. It means that EEADMD 100 and EEDMD 100 were able to increase monocytes in cyclophosphamide-induced rats and imunosupressed rats. Eosinophils parameter shows that EEADMD 100 and EEDMD 100 groups differed significantly from the negative control. It means that EEADMD 100 and EEDMD 100 were able to increase

eosinophills in cyclophosphamide-induced rats and imunosupressed rats.

Basophils parameter shows that EEADMD 200 and EEDMD 100 groups differed significantly from the negative control. It means that EEADMD 100 and EEDMD 100 were able to increase basophills in cyclophosphamide-induced rats and imunosupressed rats.

Ethyl Acetate Extract dose of 400 mg/kg BW in normal rats and Ethanol Extract of *P. macrocarpa* leaves dose of 100 mg/kg BW with cyclophosphamide induction increased the total leukocyte count and differential leukocytes with a significant difference to the negative group ($p < 0.05$).

Leukocytes are part of the body's defense system. The advantage of Leukocytes is that most of these cells are specifically transported to areas of severe inflammation to provide a quick and strong defense against possible infection. The ethanol extract of *P. macrocarpa*, which contains flavonoids and saponins, can increase immunomodulation by increasing the effectiveness of the proliferation of lymphokines produced by T cells, leading to an increase in phagocytosis cells stimulation to increase phagocytosis; this increases the number of leukocytes [9].

Table 5 Effect of ethyl acetate and ethanol extracts of *P. macrocarpa* leaves on the volume of rat feet (slow type hypersensitivity)

Treatment Group	Rat foot volume (Mean ± SD)	
	Without Cyclophosphamide Induction	Cyclophosphamide Induced
Negative control	$0{,}32 \pm 0{,}04^{bc}$	$0{,}14 \pm 0{,}08^{bc}$
Positive control	$2{,}00 \pm 0{,}03^{ac}$	$1{,}75 \pm 0{,}17^{ac}$
EAADMD 100 mg/kg BW	$0{,}75 \pm 0{,}45^{ac}$	$0{,}46 \pm 0{,}08^{ac}$
EAADMD 200 mg/kg BW	$0{,}92 \pm 0{,}06^{ac}$	$0{,}81 \pm 0{,}16^{abc}$
EAADMD 400 mg/kg BW	$1{,}73 \pm 0{,}19^{a}$	$1{,}54 \pm 0{,}16^{b}$
EEDMD 100 mg/kg BW	$0{,}94 \pm 0{,}14^{ac}$	$0{,}85 \pm 0{,}09^{abc}$
EEDMD 200 mg/kg BW	$1{,}09 \pm 0{,}29^{c}$	$0{,}96 \pm 0{,}09^{bc}$
EEDMD 400 mg/kg BW	$1{,}34 \pm 0{,}10^{c}$	$1{,}19 \pm 0{,}30^{bc}$
Normal control	$1{,}49 \pm 0{,}10^{ab}$	$1{,}49 \pm 0{,}10^{ab}$

Notes :

Effect of ethyl acetate and ethanol extracts of *P. macrocarpa* leaves on leukocyte differential: a. (p) <0.05, there is a significant difference with the negative control group; b. (p) <0.05, there is a significant difference with the positive control group; c. (p)<0.05, there is a significant difference with the normal control group.

The rat group that received EEADMD 100 mg/kg BW and EEDMD 100 mg/kg BW, which received cyclophosphamide, had significantly different leg volumes in the negative control

class. There was no significant difference between the normal and positive control groups. Ethyl acetate extract dose of 100 mg/kg BW, ethanol extract of *P. macrocarpa* leaves dose of 100 mg/kg BW without cyclophosphamide induction, and the cyclophosphamide-induced group could increase the leg volume of rats from the delayed hypersensitivity response test with a significant difference to the negative group ($p < 0.05$).

The DTH response is a response that involves the activation of Th cells, which will release proinflammatory cytokines and increase macrophage activity which is characterized by swelling of the animal's feet. The increased volume of the rat's feet was due to the *P. macrocarpa* extract containing flavonoids and saponins, which have immunostimulating activity. Flavonoids activate Th cells, which release proinflammatory cytokines and increase macrophage activity, characterized by swelling of the animal's legs [10].

