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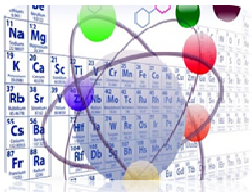

A Review: Immunomodulator Effects of Plants Extract on Rats with Haematological Parameter include White Blood Count and Differential Leucocytes Count

Rafika Hayu Pratiwi *, Tita Juwitaningsih

Department of Chemistry, Faculty of Mathematics and Natural Science, State Universitas Negeri Medan, Medan 20221, Indonesia
**Corresponding author : rafika.hayu28@gmail.com*

ABSTRACT

Plants extract as immunomodulatory is substance which can stimulates or suppresses the component of immune system including white blood count and differential leucocytes count. The modulation of immune system by various plants extract has become subject for scientific investigation currently worldwide. Some of these plants have been claimed to increase immune system by increasing the total white blood count and differential leucocytes count are Achyranthes aspera Linn*, Cermai Leaves* (*Phyllanthus acidus (L.)*) *Skeels, Moringa oleivera and Poguntano herb* (*Picriafel-terrae*Lour).. *Plants extract were recommended for us as immunomodulatory because there are several botanical products with potential therapeutic applications such as high efficacy, low cost and low toxicity*.

Keywords: immunomodulatory, plants extract, leucocytes, differential leucocytes

1. INTRODUCTION

Covid-19 was first detected in Wuhan, Hubei Province, China in December 2019 due to transmission of acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which spread quickly to other areas around the world.[1] Based on Covid-19 distribution data from the World Health Organization (WHO), globally Covid-19 has infected 657,430,133 people and caused 6,676,645 deaths as of January 6 2023. Meanwhile in Indonesia, Covid-19 was recorded as infecting 6,722,227 people spread throughout the province with 160,665 deaths as of January 6, 2023. Low immunity will make it easy to be exposed to the Covid-19 virus. To reduce Covid-19 cases, each region applies many methods, one of the methods used is to increase the immunity of each individual through the use of immunomodulators.[2]

Diseases that are immune system-mediated were the major issue in developing nations. Human's infection can bring on by environments that are abundant in different kinds of harmful microorganisms, such

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as bacteria, viruses, fungi, protozoa, and parasites[3]. By using cellular and humoral mechanisms, the immune system of the human body fundamentally guards against and manages neoplasia and infection. While humoral immunity primarily relies on the production of particular antibodies by B lymphocytes in response to antigen presentation, cellular immunity primarily relies on T cells and B lymphocytes. Many factors, including starvation, oxidative stress, exogenous infections, and antigens, can harm immunological functions.[4]

Clinically an immunomodulator is used in patients with impaired immunity, including in cases of cancer, HIV / AIDS, malnutrition, allergies, and others. However, its use results in many undesirable effects, such as nonsteroidal anti-inflammatory group (microscopic bleeding of the gastrointestinal tract, decrease in platelet levels, respiratory depression, and so on), immunostimulants (increased acid levels tendons, urticaria, agranulocytosis, and others), immunosuppressants (toxic to the liver, disorders of the gastrointestinal tract, and miscellaneous).[5] To achieve the desired impact on disease prevention, the immunomodulating properties of plants are extensively studied. As a result, herbal treatments have been using for millennia due to their security, potency, lack of serious adverse effects, and cultural acceptance. Therefore, plants and their products are safe, and as a result, this method of treating patients has used since ancient times. Plant products are applied as an alternative frequently.[6]

Cyclophosphamide is a popular alkylating agent used to treat a variety of malignancies, such as lymphoma, myeloma, and chronic lymphocytic leukemia. The DNA of cells that were actively proliferating had crosslinked by immunosuppressive agents, which suppressed both cellular and humoral immune responses. Immunosuppression brought on by cyclophosphamide has been linking to a number of infections. Cyclophosphamide can be used as an immunosuppressive agent to research the immunomodulatory effects of plant extracts, as per researchers.[7] One of the earliest immune responses can be observed and analyzed by studying the hematological parameters of an animal. Leukocyte counts were subsequently measured. Invading non-self materials are recognized first by blood cells, which are the first cells to react. Any immunological substance's immunomodulatory action manifests itself initially as a shift in leukocyte count. By administering the extract, the leukocyte count significantly increased.[8]

2. PLANTS EXTRACT AS IMMUNOMODULATOR TO INCREASING THE TOTAL LEUCOCYETS AND DIFFERENTIAL LEUCOCYTES

2.1. Achyranthes aspera Linn

Achyranthes aspera Linn is widespread in the world as a weed, in Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America. Up to a height of 2100 m, it grows as a weed on road sides, field boundaries, and waste areas across India, as well as in the South Andaman Islands. The plant is highly esteemed by traditional healers and used in treatment of asthma, bleeding, in facilitating delivery, boils, bronchitis, cold, cough, colic, debility, dropsy, dog bite, dysentery, ear complications, headache, leucoderma, pneumonia, renal complications, scorpion bite, snake bite and skin diseases etc[9].

