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Original Article

The Effect of Ethanol Extract of Moringa Leaf (*Moringa oleifera* Lam) Against the Activity and Capacity of Phagocytosis of Macrofag Cells and the Percentage of Leukosit Cells of White Mice

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ABSTRACT

Aim: This study aims to determine the activity, capacity of macrophage phagocytosis, and percentage of leukocyte cells of male mice treated with the ethanol extract of moringa leaves (*Moringa oleifera* Lam). **Methods:** Twenty male mice were divided into four equal groups. The extract was administered orally for seven days at a dose of 10; 30; 100 mg/kg; and 0.5% CMC Na suspension as a negative control. On the 8th day, the percentage of blood cell leukocytes in mice tail was calculated using a microscope. Suspension of *Staphylococcus aureus* was injected intraperitoneally. The peritoneal fluid was taken to figure the activity and capacity of macrophage cell phagocytosis. The activity and capacity of macrophage cells are calculated using a microscope. The data were statistically analyzed by the one-way variance analysis (ANOVA) method and Duncan test. **Results:** The results showed that giving oral ethanol extract of Moringa leaves on male could increase macrophage activity and capacity, increasing dose, macrophage activity number, and capacity increased. The highest activity and capacity was achieved at a dose of 100 mg/kg. Moringa leaf extract can also increase the percentage of banded neutrophil, lymphocytes, eosinophil cells and decrease the percentage of neutrophil cell segments and monocytes from male white mice. The highest percentage of banded neutrophil, lymphocytes, eosinophils, and reductions in the percentage of neutrophil cell segments, monocytes present at doses of 100 mg/kg. **Conclusion:** From the result, it can conclude that ethaol extract of Moringa Leaf can increase the activity and capacity of macrophages and percentage of neutrophils.

Key words: Phagocytosis, macrophage cell, *Staphylococcus aureus, Moringa oleifera* Lam.

INTRODUCTION

The immune system is an essential part of the body's defense system[1]. This defense aims to protect the body from noxious agents, foreign objects that can be infectious or not. The human body will always be threatened by pathogens such as bacteria, viruses, parasites, solar radiation, and pollution that can cause disease. Therefore, the immune system is needed to respond to these pathogenic elements[2].

Immune defense consists of a non-specific (natural) immune system and a specific (adaptive) immune system. The non-specific immune response is the body's leading defense in the face of attacks by various foreign substances. Cells that play a role in non-specific (natural) immune responses consist of phagocyte cells (macrophages and neutrophils) and NK cells (natural killer). Macrophages are phagocytic cells that play their function in the immune system by phagocytosis of foreign substances that enter the body. Macrophage response to microbes is almost as fast as neutrophils, but macrophages live longer than neutrophils. Macrophage phagocytosis is also more active in dealing with pathogens such as microorganisms or other antigens, and even cells or tissues themselves are damaged or dead so that macrophages can be categorized as primary effector cells in the natural immune response[3].

The body's defense mechanism can be enhanced with certain compounds that are immunostimulant. Immunostimulants are generally defined as a compound that can increase the body's defense mechanisms specifically and non-specifically, both defense mechanisms cellular and humoral[4]. Therefore, the presence of chemical compounds that can increase the immune system's activity helps overcome the decrease in the immune system, and these compounds can be obtained from plants.

Indonesia is already known as a country rich in natural materials. One of the natural ingredients that can be investigated as enhancing the body's defense system is moringa leaf (*Moringa oleifera*Lam). Concerning the immune system, Moringa leaves have been studied. The state can increase the number of CD4 + T cells and CD8 + T cells in all mice groups and give high doses of Moringa leaf extract, causing immunosuppression. Moringa leaf extract can function as an immunostimulant and immunosuppressed on CD4 + T cells and CD8 + T cells of mice[5].

During this time, Moringa leaves are widely used traditionally, including treating conjunctivitis, coughing, diarrhea, eye and ear infections, fever, swollen glands, scabies, sore throat, respiratory disorders, asthma, blackheads, and tuberculosis[6]. Considering its use is quite broad-spectrum,

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especially in infectious diseases, the effect is likely positive as an immunostimulator. To test this, researchers were encouraged to look at the impact on phagocytic macrophage cells' activity and capacity and calculate the percentage of white mice male leukocyte cells.