4. Conclusion

P. macrocarpa (L.) is a plant often found in Indonesia. The people of Indonesia widely use this plant as a medicinal plant. *P. macrocarpa* (L.) is a plant rich in chemical compounds, including flavonoids, tannins, and glycosides. Flavonoids in *P. macrocarpa* can provide an immunomodulatory effect by increasing IL-12 activity and lymphocyte proliferation. CD4+ cells will affect lymphocyte proliferation and then cause Th-1 cells to be activated. An in vivo study of the immune response was carried out to investigate the immunomodulatory effect of *P. macrocarpa* leaf extract on the increase in total leukocytes, leukocyte differential, and DTH response [11].

EEDMD 400 mg/kg BW in normal rats and EEDMD 100 mg/kg BW in cyclophosphamide induced rats could increase total leukocytes and leukocyte differential with a significant difference to the negative group ($p < 0.05$). The results of the delayed hypersensitivity response test of EEADMD and EEDMD at a dose of 100 mg/kg BW with cyclophosphamide induction and normal rats could give an increase in rat paw volume with a significant difference to the negative group ($p < 0.05$). This study shows that EEADMD and EEDMD have an immunomodulatory effect on increasing total leukocytes and leukocyte differential and delayed type hypersensitivity response. The results of this study suggest that the extract of *P. macrocarpa* leaves has immunomodulatory activity by increasing total differential leukocytes, leukocytes, and delayed hypersensitivity.

References

[1] Bratawidjaja KG, Iris R . Imunologi dasar. Edisi ke-10 Jakarta: Badan Penerbit Fakultas kedokteran Universitas Indonesia; 2012

[2] Ali RB, Atangwho I.J, Kaur N., Abraika O.S., Ahmad M., Mahmud R., Asmawi M.Z., Bioassay-guided antidiabetic study of Phaleria macrocarpa fruit extract. Molecules. 17(5):4986-5002. April 2012.

[3] Alara, O.R., Mudalip, S.A. and Olalere, O.A., Optimization of mangiferin extracted from Phaleria macrocarpa fruits using response surface methodology. *Journal of Applied Research on Medicinal and Aromatic Plants*, *5*, hal: 82-87, May 2017.

[4] Depkes RI. *Sediaan Galenik*. Jakarta: Departemen Kesehatan RI. Halaman 19, 1985

[5] Depkes RI. . *Farmakope Herbal*. Edisi I. Jakarta: Departemen Kesehatan Republik Indonesia. Halaman. 164-175, 2013

[6] Hendra R, Ahmad S, Sukari A, Shukor MY, Oskoueian E. Flavonoid analyses and antimicrobial activity of various parts of Phaleria macrocarpa (Scheff.) Boerl fruit. *International journal of molecular sciences*. 12(6):3422-31. May 2011.

[7] Ibrahim, A., & Rusli, R. Potensi Antibakteri ekstrak Diethyl ether daun Mahkota Dewa (Phaleria macrocarpa (scheff.) Boerl) terhadap Bakteri Pseudomonas aeruginosa dan Staphylococcus aureus. *Journal of Tropical Pharmacy and Chemistry*, 1(1): 17-23, Dec 2010

[8] Shivaprasad, H.N., Gupta, M. S. Kharya, M. d., dan Rana, A. C. Preliminary Immunomodulatory Activities of Aqueous Exstract of Ternibaia chebula.J. Pharm. Biol 44(1) hal 32-34. Jan 2006.

[9] Mufidah, Z., Rifa'i, M., dan Rahayu, S., (2013), Aktivitas Imunomodulator Ekstrak Buah Mengkudu pada Mencit yang Diinfeksi *Staphylococcus aureus*. *Jurnal veteriner.* 14(4): 501-510. March 2013

[10] Sudha P, Asdaq SM, Dhamingi SS, Chandrakala GK. Immunomodulatory activity of methanolic leaf extract of Moringa oleifera in animals. Indian J Physiol Pharmacol. ;54(2):133-40, June 2010

[11] Emadi A, Jones RJ, Brodsky RA. (2009) Cyclophosphamide and cancer: golden anniversary Nature Reviews Clinical Oncology 6(11):638-47, September 2009