International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Capacity and Activity Test of Mice Peritoneal Macrophagy Phagocytic from Polyherbal Formula X IN VIVO

Lusi Nursilawati Syamsi[1], Letitia Desiree[2], Syamsudin[3], Bambang Pontjo Priosoeryanto[4]

[1]Pharmacy Department of University Pancasila, Doctoral Study Program

[2]Pharmacy Department of University Pancasila, Undergraduate Program

[3]Pharmacy Department of University Pancasila, Dean of the Faculty of Pharmacy, Pancasila University

[4]Bogor Agricultural Institute of Veterinary Medicine, Head of the Pathology Division of the Clinical Department, Reproductive Pathology (KTP)

Abstract: ***In the human body there are many systems, one of which is the immune system. Compounds that can improve the immune system are called immunomodulators and can be found in plants and animals, including polyherbal X which contains extracts of snakehead fish, curcuma xanthorriza, and moringa. This experimental study aimed to observe the potential of Polyherbal X in increasing the phagocytic capacity and activity of macrophages in DDY male mice in vivo. Mice were divided into 5 groups with two control groups and three test groups, all of which were given non-A Staphylococcus aureus induction on the eighth day after administration of the test drug. The first control group (I) was given aquadest, while the second control group (II) was given a syrup supplement containing 254 mg/kg BWE. Purpurea L. Moench extract. The third group (III) was given polyherbal X 2.176 mg/kgBW, the fourth group (IV) was given polyherbal X 4.352 mg/kgBW, and the fifth group (V) was given polyherbal X 8.705 mg/kgBW orally administered for 7 consecutive days. After the induction on the eighth day, surgery was performed to take the peritoneal fluid of the mice and make a smear. The phagocytic capacity and activity were observed and counted from the smear. The results showed an increase in the capacity and phagocytic activity of macrophages from the test group as evidenced by ANOVA analysis compared with the control group, obtained a Sig value of 0.000 (P<0.05). The conclusion from this study was that the group that had the highest effectiveness in increasing the average data of phagocytic capacity and activity of macrophages was the group that received polyherbal X at a dose of 8.705 mg/kgBW with a capacity of 18,31 and an activity of 79,67% when compared to other groups.***

Keywords: Polyherbal X, snakehead fish (*Channa striata*), curcuma (*Curcuma xanthorrhizae* Roxb.), moringa (*Moringa oleifera* Lam), phagocytic capacity and activity, *Staphylococcus aureus*

1. Introduction

In the human body there are various systems, one of which is the immune system. The immune system or immunity is the mechanism and function of the immune system against foreign creatures and the efforts to neutralize, eliminate, and metabolize these creatures or the substances they produce. The immune system aims to protect the body from foreign creatures that enter the body such as bacteria, viruses, and parasites. (1)The immune system is divided into two types, namely the specific and non-specific immune systems. The specific immune system is an immune system that can recognize specific foreign creatures that enter the body, while the non-specific immune system cannot recognize what foreign creatures are attacking the body. (1)One of the mechanisms of the nonspecific immune system is phagocytosis by macrophages which then change shape or polymorph as the final phase of antigen destruction(2). Macrophages have the most extensive pathogen-recognition receptors, leading to efficient phagocytic action and can reduce the production of proinflammatory cytokines(3).Snakehead fish (C. striata) is an animal that is often consumed in Indonesia and contains several secondary metabolites and bioactive compounds that have the potential to increase immunity or as immunomodulators and snakehead fish extract can increase the number of macrophages. Curcuma (curcumin) and Moringa (carotenoids) are also sources of natural immunomodulators that are generally consumed in Indonesia that increasing the capacity and phagocytic activity of macrophages has been proven (4) (5) (6)

2. Materials and Methods

1) Initial preparation

All tools to be used are cleaned and sterilized.

2) Preparation of Phosphate Buffered Saline (PBS) solution

Phosphate Buffered Saline solution was made with a pH of 7.4 by weighing 0.8 grams of sodium dihydrogen phosphate as solution I, weighing 3.79 grams of sodium dihydrogen phosphate as solution II, and weighing 2.2 grams of sodium dihydrogen phosphate as solution III. Then dissolved solutions 1, 2, and 3 into 100 mL of distilled water and mixed. The mixture was diluted to a volume of 500 mL with distilled water.