Based on phytochemical tests, *Moringa oleifera* Lam leaves contain tannins, steroids, triterpenoids, flavonoids, saponins, anthraquinones, and alkaloids, which are all antioxidants. Flavonoids can also function as immunomodulators, in addition to alkaloids, tannins, and saponins[7,8]. Immunity can also be enhanced by several compounds that have antioxidant effects, such as phenolic compounds or polyphenols. Phenolic and polyphenol compounds are known to increase the ability of phagocytosis of peritoneal macrophages in mice[9].

This study was conducted to determine the effect of *Moringa oleifera* Lam leaf on the phagocytosis ability of macrophage cells of male white mice infected by Staphylococcus aureus bacteria concerning an increase in the body's defense system. The experimental parameters are the activity ability and phagocytic capacity of macrophages with count the number of activated macrophages in 100 total macrophages and count the number of bacterial cells phagocyted by 50 active macrophages. The percentage of leukocyte cells is determined by measuring leukocyte cell types (neutrophil segments, banded neutrophils, monocytes, lymphocytes, and eosinophils) in 100 total leukocytes. This research is expected to inform the public that Moringa leaf extract can be useful as an immunostimulant. It also can complement pharmacological data from *Moringa oleifera* Lam extracts.

MATERIAL AND METHODS

Tool

The tools used are vials, vessels, filter paper, pipette drops, syringes, measuring cups, animal scales, spatters, analytical scales, microscopes (ZEISS), containers (bottles), mortars, and stamper. Object glass, surgical scissors, gloves, mask, ose needle, test tube, TLC-scanner, rotary evaporator, syringe, sonde needle, petri dish, Erlenmeyer, test tube, incubator (Memmert), centrifuge (Hettich).

Material

The ingredients used are *Moringa oleifera* Lam extract, aqua dest, and chemicals such as 70% ethanol, methanol, chloroform, ethyl acetate, distilled water, toluene, Staphylococcus aureus (BPOM Padang), nutrient agar (Merck), nutrient broth (Merck), physiological NaCl, CMC Na, Giemsa dyes, and male white mice.

Procedure

Sampling

Fresh *Moringa oleifera* Lam leaf samples were taken in Lubuk Basung, Agam Regency, West Sumatra

Plant identification

Moringa leaf plant identification has been carried out at Andalas University Herbarium, Biology Department, Faculty of Mathematics and Natural Sciences (FMIPA), Andalas University, Padang.

Making moringa leaves ethanol extract

Make an extract from dried Simplicia powder by maceration using 70% ethanol solvent. Add 200 g of dried Simplicia powder to the macerator, add 10 L of solvent. Soak for the first 6 hours while stirring occasionally, then let stand for 18 hours. Filtered then obtained macerate I. Repeat the search process with at least the same type and amount of the solvent so that it can get macerate II and III. Collect all macerate, then evaporate with vacuum evaporation or low-pressure evaporation so that thick extract is obtained. Calculate the yield obtained, namely the percentage weight (b / b) between the yield and the simplicia powder's weight with weighing. Yields must reach at least the rates specified in each extract monograph[10].

Preparation of experimental animals

This study's experimental animals were male white mice aged 2-3 months with 20-30 grams. Before being used as experimental animals, mice were acclimatized in the study room for one week. It aims to adjust the environment, control health and weight and homogenize the food. Diseased animals with signs of standing fur, decreased motor activity, and weight was not used in the study. The animals used were healthy mice, body weight during acclimatization, which did not change more than 10% and visually showed normal behavior.

Dosage planning

Dose Moringa leaf extract given 25 mg / kg BW, 30 mg / kg BW and 100 mg / kg BW given orally.

Grouping of experimental animals

Experimental animals were divided into four groups, with each group consisting of five mice, namely:

a. Group 1 (negative control) group of mice was given a 0.5% NaC CMC solution orally once a day for seven days.

b. Group II is the group of mice given Moringa leaf extract, a dose of 10 mg/kg B.W. orally one time a day for seven days.

c. Group III is the group of mice given Moringa leaf extract, a dose of 30 mg/kg B.W. orally one time a day for seven days.

d. Group IV is a group of mice given Moringa leaf extract, a dose of 100 mg/kg B.W. orally one time a day for seven days.

