Immunostimulant Effect Test Of Curcuminoid Extract From Turmeric Rhizome (*Curcuma Domestica* Val.) In VCO On Rats Induced Cyclophosphamide Againts An Increasing Total Leukocytes And Leukocyte Differential

Damayanti S[1], Yuandani[2*], Denny Satria[3]

[1]Graduate School of Pharmaceutical Science Program, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
[2]Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
[3]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
*Corresponding Author:
Email: yuandani@usu.ac.id

Abstract.
Turmeric (Curcuma domestica Val.) is a traditional Indonesian plant that has many benefits. Curcuminoids are turmeric compounds known to have effects as immunomodulators and have been proven in several in vivo tests. Immunosuppressive drugs such as cyclophosphamide are widely given to improve clinical symptoms in autoimmune patients and cancer treatment which can cause susceptibility to infection.Therefore,this study aimed to determine the immunostimulating effect of curcuminoid extract from turmeric rhizome on cyclophosphamide-induced white rats. Proof of the immunostimulating effect of curcuminoid extracts was by testing the total leukocytes and leukocytes differential. The test results showed that curcuminoid extracts of 1 mL, 0.5 mL, and 0.25 mL increased total leukocytes and leukocyte differential (lymphocytes, neutrophils, monocytes, basophils, eosinophils) with a significant difference ($p<0.05$) to the Na group CMC 0.5% as a negative control. Therefore, curcuminoid extract had the potential to have an effect as an immunostimulant.

Keywords: *Curcuma domestica, Curcuminoid, Leukosit, Immunostimulant, and Siklofosfamid.*

I. INTRODUCTION

Compounds or drugs that can stimulate and improve the function of the immune system or increase the activity of the immune system are referred to as immunomodulators. There are three types of immunomodulators: immunorestoration, immunostimulation, and immunosuppression [1]. Cyclophosphamide is an immunosuppressive drug that is widely used to improve autoimmune clinical symptoms and cancer treatment, which can make a person's body susceptible to infection [2]. The suppressive effect of cyclophosphamide on the bone marrow will inhibit the formation of blood cells, including leukocytes [3]. One of the synthetic immunostimulant drugs used to treat this problem is levamisole. However, levamisole has side effects such as nausea, vomiting, urticaria, and agranulocytes [1]. Therefore, alternative immunostimulants are needed which are derived from plants so that they have relatively low side effects and toxicity to the body.Turmeric contains curcuminoids which act as immunomodulators and their effectiveness has been proven in several in vivo tests. Curcuminoids can significantly stimulate the primary humoral immune response and secondary humoral antibody titers in rats. Curcumin's function on natural killer cells and its antioxidant functions on macrophages accompanied by mitogen and antigen-induced T cell proliferation [4].

Based on several recent studies evaluating a cleaner extraction method to reduce environmental impact compared to conventional methods while still producing high-quality herbal extracts, namely the Green Extraction method [5]. One of them is the Microwave Assisted Extraction (MAE) technique which provides several attractive advantages, including high extraction yields, faster heating, reduced thermal gradients, and shorter reaction and preparation times [6]. The latest technology has been developed to extract turmeric without chemical solvents but using vegetable oils, one of which is the use of VCO [7].Based on research, curcuminoids dissolve in non-polar solvents where VCO is non-polar. The choice of using natural solvents is expected to provide better and safer results because VCO does not leave harmful residues and can be eaten immediately if curcuminoid preparations are processed in food form [8]. VCO contains the active

substances monolaurin, glycerol, and lauric acid products which are known to be immunomodulatory and antimicrobial, thereby increasing the immune system against bacterial infections as seen from lymphocyte proliferation [9].Based on these descriptions, this study aims to determine the immunostimulating effect of curcuminoid extract on cyclophosphamide-induced rats by observing the increase in total leukocytes and leukocyte differential.

II. METHODS

2.1 Reagents

The reagents used was virgin coconut oil (Palem Mustika, Indonesia). *Staphylococcus aureus* bacteria were obtained from the Microbiology Laboratory, Faculty of Pharmacy, the University of Sumatera Utara. Phosphate buffer saline (PBS; Sigma, USA), sodium carboxymethyl cellulose (Na CMC; Sigma, USA) levamisole (Askamex®; Konimex, Indonesia), and cyclophosphamide (Kalbe, Indonesia).

