
THE EFFECT OF MANGO LEAVES EXTRACT VAR ARUM MANIS (*Mangifera indica* L.) AS IMUNOSTIMULANTS ON MICE MODEL**Oleh****Ifmaily¹⁾ & Putri Rizki Fitriani²⁾****¹Fakultas Farmasi, Univesitas Perintis Padang****²Fakultas Kedokteran, Universitas Andalas Padang****Email: [1ifmaily.72@gmail.com](mailto:ifmaily.72@gmail.com)****Abstract**

Background : Every time our body is always exposed to microorganisms that can cause infection. Usually we are immune to infection because of the immune system that protects us, but when the body is exposed to antigens while the immune system activity decreases, then we need immunostimulants. One of traditional plant always get to use as the traditional drug is mango plant, Previous research it have effect as antioxidant, antidiabetes type II, antiinflammatory, anticancer, antihypertensive, and antimicrobial. It contains the flavonoid, alkaloid, phenolic, and steroid compounds. This study aims to determine the effect of mango leaves extract var arum manis against the activity and phagocytosis capacity of macrophage cells, and the percentage of leukocyte cell types, as well as the effect of dose variations on the administration of var arum manis mango leaves extract. Methods :Twenty mice were divided into 4 groups. Consisting of group I getting 0.5% Na.CMC, groups II, III and IV getting mango leaves extract var arum manis with consecutive doses of 30 mg / kgBW, 60 mg / kgBW, and 120 mg / kgBW which were given respectively. orally once a day for 7 days. On the eighth day, *Staphylococcus aureus* bacteria were injected intraperitoneally and after one hour, the activity and capacity of macrophage cell phagocytosis and the percentage of leukocyte cell types were calculated. Result and Discussion: The activity and phagocytosis capacity of macrophage cell was determined by calculating the phagocytosis index where the results obtained were the higher the dose, the increased activity and phagocytosis capacity of macrophage cell which affected the percentage of leukocyte cell types. The data were then processed by statistical analysis of one-way ANOVA. Conclusion of research showed that the mango leaves extract var arum manis at a dose of 30 mg / KgBW, 60 mg / KgBW, 120 mg / KgBW was significant ($p \leq 0.05$) and affected activity and phagocytosis capacity of macrophage cells and the percentage of leukocyte cell types significantly ($p \leq 0.05$), where the most effective result was a dose of 120 mg / KgBW

Keywords: Leaves Extract, *Mangifera indica*, Phagocytosis Cells, Immunostimulants, Mice**INTRODUCTION**

Every time our bodies are always exposed to microorganisms that can cause infections, generally we are immune to these infections because of the immune system that protects our bodies (Radji, 2010). The body has a defense known as the immune system, this thing very important in protecting the body from attacks by foreign objects that enter the body (Wahyuni, 2017). When the immune system in the body is exposed to substances that are considered foreign, there are two

possible immune responses occurs namely non-specific immune response and specific immune response.

The process of phagocytosis is part of the foremost non-specific immune response against infectors that enter the body and will carry out cell activities, namely in the form of ingestion of particles. The group of cells that carry out this function are called phagocytic cells. Phagocytic cells are produced by stem cells in the bone marrow which then develop into mononuclear phagocytes and

polymorphonuclear phagocytes. Mononuclear phagocytes consist of monocytes in the blood circulation and macrophages found in various body tissues. Meanwhile, polymorphonuclear phagocytes consist of three types of cells, namely neutrophils, eosinophils, and basophils.

Immune system activity can decrease due to various factors, including age and disease. Immunomodulators are drugs / chemical compounds that can restore and repair a compromised immune system or suppress one that is overworked. Therefore, the presence of chemical compounds that can increase the activity of the immune system is very helpful for overcoming the immune system and these compounds can be obtained from plants (Nugroho et al, 2012).