Making test preparations

A 0.5% Na CMC suspension was made through a 0.5% CMC Na weighed 500 mg developed with hot water 20 times, after being crushed then added the ethanol extract of Moringa leaves according to the planned extract concentration, then crushed homogeneously and sufficient with aqua dest until the volume of 100 mL. Moringa leaf extract concentrations made were 0.1%, 0.3% and 1%.

$$\text{Concentration (mg / mL)} = \frac{\text{Dose (mg / kg) x Weight (kg BW)}}{\text{Drug administration volume (ml)}}$$

Manufacture of *Staphylococcus aureus* (S.A.) culture

Staphylococcus aureus (S.A.) is cultured on nutrient agar (N.A.). One ose S. A culture was inoculated into new N.A. media, after which it was incubated at 37 °C for 24 hours in an incubator. Staphylococcus aureus that grows on N.A. media are transferred into nutrient brooth (N.B.) media, incubated 24 hours at 37 °C, then centrifuged 2500rpm for 25 minutes, then pellets and siresensensension with 0.9% physiological NaCl solution equivalent to Mc Farland's solution. 0.5

Calculate the percentage of the number of leukocyte cells

On the 8th day, the mice's tail was moistened using 70% ethanol so that the blood vessels of the mice's tail veins dilated, then the tip of the mice's tail veins was cut, and fresh blood was dropped by one drop of the slide. Then flatten with another slide so that a homogeneous blood layer (blood smear) is obtained, then dry it. After dry drops with methanol so that the entire blood smear coat leaves for 5 minutes. Add one drop of Giemsa solution that has been diluted with distilled water

(1:20) and leave for 20 minutes. Wash with distilled water, dry it and add emersion oil and observe it under an ocular microscope. Count the number of oesinophils, banded neutrophils, neutrophils of segments, lymphocytes and monocytes at 100x magnification using a microscope.

Analysis of macrophage cell phagocytosis

On the 8th day, mice in each group were infected with 0.5 ml injection of Staphylococcus aureus in physiological NaCl 0.9% intraperitoneal, then left for 1 hour. After administration of Staphylococcus aureus, mice were sacrificed using a pen, in the chest and abdomen moistened with 70% ethanol, and mice dissected, add heparin to the peritoneal fluid. Peritoneal fluid is taken using a micropipette. The peritoneal liquid was made, smear on the slide and fixed with methanol for 5 minutes, then stained with Giemsa staining, allowed to stand for 20 minutes, rinse with running water, and dried. After the preparation is dry,

Phagocytic activity is determined based on the percentage of phagocytes that carry out phagocytosis from 100 phagocytic cells[11,12]. Phagocytic capacity is determined based on the number of Staphylococcus aureus phagocytosis by 50 active phagocytic cells[12].

Data analysis

The data obtained were statistically analyzed by one-way analysis of variance (ANOVA) and then followed by Duncan's Multiple Range Test (DMRT) analysis.

RESULTS AND DISCUSSION

After researching the activity and capacity of *Moringa oleifera* Lam extracts on several parameters of macrophage cell activity and the percentage of leukocytes in male white mice, the following results were obtained:

Organoleptic observations showed that Moringa leaf extract has a thick shape, blackish-brown color, characteristic odor, and flavor. From maceration, as much as 1 Kg of *Moringa oleifera* Lam dried powder with 70% ethanol obtained thick extract as much as 217.3 g with 21.73% amendment.

Based on the study, the water content of the extract was 9.63%. Moringa leaf extract also reacts positively with alkaloid reagents, flavonoids, saponins, phenols, carbohydrates, glycosides, tannins, phytosterols, and proteins.

Observation of phagocytic activity and capacity of macrophage cells after administration of ethanol extract of Moringa leaves for seven days and induced with Staphylococcus aureus bacteria:

a. The results of determining phagocytic activity in the control group, the dose of 10 mg/kg, 30 mg/kg, and 100 mg/kg body weight after administration of *Moringa oleifera* Lam extracts were 59%; 76%; 79.8%; and 87.6%

b. The results of determining the peritoneal macrophage capacity in the control group, extract dose of 10 mg/kg, 30 mg/kg, and 100 mg/kg after administering *Moringa oleifera* Lam extracts were 90.2%; 94.4%; 101.4%; and 106%.