2.2 Extraction Procedure

The sample used was curcuminoid extract from turmeric (*Curcuma domestica* Val.) rhizome. Sample extraction used the Microwave Assisted Extraction (MAE) method, in which 6 grams of Simplicia powder was added to 60 mL of VCO as a solvent, then stirred until it was homogeneous. After that, it was placed in the microwave for 10 minutes at a medium temperature (270 watts). Let the mixture cooled, then separated the filtrate and residue.

2.3 Staphylococcus Aureus Bacteria Preparation

Staphylococcus aureus bacteria to be used were regenerated on nutrient agar media and incubated for 24 hours at 37^0C. After 24 hours of incubation, the growing bacterial colonies were aseptically transferred to liquid nutrient solution using a sterile ose and incubated again for 24 hours at 37^0C. Next, 1 mL was taken and 9 mL of new nutrient solution was added. Then the cell concentration was calculated using the spectrophotometry method up to a concentration of 10^9 cells / mL. After getting the bacterial cell concentration of 10^9 cells / mL, then it was centrifuged at 2500 rpm for 25 minutes. The supernatant was discarded. Then, the cells were resuspended with 1 mL of PBS

2.4 Preparation of Experimental Animals

The test animals were male rats weighing 150 – 200 grams which were divided into 7 groups. Group I was given a curcuminoid extract dose of 1 mL, group II was given curcuminoid extract 0.5 mL, group III was given curcuminoid extract 0.25 mL, group IV was given curcumin suspension 200 mg/kgBB, group V was given levamisole suspension 25 mg/kgBB, group VI was given VCO, and group VII was given 0.5% Na CMC suspension as a negative control. Treatment was given to each group orally for 14 days. On day 4, all test animals were infected by Staphylococcus aureus bacteria, then induced with cyclophosphamide 30 mg/kgBB on days 4, 8, and 12. On day 15, the animals were anesthetized using ketamine, their blood was taken from the heart and was collected in a tube containing anticoagulants for blood plasma sampling. Then the immunostimulatory effect was carried out by measuring the total leukocytes and leukocyte differential.

2.5 Measurement of Total Leukocyte and Leukocyte Differential

Total leukocytes and leukocyte differential (lymphocytes, neutrophils, monocytes, basophils, eosinophils) were tested by taking 1 mL of blood plasma and putting it into a tube containing anticoagulants, then measured using a hematology analyzer in the Integrated Laboratory of Prof. dr. Chairuddin Panusunan Lubis USU Hospital.

2.6 Data Analysis

The data were presented as mean ± SD and analyzed with SPSS software using a one-way analysis of variance (ANOVA) followed by the post hoc Tukey test. Value of $P<0.05$ was considered significantly different.

III. RESULT AND DISCUSSION

3.1 Result of Total Leukocyte Measurement

Based on the results of the study shown in Figure 1, there is a significant difference ($p < 0.05$) in the increase in the number leukocytes after administration of curcuminoid extract compared to the negative

control group. In 1 mL curcuminoid extract, the increase in leukocytes was 5.57 ± 0.13 10^9/L. In curcuminoid extract 0.5 mL the increase in leukocytes was 4.37 ± 0.18 10^9/L. In curcuminoid extract 0.25 mL the increase in leukocytes was 3.75 ± 0.17 10^9/L.