3) Preparation of test animals

The test animals used were healthy male mice (Mus musculus) DDY strain (Deutchland Denken Yonken) weighing 20-30 grams and 2-3 months old from the Non-Ruminant and Animal Hope Laboratory. The number of mice was 20, divided into three test groups and two control

groups with 4 mice in each group. Acclimatization was carried out for 14 days to adapt to the environment, physical control, and uniformity of activity and food. To prevent disease and infection, mice were given worm medicine (Combantrin) for 1 day and antibiotics (Co-trimoxazole) for 3 days. Monitored the need for eating and drinking mice to prevent thirst and hunger. To minimize pain in mice during surgery, a combination of anesthetic and analgesic (Ketamine 10% and Xylazine 2%) was given as a euthanasia measure

4) Preparation of S. aureus bacteria suspension

S. aureus bacteria from the Microbiology Laboratory of the Faculty of Veterinary Medicine, Bogor Agricultural University. S. aureus bacteria rejuvenated by culturing in TSA medium for 24 hours. then suspended in BHI Broth media for 24 hours at 36 C. centrifuged for 10 minutes at 3000 RPM, the supernatant was rinsed 3 times with 5 mL NaCl and homogenized. Then McFarland 2 solution was added until the bacterial concentration was 108 cells/mL. S. aureus bacteria suspension was injected into mice on the eighth day with dosing and positive control for 7 days.

5) Dosing

Prepared 20 test animals which were divided into five groups with each containing 4 mice. Calculated by the formula Federer (n-1) (t-1) 15 with t as the number of groups and n as the number of test animals. In this study, t as many as 5 groups and n at least 4 individuals. The test animals were grouped randomly as many as 20 tails. Negative Control (I) used aquadest, Positive Control (II) was given a supplement containing E. purpurea 254 mg/kgBW, groups III,IV, V were each given Polyherbal X at a dose of 2.176 mg/kgBW, 4.352 mg/kgBW, 8,705 mg/ kgBB Once a day, seven days in a row.

6) Phagocytosis test

Each mouse was induced by 0.5 mL of S. aureus bacteria intraperitoneally on the eighth day for one hour. Then euthanasia was carried out. Peritoneal fluid was taken with a micropipette, 1-2 mL of sterile phosphate buffered saline was also added and then stirred. The liquid was poured into an Eppendorf tube and vortexed until homogeneous. smear preparations were made with Giemsa staining. Observations were made under a microscope by calculating the phagocytic capacity and activity of macrophages.

7) Determination of phagocytic capacity and activity

Phagocytic capacity

The value of phagocytic capacity was obtained from the calculation of the number of S. aureus bacteria that were ingested or phagocytized by 50 macrophage cells with the number of active macrophages.

Phagocytic capacity = (Number of phagocytized S. aureus bacteria)/(Number of active macrophages)

Phagocytic activity

The value of phagocytic activity was obtained from the calculation of the number of active macrophages that carried out phagocytosis from 100 macrophage cells and expressed in percent units.

Phagocytic activity = (Number of active macrophages)/(100 observed macrophages) x 100%

3. Results and Discussion

The phagocytic capacity of macrophages was calculated from 50 observed macrophages that were active in phagocytosis. In each group each had 4 mice, so that there were 200 cells observed with each mouse observed 50 macrophage cells. Then look for the average capacity data from each group. While the phagocytic activity was calculated from macrophage cells that were actively phagocytosing from 100 observed macrophages. Each group had 4 mice, so that there were 400 cells in which 100 macrophage cells were observed in each mouse. As in pictures V1 and V2.

