Asean Journal of Pharmaceutical and Clinical Research

by Artikel 1 Immunomodulatory Effect Test From Moringa Leaf Ex

Submission date: 04-Nov-2018 11:38PM (UTC+0800)
Submission ID: 1032494068
File name: kel_1_IMMUNOMODULATORY_EFFECT_TEST_FROM_MORINGA_LEAF_EXTRACT.pdf (661.35K)
Word count: 3711
Character count: 18481

ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 9, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

IMMUNOMODULATORY EFFECT TEST FROM MORINGA LEAF EXTRACT (*MORINGA OLEIFERA* L.) WITH CARBON CLEARANCE METHOD IN MALE WHITE MICE

DWISARI DILLASAMOLA[1]*, YUFRI ALDI[1], MUTIA FAKHRI[1], SKUNDA DILIAROSTA[2], BIOMECHY OKTOMALIO P[3], NOVERIAL[3]

[1]Department of Pharmacy Faculty of Pharmacy, Andalas University, Padang, Indonesia. [2]Department of Math and Science, United of State, Padang, Indonesia. [3]Department of Medicine, Faculty of Medicine, Andalas University, Padang, Indonesia.
Email: dwisaridillasamola@phar.unand.ac.id

Received: 16 April 2018, Revised and Accepted: 24 May 2018

ABSTRACT

Objective: *Moringa oleifera* leaf has chemical compounds that have been utilized by the community to cure health problems. One of its activities is an immunomodulator. The aim of this study is to determine the immunomodulatory effect from *M. oleifera* leaf using a carbon clearance method to measure the activity of phagocytic cells in exterminating pathogens that enter the body then followed by calculating the total leukocyte cells. The parameters of this test are phagocytosis index and total leukocyte cells.

Methods: Twenty white male mice were divided into four groups. Group I (vehicle control) was treated with sodium-carboxymethyl cellulose (NaCMC) 0.5%, Group II-IV were treated by *M. oleifera* leaf extract given to the mice for six consecutive days orally in doses of 10, 30, 100 mg/kg. On the seventh day, white male mice were given with intravenous carbon suspension through their tails. The value of phagocytosis index (PI > 1) indicated immunostimulant activity. The data were analyzed by using one-way analysis of variance and Duncan test.

Results: The analysis of variance results showed that the groups treated with *Moringa* leaf extract are significantly different with the vehicle groups (NaCMC 0.5%) ($p<0.05$). Increased doses of *Moringa* leaf extract are effective to improve the immunomodulator effect. It was included that *Moringa* leaf extract had the immunomodulatory capabilities as an immunostimulant.

Conclusion: Immunomodulatory effect test of *M. oleifera* Lam. Based on the result of the research about immunomodulatory effect test from *Moringa* leaf extract (*Moringa Oleifera* L.) with carbon clearance method in male white mice, it can be concluded that Moringa leaf extract (Moringa Oleifera L.) has effect as an Immunomodulator.

Keywords: *Moringa leaf* extract, Immune system, Immunomodulator, Carbon clearance.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i9.26703

INTRODUCTION

The human body develops complex mechanisms to deal with pathogens that have the ability to enter the body and have the potential to disrupt the body's balance [1]. The immune system is a system that served to prevent the occurrence of body damage or the onset of disease, an immune system that works well is necessary for human survival [2].

The optimal immune system is needed to protect the body against invading pathogenic microorganisms [3]. Through the optimal immune system, the body is not vulnerable to invasion of microorganisms. Microorganisms that have the potential to invade the body exist in the environment of human life such as protozoa, viral, and bacterial fungi [4]. The immune system has responsibilities to protect the body and defend itself against the invasion of the pathogenic microorganisms through non-specific and specific mechanisms [5]. The immune system consists of specific immune systems and non-specific immune systems. The non-specific immune system is the outermost defense in the form of skin, mucous membranes, skin secretions, and mucosa, while the non-specific internal immune system in the form of phagocytosis by white blood cells, antimicrobial protein, and inflammatory response [6]. Specific and comprehensive (systemic) body defenses that can be non-specific and specific to humoral and cellular mechanisms [7]. The dual body defense mechanisms cause complex roles to find an infection if other invading microbes or parasites fail to penetrate the body's defenses because they cannot cause manifestations of real pain [8].

Immunomodulators are certain compounds that can affect the quality and intensity of the immune response. Immunomodulators have a function to improve the immune system by stimulation (immunostimulant), restore immune system function (immunorestoration), or by suppressing/ normalizing an abnormal immune reaction (immunosuppressant) [8].