The percentage of activity and the amount of macrophage cell capacity after treating by Moringa leaf ethanol extract with varying doses have been carried out statistical analysis tests shown in Figures 1 and 2.

The results of the calculation of the percentage of blood leukocyte cell types of mice, namely neutrophil segment cells, banded neutrophils, monocytes, lymphocytes and eosinophils after administration of Moringa leaf extract in the control group was 43.8%; 5.8%; 6.4%; 42.2%; 1.8%, at a dose of 10 mg / kg is 42%; 3.6%; 5.8%; 46.6%; 2%, at a dose of 30 mg / kg 38%; 4.6%; 4.4%; 50.6%; 57%, and at a dose of 100 mg / kg 31%; 5%; 4%; 57%; 3% and a statistical analysis test has been performed which can be seen in Figure 3.

In this study, the sample used was *Moringa oleifera* Lam leaf. Moringa leaves that obtained from Pariaman city, West Sumatra. Moringa leaves have been identified at Herbarium, Andalas University, where the results state that the sample was *Moringa oleifera* Lam leaves. Thus, the sample can be used for further research.

The purpose of this study was to determine the effect of *Moringa oleifera* Lam extract on the activity and capacity of phagocytosis of macrophage cells and the percentage of leukocytes. Standardization of Moringa leaf extract was done before experimenting with the animal. The purpose of standardization is to get a good quality of extract, standardized and tested its stability.

The method of extraction used in this study was maceration. Maceration aimed to make the active substance in plant cells dissolve so that the active substance will be pushed out of the cell. The maceration method was chosen because the process is easy. The equipment is quite simple, and it does not require too much solvent. This method is also suitable

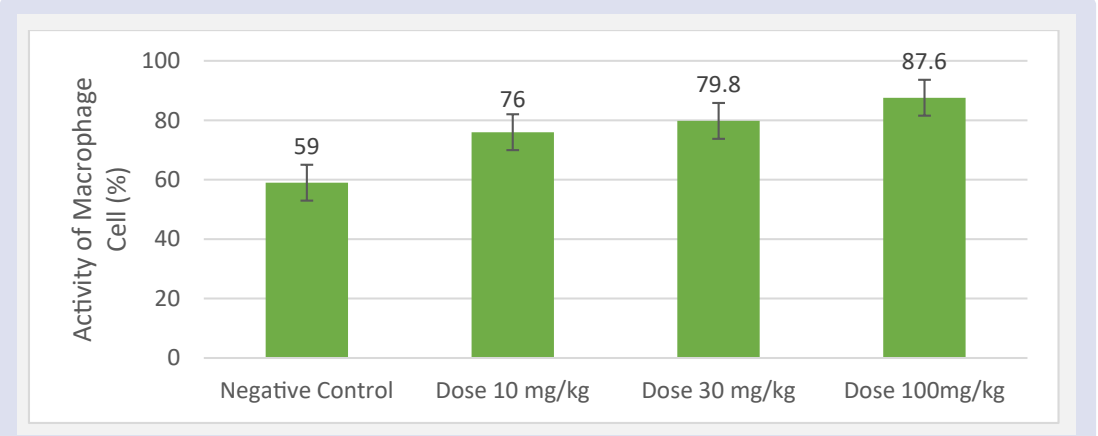

Figure 1: Bar diagram of macrophage cell activity in peritoneum fluid of male white mice against mg/kg dosage.

Figure 2: Bar diagram of macrophage cell capacity in peritoneum liquid of white mice.