One of the plants that has the potential to be studied is the leaves of mango (*Mangifera indica L*). The traditional use of mango leaves is as antioxidant, antidiabetic, antiinflammatory, antihypertension, cancer and analgesic. Mango leaves contain the flavonoid, phenolic, alkaloid, and saponin. Researcher was interesting to research about mango leaves extract var arum manis indicated as immunostimulant

METHODS

Tools and Materials

Tools

The tools used is a rotary evaporator (BÜCHI, Switzerland), maceration bottle, cotton swab, syringe (Terumo), oral needle (Terumo), scissors, animal scale, animal enclosure, test tube (IWAKI), measuring cup (Pyrex), porcelain crucible, oven (Memmert), desiccator, slide (Slider), drop plate, dropper pipette, micropipette (Eppendorf), mortar and pestle, test tube rack, vial, spatel, parchment paper, analytic scales (Precisa), microscope (Smic).

Material

The materials used are mango leaves var arum manis, 70% ethanol (Bratachem), 96%

ethanol (Bratachem), aquadest (Novalindo), Staphylococcus aureus bacteria, physiological NaCl (Widarta), Na.CMC (Merck), emersion oil (Merck.), giemsa solution (D6 100-darstadt). The animals used were female white mice weighing 20-30 grams.

Procedure

A. Mango Leaves Sample Extraction

Fresh samples of mango leaves var arum manis weighing 2 kg are cleaned, and washed. Then dry in open air protected from direct sunlight. After drying, it is weighed and then macerated to obtain the thick extract. The trick is to put a dry sample of leaves mango var arum manis into a dark maceration bottle then soaked with 70% ethanol solvent until the sample is submerged and then left to stand for 3x 24 hours while stirring occasionally. The maserate was filtered with filter paper and then added with 96% ethanol, then repeated until a clear macerate was obtained. Then combine all the maserate. To obtain a thick extract, maserate is concentrated with a rotary evaporator (Harborne, 1987)

B. Characterization of Mango Leaves Extract (*Mangifera indica L*)

1. Organoleptic examination is carried out by visual observation which includes shape, smell, color and taste

2. Determination of Extract Yield

The yield is the ratio between the extracts obtained by initial simplicia (DepKes RI, 2000). Put it in the porcelain crucible then weigh it, then slowly shake it so that the extract is evenly distributed. Put it back in the oven, open the lid and leave the lid in the oven

3. Determination of Drying Shrinkage (Ministry of Health, Republic of Indonesia, 1995)

Tara porcelain crucible that has been dried for 30 minutes in an oven at 105 °C, weighed the extract as much as 1 g to 2 g,

4. Phytochemical Test of Mango Leaves Extract (Yufri Aldi, 2016)

The thick extract of mango leaves var arum manis is put into a test tube, then add 5 ml of aquadest and 5 ml of chloroform, leaving it until a water layer and chloroform layer are formed.

a. Flavonoid Examination ("Cyanidine Test" Method) Take a layer of 1 - 2 drops of water, Drop it on a drop plate then add concentrated Mg and HCl powder, a red color appears indicating the presence of flavonoids

b. Phenolic Examination

Take a layer of 1-2 drops of water, drop it on the drop plate then add, reagent FeCl₃, arise blue color indicates the presence of phenolic content

c. Saponin examination

Take a layer of water, put into the test tube and shake vigorously, the formation of permanent foam (\pm 15 minutes) indicates the presence of saponins

d. Examination of Terpenoids and Steroids ("Liebermann Buchard and Simes" Method) Take a little layer of chloroform with a dropper which has been coated in cotton and norit inside. The colorless filtrate will come out, then drop the filtrate on the drop plate and dry it. The residue is added with 1 drop of anhydrous acetic acid and 2 drops of concentrated H₂SO₄, if a red color appears, it indicates the presence of terpenoids, whereas if a blue purple color appears, it indicates that there is steroid content

e. Alkaloid Examination (Method "Culvenore - Fristgerald")

Take a little layer of chloroform, put it in a test tube, add 10 ml of chloroform ammonia 0.05 N, stir gently, add 2-3 drops H₂SO₄ 2N then shake gently, let it separate. Take the acid layer and put it in another test tube then add 2 drops of mayer reagent, the positive reaction of the alkaloid is indicated by the presence of a white mist to a white lump.