Kelor (*Moringa oleifera* Lam.) is an Indian plant that has various chemical compounds useful in the world of health especially to cure various diseases. Martatino (2014) stating that the pores of nutrients leave kelor can reduce the incidence of infection in HIV/AIDS patients. The results of phytochemical studies of *Moringa* leaf mention that the leaf kelor contains secondary metabolite compounds flavonoids, alkaloids, and phenols that can inhibit bacterial activity. The composition and concentration of phytochemical compounds undergo changes during plant growth. Younger *Moringa* leaves contain the highest phytochemical compounds [9].

METHODS

Animal test preparation

Because this research involved animal, ethical clearance is needed for this research in order to prevent undue suffering, the sertificate of the ethical clearance is shown in Appendix 1. The experimental animals used were 20 male white mice aged 2–3 months with weight 20–30 g and had never experienced treatment of previous drugs. Before being used as experimental animals, all mice were adapted for a week or so for environmental adjustment, health and weight control, and food uniformity.

Determination of dosage

The doses of moringa leaf extract (*M. oleifera* L.) that tested on male white mice in this study were as follows:

a. A dose of 10 mg/kg extract
b. A dose of 30 mg/kg extract
c. A dose of 100 mg/kg extract.

Preparation of *Moringa* leaf extract suspension

The 0.5% sodium-carboxymethyl cellulose (Na-CMC) suspension was prepared by developing 50 mg of Na-CMC with hot water for 20 times. After expand crushed until homogeneous, the suspension was added with the extract of *Moringa* leaf in accordance with the planned concentrations for the experimental animals. Extracts were homogeneously crushed with sufficient amount of Aquadest to a volume of 10 mL. The extract concentrations made were 0.1%, 0.3%, and 1%.

This concentration can be determined by the formula:

$$\text{Concentration} = \frac{\text{Dose}\left(\frac{\text{mg}}{\text{Kg BB}}\right)}{\%\text{VAO}\left(\frac{\text{ml}}{\text{g}}\right)}$$

The percentage of oral administration of drugs is 1%.

Provision of test compounds

The experimental design used in this study was a randomized block design, this design was shown in Table 1, 20 mice were picked randomly into four treatment groups, which consist of five mice each, and those were given different treatments:

1. Vehicle control (Na-CMC 0.5%)
2. Group of extract I, Moringa leaves dose 10 mg/kg
3. Group of extract II, Moringa leaves of dose 30 mg/kg
4. Group extract III, Moringa leaves dose 100 mg/kg.

Mice were given with test and control compounds on an oral basis for 6 days.

Mice were given with test and control compounds on an oral basis for 6 days. The volume of test compound that given to mice is 20 g based on the calculation of Drug Administration Volume (VAO):

$$\text{VAO} = \frac{\text{Dose} \times \text{body weight}}{\text{Concentration}}$$

$$= \frac{10\frac{\text{mg}}{\text{kg}}\text{BW} \times 0.02\ \text{kg BW}}{1\frac{\text{mg}}{\text{mL}}}$$

$$= 0{,}2\ \text{mL}$$

Determination of carbon content

Weigh carefully 5 g of Chinese ink then dry in the oven at 105°C for 30 min and the drying process is continued in the desiccator until the weight of the Chinese ink is constant.

Preparation of carbon standard curve

The dried Chinese ink then weighted as much as 100 mg, dispersed in 100 mL acetic acid to obtain a concentration of 1000 ppm. Each solution was taken as much as 2, 3, 4, 5, and 6 mL then sufficiently added with 1% acetic acid to a volume of 50 mL so that the concentrations of carbon content were 40, 60, 80, 100, and 120 ppm. Each concentration was plated as much as 4 mL, then added with male white mouse blood that taken from its vein as much as 75 µL. Once homogenized the absorbance level was measured by an ultraviolet (UV)-visible spectrophotometer at a wavelength of 650 nm which is an absorption area for carbon. The absorbance plot obtained with carbon content is used to create the calibration curve. As for the marker, only white, male, and Aquadest mouse blood were used.

Preparation of colloidal carbon suspension

A total of 1.6 g of dried Chinese ink suspended with 25 mL tween 80.1% (w/v) and 0.9% (w/v) sodium chloride (NaCl) physiological solution was added to obtain 64 mg/mL concentration (6.4%).

Test of immunomodulatory effects

Activity testing (method of carbon clearance)

Male white mice were divided into four groups which consist of one negative control group that was given with Na-CMC and the rests three test groups that were given with three dose variations. Each test group was given a sample of suspension orally with a dose regimen of once daily for six consecutive days.