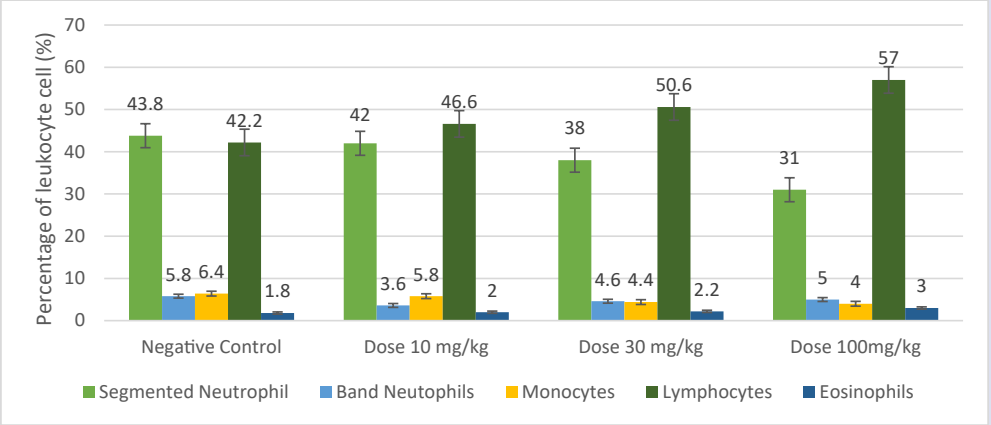

Figure 3: Graph of percent of blood leukocyte cell types to the Moringa leaf extract dose given to mice for seven days orally.

for attracting compounds that are not resistant to heating[13]. Maceration was done by immersing Moringa leaf powder with 70% ethanol for 3x24 hours. We chose ethanol because it is more selective, molds are challenging to grow in ethanol 20% and above, non-toxic, neutral, good absorption, requires less heat for the concentration process. The Maserati obtained was evaporated with a rotary evaporator until a thick extract of 217 was obtained.

The extracts were tested for non-specific water content. Moisture content determines the stability of the extract. The requirement of water content is less than 10% to avoid the rapid growth of fungi in the extract. The water content in the extract was 9.63% that less than 10%.

Specific characterizations include organoleptic for simple initial recognition. We can find that the extract was blackish brown color, bitter taste, distinctive aromatic odor, and thick shape from the organoleptic study.

In this study, the animals used were 20 male mice (Mus muculus) aged 2-3 months with a bodyweight of 20-30 grams. Mice were chosen because the nature of mice's anatomy and physiology is almost the same as humans, easily obtained and handling. Before being treated, the animal must be acclimatized for one week and satisfied for 18 hours (drinking is still given) before the experiment. Healthy mice i.e. body weight during acclimatization, do not experience changes of more than 10% and visually exhibit normal behavior[14].

Mice are grouped into four groups, each of which is given a different treatment. In this study, the effects of ethanol extract of Moringa leaves were observed divided into several doses. Observation of extracts' immunomodulatory effects on the increase in macrophage cells' phagocytic activity and capacity was carried out by administering the extract for seven consecutive days. On the eighth day, the mice were injected with Staphylococcus aureus which had been suspended with a 0.9% physiological NaCl solution as an antigens Intra peritoneally. Staphylococcus aureus is the first type of microbial antigen captured by macrophages. Phagocytosis is the primary mechanism against Staphylococcus aureus. Generally, microbes are presented to T cells by macrophages. Besides, Staphylococcus aureus has advantages,

Calculation of phagocytic activity and macrophage cells' capacity in which phagocytic activity shows the number of active macrophage cells phagocytosis of bacterial cells in 100 macrophage cells expressed in percent. Simultaneously, the value of phagocytic capacity is obtained by counting the number of bacterial cells phagocyted by 50 macrophage cells. The results of the calculation of phagocytic activity of macrophages in peritoneal fluid of male white mice were obtained. For the negative control animal group, an average of 59%, for the dose group 10 mg/kg body weight an average of 76%, for the group a dose of 30 mg/kg body weight has obtained an average of 79.8%, and for a dose of 100 mg/kg body weight an average of 87.6%.

On the statistical analysis of one-way variant analysis, macrophages' phagocytic activity after administration of *Moringa oleifera* Lam. Extracts increased significantly (p <0.05). Furthermore, Duncan's further test analysis is performed to determine the different effects of each dose. There was no difference (P> 0.05) between the 10 mg / kg BW and 30 mg / kg BW dosage groups, also the 30 mg / kg BW dosage group and the 100 mg / kg BW dose. The dosage groups of 10 mg/kg body weight and 100 mg/kg body weight had significant differences. This shows that Moringa leaf extracts an effect on macrophages' phagocytic activity, and different dosages also have different effects. Moringa leaf extract dose of 100 mg/kg gives the best effect.