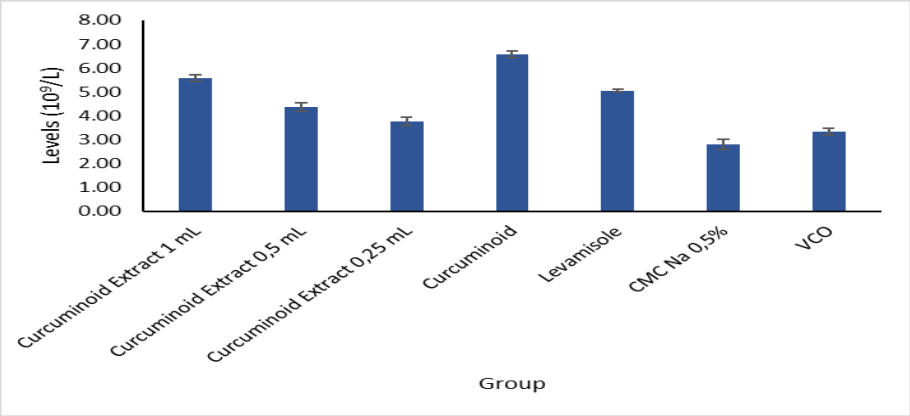

Fig 1. Effect of curcuminoid extract from turmeric (*Curcuma domestica* Val.) in VCO on the increase in total leukocytes.

The effect of cyclophosphamide administration in this study was to reduce the total leukocyte count. This was due to the suppressive effect of cyclophosphamide on the bone marrow which inhibits the formation of blood cells including leukocytes [3]. An increase in the number of leukocytes is the body's defense response to antigens. This is also in accordance with other studies which explain that curcuminoids can increase leukocyte proliferation resulting in an increase in the number of leukocytes in the blood circulation [11].

3.2 Result of Leukocyte Differential Measurement

Based on the results of the study shown in Figure 2, there is a significant difference ($p < 0.05$) in the differential increase in leukocytes, namely neutrophils, lymphocytes, monocytes, eosinophils, and basophils after administration of curcuminoid extract compared to the negative control.Neutrophil content in 1 mL curcuminoid extract was 20.66 ± 0.18%, in 0.5 mL curcuminoid extract was 15.65 ± 0.23%, and in 0.25 mL curcuminoid extract was 11.24 ± 0.14% higher compared to the negative control of 4.35 ± 0.17%. The lymphocyte level in 1 mL curcuminoid extract was 81.56 ± 0.31%, in 0.5 mL curcuminoid extract was 77.64 ± 0.16%, in 0.25 mL curcuminoid extract was 11.24 ± 0.14% higher than the negative control of 63.57 ± 0.18%. Monocyte content in 1 mL curcuminoid extract was 4.41 ± 0.09%, in 0.5 mL curcuminoid extract was 3.51 ± 0.10%, in 0.25 mL curcuminoid extract was 2.75 ± 0.16% higher than the negative control of 1.59 ± 0.12%. The eosinophil level in 1 mL curcuminoid extract was 4.28 ± 0.07%, in 0.5 mL curcuminoid extract was 3.36 ± 0.14%, in 0.25 mL curcuminoid extract was 2.86 ± 0.14% higher than the negative control of 1.48 ± 0.08%. The basophil level in 1 mL curcuminoid extract was 4.39 ± 0.10%, in 0.5 mL curcuminoid extract was 3.36 ± 0.14%, in 0.25 mL curcuminoid extract was 2.86 ± 0.14% higher than the negative control of 1.54 ± 0.09%.

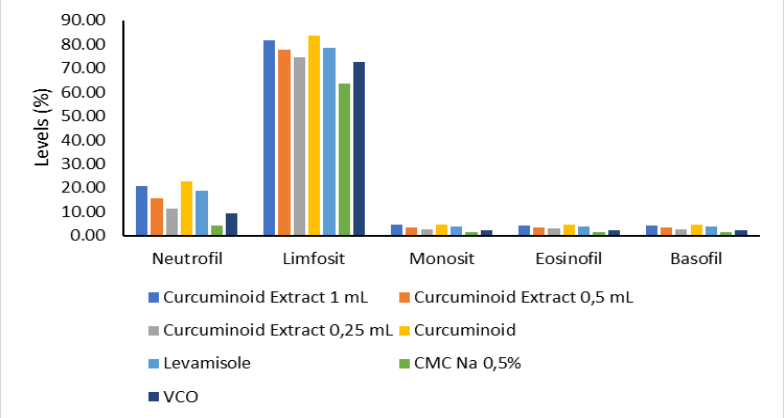

Fig 2. Effect of curcuminoid extract from turmeric (*Curcuma domestica* Val.) in VCO on the increase in differential leukocytes.