Kolli et al, (2021) have reported immunomodulatory effect of methanolic and aqueous extracts of whole plant of *Achyranthes aspera* Linn. in rats induced by cyclophosphamide. Cyclophosphamide significantly decreases WBC counts. Cyclophosphamide at the dose of 30 mg/kg BW treatment for the period of 3 days intravenously showed a significant reduction in total WBC count and thereby exerted immunosuppressant effect when compared to control animals in Table 1. Treatment with the extracts after administration of cyclophosphamide resulted in significantly higher counts on various hematological parameters when

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compared to the group that was administered only cyclophosphamide showed in Table 2. Both of AEAA and MEAA at dose 200 mg/kg BW have shown statistically significant higher count of total leucocytes and differential leucocytes when compared to the untreated cyclophosphamide group. The findings indicate that MEAA is a more effective immunomodulator than AEAA in increasing total leucocytes count and differential leucocytes count. The presence of carbohydrates, steroids, phenolic compounds, tannins, alkaloids, saponins, flavonoids, proteins, and glycosides in the methanolic extract causes the activity of immunomodulatory.

Tabel 1. White Blood Count on rats after cyclophosphamide induced myelosuppression.

Group	WBC (10^9/L)
Control	5.11±0.12
Cyclophosphamide	2.95±0.25
AEAA 200 mg/kg bw	3.93±0.28
MEAA 200 mg/kg bw	4.26±0.18

Tabel 2. Immunomodulatory effect of AEAA and MEAA on White Blood Count and Differential Lecocytes Count on rats induced by cyclophosphamide

Group	Neutrophil (%)	Lymphocyte (%)	Monocytes (%)	Eosinophil (%)	Basophil (%)
NControl	21.72±0.47	71.58±0.47	2.02±0.20	2.02±0.20	1.00±0.13
Cyclophosphamide	13.53±0.84	61.95±0.54	1.10±0.23	1.10±0.23	0.38±0.12
AEAA 200 mg/kg bw	15.69±0.37	64.62±0.79	1.87±0.19	1.87±0.19	0.47±0.17
MEAA 200 mg/kg bw	17.07±0.47	64.98±0.50	2.10±0.22	2.10±0.22	0.57±0.12

AEAA : aqueous extract *Achyranthes aspera* Linn.
MEAA : methanolic extract *Achyranthes aspera* Linn.

2.2. Cermai Leaves (Phyllanthus acidus (L.)) Skeels

P. acidus (L.) plant is one of the Indonesia's leading medicinal plants; this can be seen from the benefits and effectiveness of these medicinal plants in curing several diseases, including allergic asthma. *P. acidus* (L.) is a plant that has rich of various chemical constituents, including flavonoids, tannins, and saponins. Nurfadhilah et al (2022) have reported immunomodulatory effect of Phyllanthus acidus (L.) Skeels ethanol extract in rats induced by cyclophosphamide. The study was performed in normal and cyclophosphamide induced rats. Cyclophosphamide (70 mg/kg bw) was used to suppress the immune response as reported in a previous study. Cyclophosphamide was administered on days 8, and 13 in immune-suppressed groups. On day 14, the blood leukocyte cell count and leukocyte cell differential were measured. Animals were divided into 6 groups for each condition, including normal control rats without any treatment, Na CMC 0.5% suspension, *P. acidus* extract 100 mg/kg bw, *P. acidus* extract 200 mg/kg bw, and 400 mg/Kg bw, levamisole 25 mg/kg bw was used as a positive control. *P. acidus* extract suspension in Na CMC 0.5% was orally administered to animals once a day until 14 days. There are five parameters that represent white blood cell

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differentiation, namely neutrophils, lymphocytes, monocytes, eosinophils, and basophils. White blood count and differential leukocyte counts from normal rats is shown in Table 3.