Data from the calculation of phagocytic capacity after administration of Moringa leaf extract at the control dose is 90.2%, at a dose of 10 mg/kg body weight is 94.4%, at a dose of 30 mg/kg body weight is 101.4%, and at a dose of 100 mg/kg body weight, is 106%. The calculation of macrophage cell phagocytosis capacity shows an increase in macrophage cells' average phagocytic capacity.

On the results of the statistical analysis of one-way variant analysis, the phagocytic capacity of macrophages after administration of *Moringa oleifera* Lam. extracts increased significantly (p <0.05). Furthermore, Duncan's further test analysis is performed to determine the different effects of each dose. There was no difference (P <0.05) between negative control treatments and a dose of 10 mg/kg, a dose of 10 mg/kg and a dose of 30 mg/kg, and a dose of 30 mg/kg and a dose of 100 mg/kg. The dosage groups of 10 mg/kg body weight and the dose of 100 mg/kg body weight had significant differences.This shows that Moringa leaf extract has an effect on macrophages' phagocytic capacity, and different dosages also have different effects. Moringa leaf extract dose of 100 mg/kg gives the best effect.

In addition to the phagocytic activity and capacity of peritoneal macrophages, the percentage of leukocyte cells is also determined after administering Moringa leaf extract. Before being injected with Staphylococcus aureus, mice's tail blood was taken, and a smear preparation was made. Using Giemsa staining, the observed leukocytes are banded neutrophil, segment neutrophils, monocytes, lymphocytes, and eosinophils, while basophil cells are not visible because these cells dissolve in Giemsa staining[15].

On the results of statistical analysis of one-way variant analysis, it can be seen that the administration of Moringa leaf extract can significantly reduce the number of neutrophil segment cells (P <0.05). Duncan's next test was then performed to see the difference in the effects of each dose. It turns out that the difference in each dose of 10, 30, 100 mg/kg. gives a significantly different effect. The results showed that the number of neutrophil cell segments after administering test compounds decreased from normal animals. A decrease in the number of neutrophil cell segments is thought to be the process of phagocytosis which plays a more important role as macrophages or because of increased chemotaxis factors increasing phagocytic ability[15].

While the results of the one-way variant analysis of the number of banded neutrophils after Moringa leaf extract were not significantly different (p> 0.05). After further testing Duncan, it was seen that the administration of Moringa leaf extracts in both doses of 10, 30, and 100 mg/kg gave the same number of banded neutrophil as normal animals. This shows that Moringa leaf extract does not increase the number of banded neutrophils.

In the analysis of one-way variants of the number of monocyte cells after Moringa leaf extract was significantly different (p <0.05). To see the effect of each dose, Duncan continued testing. Duncan's further test results showed that the number of monocyte cells from the extract dose of 10 mg/kg was the same as the dose group 30 mg/kg and 100 mg/kg where the number of monocyte cells from the extract dose of 10 mg / kgbb was greater. Monocyte cells are thought to decrease because monocyte cells differentiate into macrophages and settle in tissues.

Analysis of one-way variants of the number of lymphocyte cells after administering Moringa leaf extract was significantly different (p <0.05). After further testing Duncan, it was seen that the administration of Moringa leaf extracts in both doses of 10, 30, and 100 mg/kg body weight gave a real difference to the number of lymphocyte cells. Data from lymphocyte count results show an increase in the number of lymphocytes which is directly proportional to an increase in dose, so it can be concluded that Moringa leaf extract can increase the number of lymphocyte cells, which are immune cells that play an important role in humoral and cellular response systems . While the results of the one-way variant analysis of the number of eosinophil cells after Moringa leaf extract were not significantly different (p> 0.05).

The results of the study of the activity and capacity of macrophage cells and leukocytes in each dose showed that the higher the dose of Moringa leaf extract, the higher the activity and capacity of macrophage cells and the percentage of leukocyte cells with the number of cells and the number of bacteria increased from the lowest to the highest dose.

CONCLUSION

Giving extracts *Moringa oleifera* Lam leaves can increase the activity and capacity of macrophages. Increasing the dose will increase the activity number and macrophage capacity. The highest activity and capacity is achieved at a dose of 100 mg/kg.