C. Treatment of Experimental Animals

In this study, the experimental animals used were 20 male white mice aged 2-3 months with a body weight of 20-30 g. Before the treatment, the mice were acclimatized for one week. Then every mice were weighed.

Animals are grouped into 4 groups randomly, each group consisting of 5 animals. Group I was the group that was only given 0.5% NaCMC, and groups II, III, IV were the treatment groups that were given mango leaves extract var arum manis based on the dosage variations that have been made. On the 1st to the 7th day the mice were given the extract by oral test and control substances. On the 8th day, mice in each group were infected by injecting Staphylococcus aureus in 0.9% physiological NaCl intraperitoneally, then left for 1 hour after administration of Staphylococcus aureus, the mice were killed and operated on, then Na₂EDTA was added to the peritoneal fluid. Then calculated activity and phagocytosis capacity of macrophage cells. Also calculating the percentage of leukocyte cell types in the same day

Phagocytosis Analysis of Macrophage Cells

On the 8th day, mice in each group were infected by injecting Staphylococcus aureus in 0.9% physiological NaCl intraperitoneally, then left for 1 hour after administration of Staphylococcus aureus, the mice were killed and operated on, then Na₂EDTA was added to the peritoneal fluid. The peritoneal fluid is taken using a micro pipette. The liquid was made smear preparations on a slide and fixed with methanol for 5 minutes, then stained with Giemsa dye, let stand for 20 minutes, rinsed with running water and dried. After the preparation was dry, the preparation was viewed under an ocular microscope using emersion oil with a magnification of 1000x. Macrophage cell phagocytosis activity and capacity were calculated. Phagocytic activity is determined based on percentage of leukocyte cell types, activity and capacity of macrophage cell phagocytosis and relative lymph weight were determined.

Calculating the Percentage of Leukocyte Cell Types

On the 8th day the mice were moistened with ethanol so that the tail veins

were dilated, then the tip of the mice's tail veins was cut off and 1 drop of fresh blood was dripped on

another glass object so that a homogeneous layer of blood was obtained (blood smear), then dried after drying the drops with methanol, so coat the entire blood smear and leave for 5 minutes. Add one drop of Giemsa solution as a dye that has been diluted with distilled water (1:20) and leave it for 20 minutes. Wash with distilled water, dry and add emersion oil to make it easier to observe under an ocular microscope. Count the number of eucinophils, stem neutrophils, segment neutrophils, lymphocytes and monocytes at 1000x magnification. the percentage of phagocytes that carry out phagocytosis of 100 phagocytic cells (Yufri Aldi, 2016). Phagocytosis capacity is determined based on the amount *Staphylococcus aureus* which is phagocytosed by 50 active phagocytic cells (Yufri Aldi, 2016).

Data Analyze

To analyze the research data obtained from all parameters used one-way analysis of variation (ANOVA), follow up to the Duncan test was chosen to see the meaning of each group (Prayitno, 2008).

RESULT AND DISCUSSION

In mango leaves extraction

White extract was obtained weighing 17.5468 g with a yield of 7.37%. Furthermore, the extract of mango leaves var arum manis was characterized which included specific parameters, namely identification and organoleptic. Organoleptic aims for early recognition

Table 1. Organoleptic Result of Mango Leaves Extract var Arum Manis

No	Examination	Observation
1	Texture	Vicous Liquid
2	Odor	Unique
3	Colour	Brownish Green
4	Taste	Bitter

Shrinkage of drying and ash content. Examination of drying shrinkage aims to determine the maximum limit of the amount of compounds lost during the drying process, while the ash content is to determine and provide an overview of the mineral content from the beginning until the extract is formed, where organic compounds and their derivatives are digested and evaporated, leaving only mineral elements and inorganic compounds (DepKesRI, 2008). The drying shrinkage is not more than 13.5% and the total ash content is not more than 0.7% (BPOM RI, 2010).