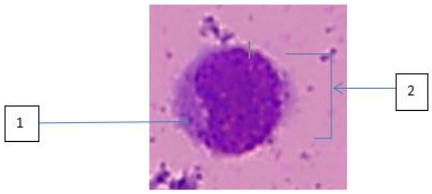


Figure 1: Active macrophages at 1000x . magnification
1. Staphylococcus aureus; 2. Active macrophage

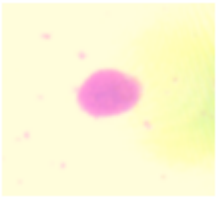


Figure 2: Inactivated macrophages at 1000x magnification

Phagocytosis capacity of macrophages

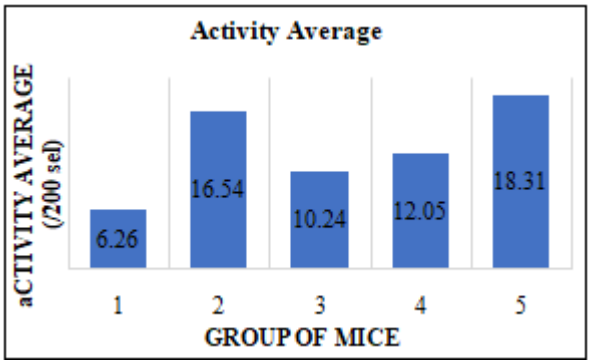
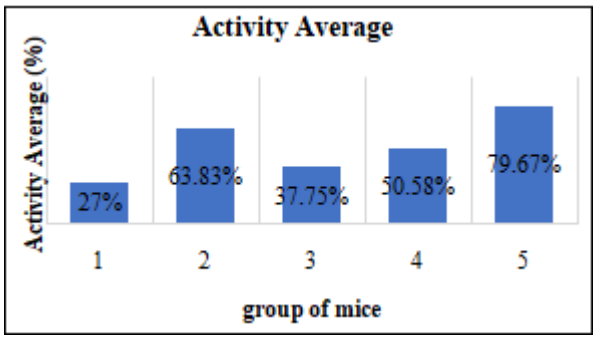


Figure 3: Phagocytic Capacity of Mice
1 = Negative control group, 2 = positive control group (supplement E. purpurea L. Moench 254mg/kgBW, 3 = Dosage group 2.176mg/kgBW, 4 = Dose group 4.352mg/kgBW, 5 = Dosage group 8.705mg/kgBB

Based on the diagram in Figure, the group that had the highest phagocytic capacity was in group 5 who received ekstrakX at a dose of 8,705 mg/kgBW. The dose of 8,705 mg/kgBW was 18.31 of 200 cells observed compared to positive control with syrup supplements containing extract of E. purpurea L. Moench which had an average of 16.54 of 200 cells observed on the macrophage phagocytic capacity data. In the ANOVA analysis with the Tukey test, the positive control group with a dose group of 8705 mg/kgBW

had a Sig value of 0.000, and the dose group of 4.352 mg/kgBW with a dose group of 8705 mg/kgBW having Sig 0.000.

Phagocytic Activity of Macrophages



Gambar 4.AktivitasFagositosisMakrofag
1 = Negative control group, 2 = Positive control group (E. purpurea supplement 254 mg/kgBW), 3 = The dose group is 2.176 mg/kgBW, 4 = Dose group 4,352 mg/kgBW, 5 = Dosage group 8,705 mg/kgBW

The average phagocytic activity data from the three test doses respectively were 37.75%, 50.58%, 79.67% observed from 400 cells of each group which were taken on average. In this study, a positive control was used to compare the test plants with the control, the positive control extract of E. purpurea L. Moench which had an average of 63,83% macrophage phagocytic activity data. In the ANOVA analysis using the Tukey test, the positive control group with a dose of 8,705 mg/kgBW had a Sig value of 0,000, and the dose group of 4,352 mg/kgBW with a dose of 8,705 mg/kgBW had a Sig value of 0,000.