Lymphocytes, neutrophils, eosinophils, basophils, and monocytes are active units of the immune system, hence the name immunocompetent cells. These immunocompetent cells can be used as an indicator of the quality of resistance or immunity. Indicators of innate immunity are represented by basophils, eosinophils, neutrophils, and monocytes, while indicators of adaptive immunity are represented by lymphocytes [12].Lymphocytes are responsible for specific defense responses against infection. Lymphocytes can be divided into three types, namely B cells, T cells, and natural killer (NK) cells [13]. Based on other studies, it is explained that curcumin acts as an immunostimulant by increasing the number of lymphocytes. Curcumin has the ability to activate T lymphocyte cells and B lymphocyte cells which are part of lymphocytes [14].Neutrophils are the first line of defense against invading microorganisms, tissue trauma, and many factors that cause inflammation [15]. The number of neutrophils increases because the body's defense system has formed so that when a bacterial infection occurs, neutrophils are produced by the lymph to be sent to the area of infection [16].

Curcuminoids can increase neutrophils. Increased neutrophils can kill bacteria by phagocytosis. In the process of phagocytosis, neutrophils kill bacteria with hydrolytic enzymes and bactericidal compounds, namely lysozyme, iron-binding protein lactoferrin, leukins, and phagocytin, as well as cationic proteins in which curcuminoid is included [17].Eosinophils are part of the differential leukocytes formed in the spinal cord which function as parasitic, inflammatory, and allergic responses. An increase in eosinophils can prevent infection from entering the body, eosinophils work with chemical functions enzymatically [18].The increase in monocytes in this study showed that it was caused by stimulation by curcumin which functioned as an immunostimulant, and the increase in monocyte cells was caused by stimulation by curcumin compounds which worked by phagocytizing bacteria [19].In this study, one of the acute symptoms of cyclophosphamide administration was a decrease in leukocytes including their components (lymphocytes, neutrophils, eosinophils, basophils, and monocytes). This was due to the suppressive effect of cyclophosphamide on the bone marrow which would inhibit the formation of blood cells including leukocytes [3].The differential increase in the leukocytes namely lymphocytes, neutrophils, eosinophils, basophils, and monocytes were caused by the presence of curcuminoid compounds contained in curcuminoid extracts which acted on lymphokines produced by T cells which stimulate phagocytic cells to carry out a phagocytic response [20].

IV. CONCLUSION

Results of the study show that curcuminoid extract from turmeric (*Curcuma domestica* Val.) in VCO could increase the amount of total leukocyte and leukocyte differential. Therefore, it can be concluded that the curcuminoid extract in VCO has the potential to have an effect as an immunostimulant.

REFERENCES

[1] K. G. Baratawidjaja, I. Rengganis, ‘Imunologi Dasar’, Edisi ke-12, Jakarta, Balai Penerbit Fakultas Kedokteran Universitas Indonesia, Halaman 96, 110, 2018.

[2] M. Buisman, T. L. V. Zwet, J. A. Langermans, M. F. Geertsma, Leenen, D.A. Cantrell, and K. A. Smith, ‘The interleukin-2 T-cell system: a new cell growth model’, Science 224 (4655), 1312–1316, 2004.

[3] Emadi, R. J. Jones, and R. A. Brodsky, ‘Cyclophosphamide and cancer: golden anniversary’, *Nature Review Clinical Oncology*, 6: 638- 647, 2009.

[4] D. Hartanti, B. A. Dhiani, S. L. Charisma, R. Wahyuningrum, ‘The Potential Roles of Jamu for COVID 19: A Le arn from the Traditional Chinese Medicine’, *Pharmaceutical Sciences and Research*, 7(4), 12-22, https://doi.org/ 10.7454/psr.v7i4.1083, 2020.

[5] Y. Yang, M. Zhao, L. Lin, ‘Effects of Extraction Methods on Structural Characteristics and Bile Acid-Binding Capacities of Moringa Oleifera Leaf Polysaccharide Fractions’, ***Int. J. Food Sci.*** Technol, 55, 1539–1546, 2020.

[6] G. Cravotto, F. Mariatti, V. Gunjevic, M. Secondo, M. Villa, J. Parolin, G. Cavaglià, ‘Pilot Scale Cavitational Reactors and Other Enabling Technologies to Design the Industrial Recovery of Polyphenols from Agro-Food By-Products, a Technical and Economical Overview’, Foods 7, 130, 2018.