Blood sampling and colloidal carbon suspension injecting (done by the top of the tail is given alcohol and then injected carbon suspension) were done at the 7th day. Blood is taken through the end of the vein of the tail of the test mice.

Blood was taken as much as 75 µL and separated in 4 mL 1% acetic acid. This first blood sample is called blank (minute 0). Then, the carbon suspension of 0.1 mL/10-g body weight was given intravenously, and the blood of the mice was taken 75 µL at 3, 6, 9, 12, and 15 min after injection of carbon. Each blood was separated in 4 mL 1% acetic acid, then the absorbance was measured with UV-visible spectrophotometer at a wavelength of 650 nm. The phagocytic constant was calculated using the following formula:

$$K = \frac{\log A\,(n) - \log A\,(n-1)}{t\,(n-1) - t\,(n)}$$

Explanation:
K: Phagocytics constants
A: Adsorbants in n time
t: Time (3,6,9,12,15) min
n: Taken period (1,2,3,4,5).

The price of the phagocytic index is calculated using the following formula:

$$\text{PI mice} = \frac{\text{Mice phagocytic constants (Z)}}{\text{Phasgocytic constants average of mice control}}$$

With:
PI: Index of phagocytosis
Z mice: The mice were known as the phagocytic constants.

If the index value of phagocytosis (PI) >1, the test substance has immunomodulatory activity that is immunostimulant.

Calculates the leukocytes total with the hemocytometer

Fresh blood was sucked with leukocyte pipette until 0.5, then sucked into the solution until reached mark 11. It was then shaken for 3 min and from inside leukocyte pipette the amount of 1–2 drops were thrown and counted in hemocytometers room drops for one drop. The liquid was being left for 2 min for the leukocyte to settle, then the number of white blood cells were calculated on all four count chambers.

RESULT AND DISCUSSION

Immunomodulatory effect test of *M. oleifera* L extract was done using carbon clearance method. This method is testing the ability of

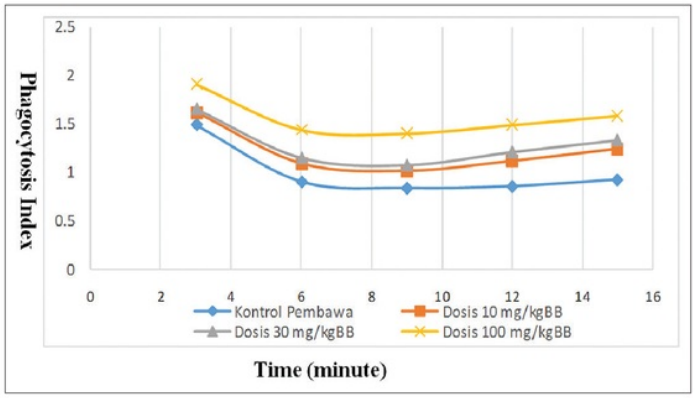

Fig. 1: Graphic of time against phagocytosis index value from mice's blood with variant of doses with *Kelor* leaf (*Moringa oleifera* L.) extract given for 6 days

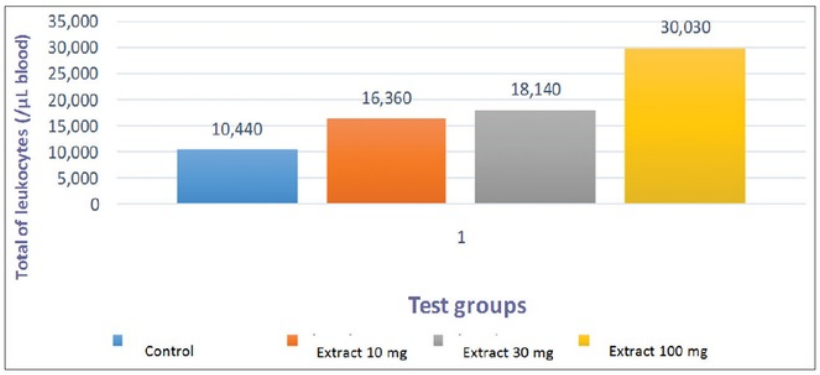

Fig. 2: Comparison graphic of the total leukocytes in white male mice's blood after *kelor* leaf (*Moringa oleifera* L.) extract given for 6 days with the doses of 10 mg, 30 mg, 100 mg

Table 1: Preparation of test animals

Preparation of animal			
Group of animal test	**Dose**	**Route of administration**	**Duration**
Vehicle control	Na CMC 0,5%	Orally	6 days
Extract Group 1	10 mg/kg	Orally	6 days
Extract Group II	Extract of kelor's leaf 30 mg/kg	Orally	6 days
Extract Group III	Extract of kelor's leaf 100 mg/kg	Orally	6 days