Tabel 3. Total White Blood Count and Differential Lecocytes Count on normal rats (before induced by cyclophosphamide)

Group	WBC (10^9/L)	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)
Normal Control	12.30±0.84	6.74±0.93	2.90±0.61	0.66±0.19	0.33±0.25	1.67±0.50
Negative Control	4.91±0.70	3.42±0.82	0.87±0.49	0.03±0.02	0.18±0.12	0.41±0.32
Levamisole	11.62±0.93	6.93±0.94	2.67±1.16	0.58±0.19	0.22±0.05	1.23±0.55
P. acidus extract 100 mg/kg bw	9.75±0.81	6.63±0.51	1.86±0.28	0.14±0.09	0.23±0.20	0.88±0.25
P. acidus extract 200 mg/kg bw	10.49±1.79	7.21±1.13	2.02±0.72	0.20±0.09	0.16±0.11	0.91±0.42
P. acidus extract 400 mg/kg bw	11.36±0.64	7.41±0.55	2.36±0.48	0.46±0.11	0.11±0.11	1.02±0.16

The effect of P. acidus ethanol extract on White blood count and differential leukocyte counts from rats induced by cyclophosphamide is shown in Table 4. The increase in the number of leukocytes is because ethanol extract of *P. acidus* contains flavonoid and saponins compounds that have the ability to increase the immunomodulatory system by increasing the effectiveness of the proliferation of lymphokines produced by T cells so that this situation stimulates phagocytic cells to carry out phagocytic responses and will increase leukocyte production[12].

Tabel 4. Immunomodulatory effect of *Phyllanthus acidus* on White Blood Count and Differential Lecocytes Counts on rats induced by cyclophosphamide

Group	WBC (10^9/L)	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)
Normal Control	12.30±0.84	6.74±0.93	2.90±0.61	0.66±0.19	0.33±0.25	1.67±0.50
Negative Control	1.07±0.45	0.58±0.37	0.29±0.17	0.13±0.06	0.01±0.01	0.06±0.06
Levamisole	7.08±1.09	4.70±1.47	1.55±0.47	0.41±0.15	0.17±0.04	0.25±0.03
P. acidus extract 100 mg/kg bw	3.45±0.81	0.79±0.13	0.79±0.13	0.23±0.05	0.09±0.03	0.11±0.05
P. acidus extract 200 mg/kg bw	6.32±1.03	1.23±0.61	1.23±0.61	0.31±0.07	0.11±0.05	0.13±0.04
P. acidus extract 400 mg/kg bw	7.05±1.67	1.49±0.55	1.49±0.55	0.36±0.13	0.14±0.04	0.17±0.06

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2.3. Moringa oleivera

M. oleifera is a widely used herb to treat various ailments in Africa and throughout the world in various disease conditions such as HIV/AIDs-related symptoms, syphilis, urinary tract infections, and malaria among others[13]. Based on Nfambi et al, 2015 rats were treated with an immunosuppressant (cyclophosphamide) at doses of 200 mg/kg bw on day 0 of the study by subcutaneous injection, and then rats administrated *M. oleifera* leaf methanol extract for 14 days. The result of this study is white blood counts in rats dosed 50 mg/kg bw of levamisole and 1000 mg/kg bwt of *M. oleifera* leaf methanol extract were significantly higher than those of the animals in the other groups with the highest counts observed in levamisole 50 mg/kg bw. Statistically significant results were observed for neutrophil and lymphocyte counts in the treatment group that received levamisole and 1000 mg/kg bw of *M. oleifera* leaf methanol extract in comparison to all the other groups in Table 5.

Tabel 5. Immunomodulatory effect of *M. oleifera* leaf methanol extract on White Blood Count and Differential Lecocytes Counts on rats induced by cyclophosphamide on day 14

Group	WBC (10^9/L)	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)
Control	12.04±1.30	2.10±0.23	8.31±1.39	1.04±0.12	0.43±0.09	0.01±0.00
Cyclophosphamide	10.76±1.34	3.17±0.43	0.45±0.04	1.61±0.33	0.11±0.19	0.01±0.00
Levamisole	22.53±0.6	11.20±0.04	9.13±0.29	1.53±0.01	0.63±0.00	0.04±0.01
MOML 250 mg/kg bw	7.66±2.19	2.86±0.47	6.50±0.95	1.01±0.36	0.03±0.01	0.01±0.00
MOML 500 mg/kg bw	16.36±0.21	0.26±0.02	3.76±1.05	0.89±0.02	0.01±0.00	0.02±0.00
MOML 1000 mg/kg bw	21.43±0.90	10.37±0.49	9.26±0.34	1.20±0.09	0.51±0.09	0.04±0.01

MOML: *M. oleifera* leaf methanol extract

*2.4. Poguntano Herb (Picriafel-terrae*Lour).