The yield obtained from drying shrinkage was 13.71% more than 13.5%. The result obtained from the total ash content is 0.83% more than 0.7%

In the phytochemical test, the mango leaves extract was carried out by adding chemical reagents based on the compound group, namely the cyanidine test method for examining flavonoids, FeCl₃ for phenolic examination, Liebermann

Buchard for testing of terpenoids and steroids, Culvenore - Fristgerald for testing alkaloids. From the results of the phytochemical test, the extract of mango leaves var arum manis contains flavonoids, steroids, and phenolic compounds. Terpenoids are negative because of evaporation during the extraction process.

Observation of the effect of mango leaves extract on the activity and capacity of phagocystosis of macrophage cells, and the percentage of leucocyte cell types were carried out by administering a suspension of temu putih rhizome extract with each dose to mice for seven consecutive days orally. the opportunity for the sample to increase the number of phagocyte cells influencing a non-specific immune response. While the control group was only given 0.5% Na.CMC suspension.

On the eighth day, *Staphylococcus aureus* bacteria were injected suspended in 0.9% NaCl as an antigen. The

Staphylococcus aureus suspension was compared with a McFarland 0.5 solution to uniform bacterial concentrations. Staphylococcus aureus suspension is injected intraperitoneally. Administered intraperitoneally due to observed macrophage cells are peritoneal macrophages. A few moments after being injected into the body of the mice, the macrophages immediately phagocytose the Staphylococcus aureus bacteria.

Although there are usually not enough numbers to deal with these attacks. Macrophages are able to withstand infection during the first one hour period (Sriningsih et al, 2006).

These considerations, the macrophage collection is carried out about one hour after bacterial induction, so that it can be seen to what extent the macrophages can ingest bacteria. Staphylococcus aureus is used as an antigen because it is a type of gram-positive bacteria that can bind the Giemsa color clearly and has a round shape making it easier to calculate under a microscope. Another advantage, these bacteria do not contain protein A, which is a protein that is antifagocytic. Lack of protein this causes Staphylococcus aureus to be unable avoid phagocytosis peritoneal macrophages (Sriningsih et al, 2006).

On smear inspection

Calculation of the percentage of leukocyte cell types, namely eucinophil cells, stem neutrophil cells, segment neutrophil cells, monocyte cells and lymphocyte cells after Giemsa staining was performed. In Giemsa's staining, basophil cells were not found because basophil cells were alkaline so that these cells were dissolved in Giemsa's dye (Yufri Aldi, 2016).

Table 2. Average Percentage of Activity Phagocytosis of Macrophage Cells in Pheritoneal Liquid After Given Mango

Leaves Extract induced by Staphylococcus aureus

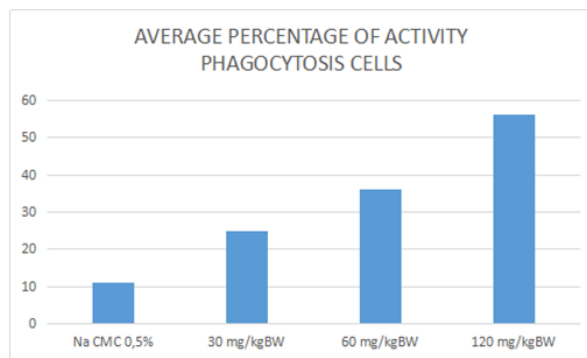


Table 3. Average of Capacity Phagocytosis of Macrophage Cells in Pheritoneal liquid after given Mango leaves extract var arum manis induced by Staphylococcus aureus