[7] M. Takenaka, T. Ohkubo, H. Okadome, I. Sotome, T. Itoh, and S. Isobe, 'Effective Extraction of Curcuminoids by Grinding Turmeric (*Curcuma longa*) with Medium-chain Triacylglycerols', *Food Science and Technology Research*, 19(4), 655–659, 2013.

[8] S. H. Suharmadi, and E. Enjarlis, 'Pemurnian virgin coconut oil menggunakan zeolit 3a sebagai bahan baku obat kulit', ***Jurnal Bahan Alam Terbarukan***, 5(2):61-67, 2016.

[9] T.C.Wallace,'Health effects of coconut oil-a narrative review of current evidence'.***J Am Coll Nutr*** 5:1, 2018.

[10] Z. Mufidah, M. Rifa'i, and S. Rahayu, 'Aktivitas Imunomodulator Ekstrak Buah Mengkudu pada Mencit yang Diinfeksi *Staphylococcus aureus', Jurnal veteriner. vo*1 4. No.4, p. 501- 510, 2013.

[11] E. Sulistyowati, B. Irma, dan S. Urip, 'Suplementasi level temulawak (Curcuma xantorrhiza roxb) yang berbeda dalam konsentrat pada sapi frisien holland: pengaruhnya terhadap total digestible nutrient (TDN) ransum', ***Jurnal Sains Peternakan,*** 5(1):20-26, 2010.

[12] Y. Belkaid, and G. Oldenhove, 'Tuning microenvironments: induction of Regulatory T Cells by dendritic cells', *Immunity journal*, 28(2): 362-73, 2008.

[13] N. Ashton, 'Physiology of red and white blood cells. Anaesthesia and Intensive Care Medicine', 11(6): 2010.

[14] Nasrullah, Isroli, and Sugiharto, 'Pengaruh Penambahan Jamu dalam Ration terhadap Profil Darah Putih dalam Darah Ayam Petelur', ***Jurnal Sains Peternakan Indonesia'***, 15(3):315-319, 2020.

[15] E. Sukmayadi, S. A. Sumiwi, M. I. Barliana, A. D. Aryanti, 'Aktivitas Imunomodulator Ekstrak Etanol Daun Tempuyung (*Sonchus arvensis* Linn.)', ***IJPST,*** 1(2):65-72, 2014.

[16] R. A. Mones, 'Gambaran Darah pada Ikan Mas (Cyprinus carpio Linn) Strain Majalaya yang Berasal dari Daerah Ciampea Bogor', Fakultas Kedokteran Hewan Institut Pertanian Bogor, Bogor, halaman 35, 2008.

[17] W. Purnamasari, P. Astuti, and, T. Ermawati, 'Viabilitas neutrofil yang diinkubasi dalam ekstrak rimpang temulawak (*Curcuma xanthorrhiza*) dan dipapar dengan Streptococcus mutans (Viability of neutrophil incubated in temulawak rhizome extract (Curcuma xanthorrhiza) and exposed by Streptococcus mutans)', ***Journal of Dentomaxillofacial Science***, *13*(3), 135-140, 2014.

[18] D. Moyes, and P. M. Schulte, 'Principles of animal physiology'. Edisi Kedua, Perarson International Edition, NewYork, 2008.

[19] K. N. Iman, M. Riauwaty, and H. Syawal, 'Diferensiasi Leukosit Ikan Jambal Siam (Pangasius hypopthalmus) yang Diberi Pakan dengan Penambahan Ekstrak Kurkumin Kunyit (*Curcuma domestica* Val.)', ***Jurnal Online Mahasiswa Fakultas Perikanan dan Ilmu Kelautan,*** 4, 2016.

[20] S.K. Kusmardi, dan E.T. Enif, 'Efek Imunomodulator Ekstrak Daun Ketepeng Cina (*Cassia alata*. L) terhadap Aktivitas dan Kapasitas Fagositosis Makrofag', *Makara Kesehatan*. 11(2): 50-51. Halaman 1-7, 2007.