Giving extracts *Moringa oleifera* Lam leaves for seven days can increase the percentage of eosinophils cells, banded neutrophils, lymphocytes and decrease the percentage of neutrophil segments and monocytes from male white mice.

CONFLICTS OF INTEREST

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

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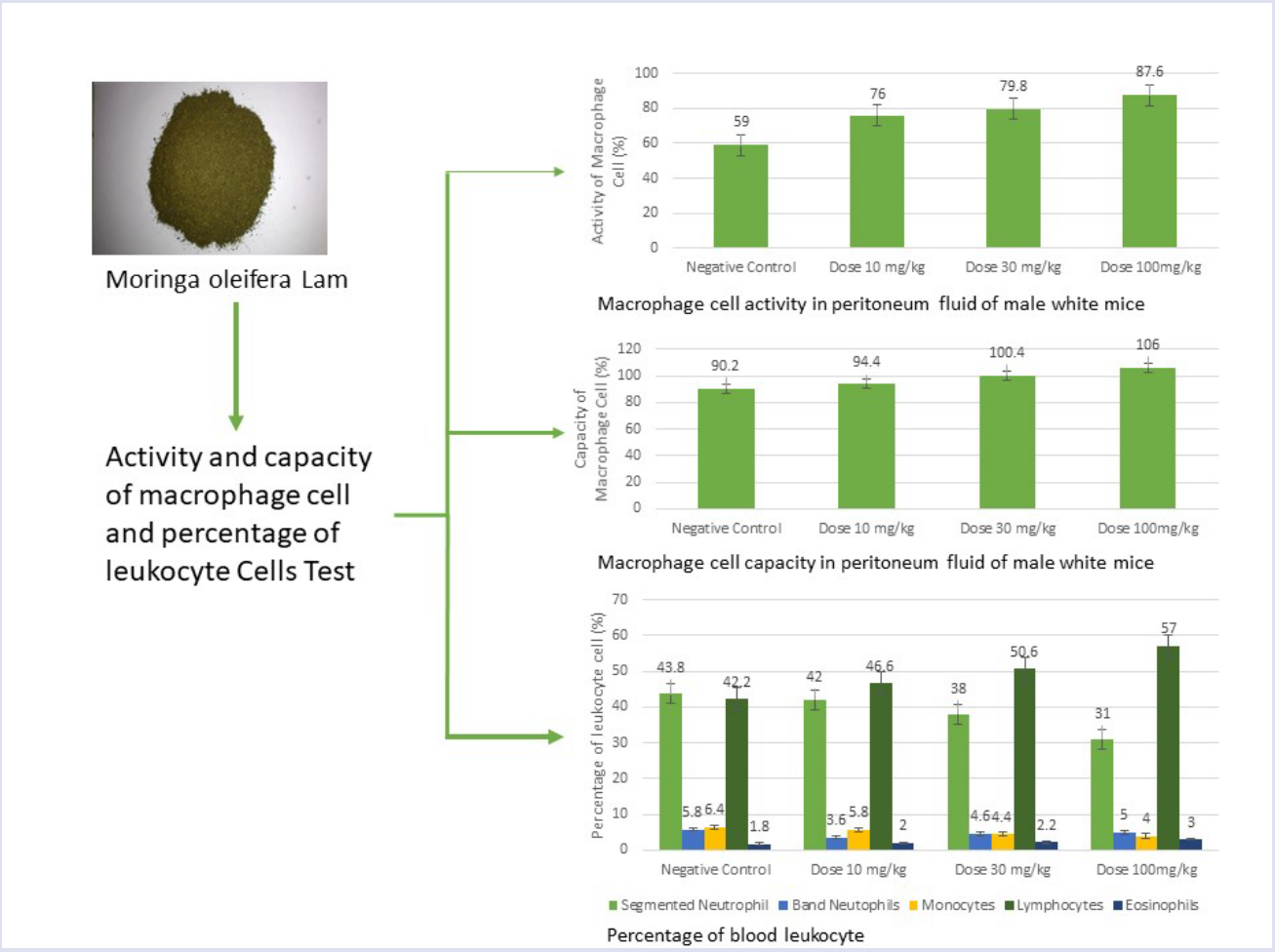
GRAPHICAL ABSTRACT



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