NaCMC: Sodium-carboxymethyl cellulose

Table 2: Phagocytosis index value from mice's blood after *M. oleifera* L. extract given for 6 days

Phagocytic index				
Time (min)	**Vehicle control**	**Extract group**		
	Na-CMC 0.5%	**Dose 10 mg/kg**	**Dose 30 mg/kg**	**Dose 100 mg/kg**
3	1.486	1.615	1.657	1.907
6	0.901	1.088	1.150	1.434
9	0.836	1.011	1.074	1.396
12	0.854	1.114	1.209	1.483
15	0.921	1.238	1.332	1.580
Avg.	1	1.213	1.284	1.560
SD	0.274	0.238	0.228	0.205

NaCMC: Sodium-carboxymethyl cellulose, *M. oleifera: Moringa oleifera*

Table 3: Total of leukocyte cells in white male mice's blood after *Kelor* leaf (*M. oleifera* L.) extract given for 6 days

No.	Total of leukocytes (µL blood)			
	Control	Extract group of *Kelor* leaf (*M. oleifera L.*)		
	Na-CMC 0.5%	10 mg/kg	30 mg/kg	100 mg/kg
1	6.900	13.550	13.400	24.450
2	7.100	15.050	15.000	25.550
3	12.300	17.300	22.150	34.200
4	13.500	17.550	17.900	33.050
5	12.400	18.350	22.250	32.900
Mean	10.440	16.360	18.140	30.030
SD	3176,16	2043,13	4042,18	4635,54

NaCMC: Sodium-carboxymethyl cellulose, *M. oleifera: Moringa oleifera*

Table 4: Statistic analysis of total leukocytes in white male mice after *Kelor* leaf (*M. oleifera* L.) extract given for 6 days

Source of variation	Sum of squares	df	Mean square	F	Sig.
Between groups	1008367375,00	3	336122458,333	25,674	0.000
Within groups	209470000,000	16	13091875,000		
Total	1217837375,000	19			

One way ANOVA statistic analysis result to total white male mouse leucocytes after giving *Kelor* leaf extract for 6 days. Duncan continued test[a], *M. oleifera: Moringa oleifera*

Table 5: Duncan continued test analysis of total leukocytes in white male mice after *Kelor* leaf (*M. oleifera* L.) extract given for 6 days

Groups	N	Subset for alpha=0.05		
		1	2	3
Control	5	10440,00	16360,00	30020,00
Dose of 10 mg/kg	5	1,000	18970,00	1,000
Dose of 30 mg/kg	5		0.271	
Dose of 100 mg/kg	5			
Sig.				

The results of Duncan's advanced test analysis of the total leukocytes of male white mice after giving *kelor* leaf extract for 6 days. *M. oleifera: Moringa oleifera*

phagocytosis using carbon as an intravenously administered marker. Carbon clearance was seen every 3rd, 6th, 9th, 12th, and 15th min. Carbon levels in the blood will decreased in time, due to phagocytic events by leukocyte cells mainly by monocyte cells, neutrophils, eosinophils, and macrophages [2]. The use of carbon as a marker has an advantage where the particle size is smaller and more stable, so the carbon does not cause blockage of blood vessels and lungs. Carbon also has a characteristic as an antigen that is alienation, and under normal circumstances, carbon is not present in the body. The carbon used is Chinese ink that had been dried. The result of the determination of Chinese ink carbon content used is 13.31%.

The carbon suspension was prepared by weighing 1.6 g of dried Chinese ink, then suspended with tween 80.1% (w/v) and physiologically added with NaCl 0.9% to obtain 64 mg/mL concentration (6.4%). The use of physiological NaCl in carbon determination aims to make the condition of the carbon suspension dosage (Chinese ink) is as the same as the animal body condition, because the carbon as the foreign matter will be phagocyted by leucocyte cells, especially neutrophils and macrophages, which present in the body of test animals [10,11]. Immunomodulatory effect test by Carbon Clearance method was seen through standard curve between carbon content in blood and absorbance value. The absorbance value was measured using a UV-visible spectrophotometer (Shimadzu®) at a wavelength of 650 nm. Wavelength 650 nm is an area of carbon uptake [12]. The result of the determination of carbon standard curve was obtained by linear regression equation between absorption and carbon concentration that is $y = 0.0049x + 0.0818$ with $R^2 = 0.9936$.