Arabinogalactan content in E. purpurea L. Moench which is efficacious in increasing the activation of macrophages and interleukin I because it has a shape or structure like bacterial lipopolysaccharide (10). Its polyphenol content is also efficacious in enhancing immune responses such as the process of bacterial phagocytosis (7).The significant difference of the test group compared to the negative control group was due to the activity of Ekstak X increase macrophage activation. The antioxidant content in C. striata is albumin and zinc, the zinc content can increase the phagocytic activity of macrophages (8). And the polysaccharide content in temulawak can increase non-specific immune responses such as the phagocytosis process through the activation of macrophage cells. During the phagocytosis process, macrophages also produce cytotoxic agents called reactive oxygen species (ROS) to fight bacteria (9). phagocytosis (non-specific) and altered antibody response (specific). (9,10). The content of saponins and flavonoids in Moringa is also efficacious in increasing CD4 cells that function in the proliferation of T cells and B cells, which produce IL-2 cytokines, and binds to Antigen Presenting Cell (APC) and enhances the immune response in the body. From the results of the study, it can be concluded that the administration of Extract X has the potential to increase the phagocytic capacity and activity of macrophages. From the results of the middle dose or the actual dose of extract X, which is 4,352 mg/kgBW in mice and 33,480 mg/kgBW and low doses of 2.176 mg/kgBW in mice and 16,740 mg/kgBW in humans have the potential to increase the capacity and phagocytic activity of macrophages. The best dose at a dose of 8,705 mg/kgBW compared to positive control, resulted in higher macrophage phagocytic capacity and activity. A high dose of 8,705 mg/kgBW in mice is equivalent to 66,960 mg/kgBW in humans. Extract X gave an immunostimulating effect as evidenced by the number of observed and active macrophages between negative controls and the test drug, the higher the dose, the greater the number. The increase in the phagocytic capacity and activity of macrophages in this study also proved the effectiveness of extract X as an immunomodulator for humans.

4. Conclusion

The preparation of extract X containing extracts of snakehead fish, Channa. Striata, (Curcuma. Xanthorrhizae Roxb, and Moringa oleifera Lam has the potential to increase the capacity and phagocytic activity of macrophages which have immunostimulant properties. The average data on the phagocytic capacity of macrophages in the test group showed a significant difference sequentially, namely 10.24; 12.05; and 18.31 of the 200 cells observed from each group. Likewise, the average data on the phagocytic activity of macrophages sequentially gave significantly different results, namely 37.75%; 50.58%; and 79.67%. The dose that gave the best phagocytic capacity and activity was the dose of 8,705 mg/kgBW in mice and 66,960 mg/kgBW in humans which gave an average capacity value of 18.31 of 200 cells observed from the group and the average value of activity was 79, 67%.

References

[1] Marliana Nina. Imunoserologi. Bahan Ajar Teknologi Laboratorium Medik (TLM): Pusat Pendidikan Sumber Daya Manusia Kesehatan.2018.
[2] Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. Science (1979). 2010; 327 (5966):656–61.
[3] Kedokteran J, Kuala S. DOI:org/10.24815/jks.v21i2.20376. 21(2):172–83.
[4] Untuk D, Persyaratan M. Pengaruh Kandungan Ekstrak Daun Kelor Paska Pencabutan Gigi Tikus Wistar (Rattus Norvegicus) : Narative Review. 2020
[5] Ratu Nft, Andrie M, Taurina W. Formulasi Salep Ekstrak Ikan Gabus (Channa Striata) Dan Madu Kelulut (Heterotrigona Itama) Dengan Cmc-Na Sebagai Bahan Pengikat. Jurnal Mahasiswa Farmasi Fakultas Kedokteran Untan. 2019;4(1):8.
[6] Pengaruh Formula Ekstrak 4 Tanaman Terhadap Aktivitas Dan Kapasitas Fagositosis Makrofag Peritoneum Ayam Yang DitantangDenganBakteri Staphylococcus Aureus Andrew Babtista Manik. 2012
[7] Rosnizar R. Nanza SRY. Eriani K. Uji Efektivitas Ekstrak Rimpang Alang-alang (*Imperatacylindria* (L.) P.Beauv) sebagai Bahan Imunostimulator. Bioleuser. 2021.5(2); p.17-21
[8] Prayugo B, Muhammad I, Yamamoto Z. Potensi Ekstrak Ikan Gabus Terhadap Kesembuhan Luka Diabetes. JKS.2021;21(2): 172-83

[9] Kim AJ, Kim YO, Shim JS, Hwang JK. *Immunostimulating Activity of Crude Polysaccharide Extract Isolated from Curcuma xanthorrhiza* Roxb.Biosci.2007.71(6); p: 1428-38
[10] Wulan H, Widagdo DP, Aulia C. Potensi Ekstrak Etanol Daun Kelor Sebagai Antiinflamasi dan Penetapan Kadar Flavonoid Total. Media Farmasi Indonesia. 2021.16(2): p.1693-7