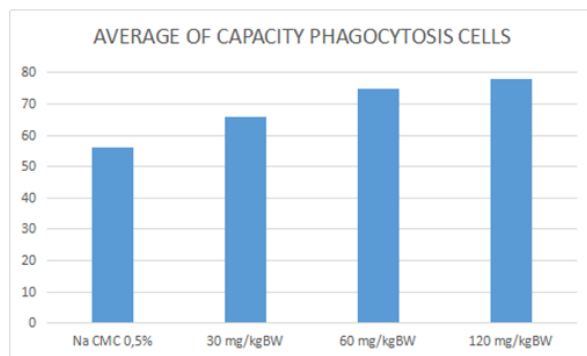


Table 4. Average Percentage of Leukocyte Cells Types After Given Mango Leaves Extract in Pheritoneal Liquid induced by Staphylococcus aureus

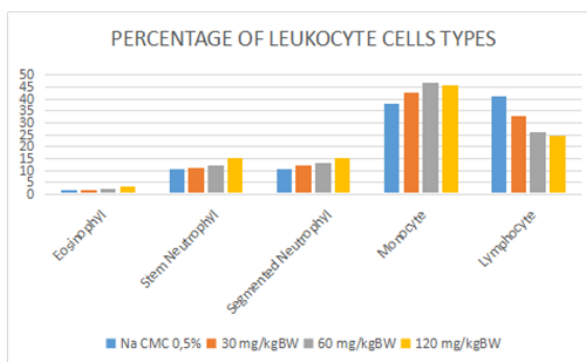




Figure 1. Mango Tree

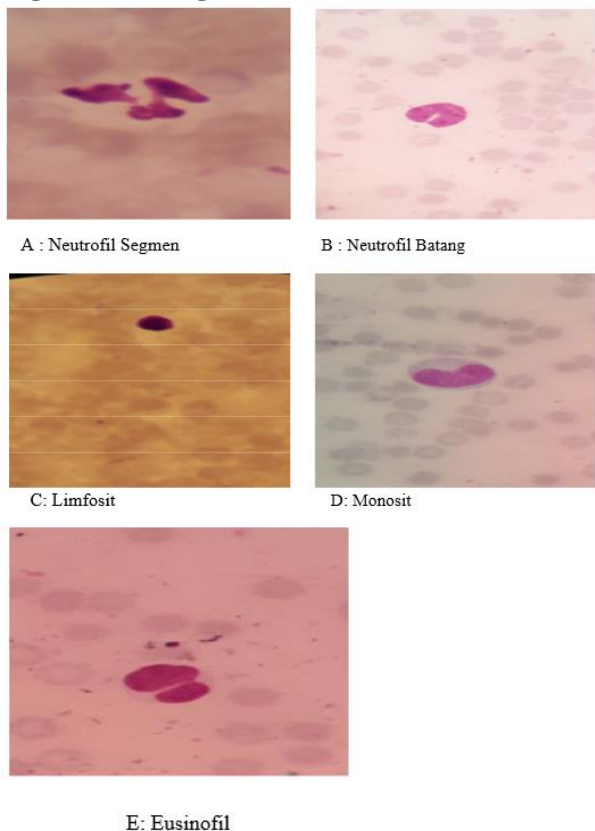


Figure 2. Leukocyte Cells Type on Blood Smear Mice

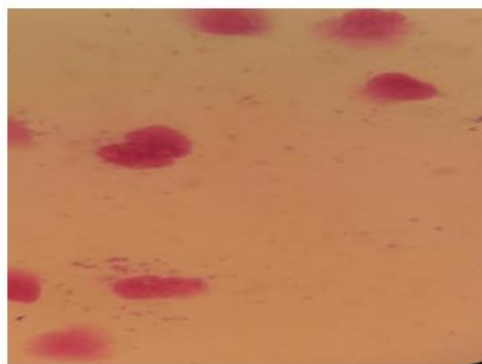


Figure 3. Phagocytosis of Macrophage Cells seen by 1000x magnification microscope

CONCLUSION OF RESEARCH

Giving extract of mango leaves, var arum manis can increase the activity and phagocytosis capacity of macrophage cell, also the percentage of leukocyte cell types along with increasing dosages. Activity, capacity, percentage of leukocyte cell types as a whole, were the most effective at a dose of 120 mg / KgBW.

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HALAMAN INI SENGAJA DIKOSONGKAN