Based on Ningtias et al, 2021 the rats were administered extract of herb Picriafel-terrae Lour once daily for 14 consecutive days, infected by Staphylococcus aureus bacteria on day 4 and induced by cyclophosphamide doses of 70 mg/kg body weight on day 8 and 13. The result showed that PHNHE cannot increase the total and differential leukocytes; PHEAE, PHEE at doses of 100 and 200 mg/kg bw were effective in increasing the total and differential leukocytes. EEAHP, EEHP has effective immunostimulating activity. The increase in lymphocytes, neutrophils, eosinophils, basophils and monocytes is caused by the presence of flavonoid compounds contained in ethyl acetate extract and ethanol extract of lymphokines produced by T cells to stimulate cells- phagocytic cells to carry out the phagocytosis response. Based on the results of research, ethyl acetate extract and ethanol extract of poguntano herbs can increase total leukocytes,

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an increase in the number of leukocytes is due to the herbal extract of poguntano containing flavonoids which can activate the lymph system so that it can increase theproduction of white blood cells in Table 6.

Tabel 6. Immunomodulatory effect of Poguntano herb n-hexane, ethyl acetate, and ethanol extract on White Blood Count and Differential Lecocytes Counts on rats induced by cyclophosphamide and Staphylococus aureus.

Group	WBC (10^9/L)	Lymphocyte (%)	Neutrophil (%)	Basophil (%)	Eosinophil (%)	Monocyte (%)
Normal control	6.79±0.40	25.00±1.41	74.50±1.20	3.25±0.95	4.25±0.57	4.50±1.29
Negative control	3.66±0.38	16.75±0.95	54.00±3.46	1.00±0.81	0.75±0.95	0.75±0.95
Levamisole	6.59±0.19	23.0±1.41	73.25±1.70	3.00±0.81	4.00±0.95	4.25±0.95
PHNHE 50 mg/kg BW	3.36±0.84	17.0±1.41	56.5±1.91	0.75±0.95	1.00±0.81	1.00±0.81
PHNHE 100 mg/kg BW	3.39±0.17	17.75±0.95	56.00±3.55	1.25±0.95	1.25±1.29	1.25±0.95
PHNHE 200 mg/kg BW	3.36±0.22	18.00±0.81	53.00±3.55	2.25±1.29	2.00 ± 1.25	1.00±1.41
PHEAE 50 mg/kg BW	5.14±0.52	21.5±1.29	67.00±3.55	2.00 ±0.8	2.00±0.81	1.50±1.29
PHEAE 100 mg/kg BW	6.04±0.35	22.25±2.21	72.50±1.29	2.75±0.95	2.25±0.95	2.00±0.57
PHEAE 200 mg/kg BW	6.08±0.42	23.00±1.63	72.75±1.25	3.00±0.81	2.75±1.25	4.00±0.81
PHEE 50 mg/kg BW	5.94±0.42	22.00±0.81	70.25±1.70	2.25±0.95	2.25±0.95	2.00±1.41
PHEE 100 mg/kg BW	6.02±0.30	22.25±0.95	72.25±2.21	3.00±0.81	3.0±0.57	3.00±0.81
PHEE 200 mg/kg BW	6.22±0.47	23.25±1.25	73.75±1.50	3.25±0.95	3.75±1.25	4.00±0.81

PHNHE: Poguntano herb n-hexane extract
PHEAE: Poguntano herb etyl acetate extract
PHEE: Poguntano herb ethanol extract

4. CONCLUSION

The use of various plants extract that administrated in specific dose after induced cyclophosphamide may be helpful in increasing of haematological parameters include total white blood count and differential leucocytes count on immune system. Plants extract were recommended for us as immunomodulatory because there are several botanical products with potential therapeutic applications such as high efficacy, low cost

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and low toxicity. And seconder metabolite that have ability to increase white blood count and differential leucocytes count are alkaloid, flavonoid, saponin, tanin, steroid, terpenoid and so on.

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