Immunomodulatory results of *M. oleifera* leaf extract (*M. oleifera* L.) were seen at the decreasing carbon absorbance in every minute in male white mouse blood which been given with the test preparation for six consecutive days. Reduced levels of carbon in the blood of test animals in every minute of testing indicated that the carbon concentration in the blood of mice is getting lower. This also indicated an increased phagocytic activity of carbon in each group of extracts.

The result of absorbance value which had been obtained can be calculated as a value of phagocytosis constant from each group of the animal test. The phagocytic constant is one of the parameters that indicate the rate of phagocytosis in immunomodulatory testing by carbon clearance method, the greater the value of the phagocytic constant, the greater the rate of carbon clearance [13].

The mean value of phagocytic constants obtained based on the calculation result of the absorbance value in the control group of the carrier is 0.027. Meanwhile, the extract group with a dose of 10 mg/kg is 0.033, the group with a dose of 30 mg/kg is 0.0357, and the group with a dose of 100 mg/kg is 0.043.

The value of the phagocytic index is calculated after the phagocytosis constant value was known. There was an experiment states that if the average index phagocytosis greater than 1 (IF <1), it indicates that the test substance has an immunomodulatory activity which is immunostimulant [14,15].

Based on calculations that had been done and shown in Table 2 and Fig. 1. the average value of phagocytosis index in the control group of the carrier is 1. Meanwhile, the extract group with a dose of 10 mg/kg is 1,213, the group with a dose of 30 mg/kg is 1,284, and the group with a dose of 100 mg/kg is 1,560. The results of phagocytosis index calculation showed that Moringa leaf extract has an immunomodulatory activity that is as an immunostimulant.

The calculation of total leukocyte value was found after leaf was extracted for 6 days and shown in Table 3 and Fig. 2. The average total leukocyte in the blood group of mice was 10,440/µL of blood, meanwhile the extract group with a given dose of 10 mg/kg was 16,360/µL of blood, dose of 30 mg/kg was 18.140/µL of blood, and dose of 100 mg/kg was 30,030/µL. The increasing total number of leukocytes showed that the immune system improved [1]. To test the real effect of increasing total leukocyte count after giving *Moringa* leaf extract (*M. oleifera* L.), Statistical analysis of variance analysis (ANOVA) one way in Table 4 showed that the total number of leukocytes in each test group were significantly different ($p<0.05$).

From the results of the analysis using Duncan's advanced test, shown in Table 5, the carrier group has given Na-CMC 0.5% was in subset 1, the dose group of 10 mg/kg and the dose of 30 mg/kg was in subset 2, and the dose group of 100 mg/kg is in a subset of 3. This indicates that *Kelor* leaf's extract given could increase the total number of leukocyte cells in the blood of test animals. Based on the research that had been done and reviewed from several test parameters namely, phagocytosis constant, phagocytosis index, and calculation of total leukocyte count, it can be concluded that *M. oleifera* L. Leaf extract has an immunomodulatory activity that is immunostimulant [12,16].

CONCLUSION

Based on the result of the research about immunomodulatory effect test from *Moringa* leaf extract (*Moringa Oleifera* L.) with carbon clearance method in male white mice, it can be concluded that *Moringa* leaf extract (*Moringa Oleifera* L.) has effect as an Immunomodulator.

AUTHORS CONTRIBUTION

All authors worked to do this manuscript, Biomechy Oktomalio P, Mutia Fakhri and Noverial were collected data, Dwisari Dillasamola wrote a final manuscript, and Yufri Aldi was edited out of the manuscript. Skunda Diliarosta wrote the first draft and collected data. All authors read and approved the final manuscript.

CONFLICT OF INTERESTS

All the authors have no conflict of interest.

REFERENCES

1. Subowo. Clinical Immunology. Bandung: Angkasa Publishers; 1993.
2. Baratawidjaja KG. Basic Immunology. Jakarta: Faculty of Medicine, University of Indonesia; 2009.
3. Almahdy A. Experimental Teratology. Padang: University of Andalas Press; 2012.
4. Aldi Y, Ogiana N, Handayani D. Immunomodulant test some subtraction ethyl acetate extract meniran (*Phyllanthus niruri* L.) on white mice with carbon clearance method. Proceedings of the National Seminar on Recent Developments of Pharmaceutical Science and Clinic III; 2013. p. 134-47.
5. Yusuf D. Immunomodulatory effect test of ethanol extract of mangosteen skin (*Garcinia mangostana* L.) and α-mangostin by carbon clearance method and total cell total leukocyte count [scripts]. Padang: University of Andalas; 2016.
6. Hamidin AS. Complete Natural Immunization Book for Children. Jogjakarta: Publisher Saufa; 2014.
7. Aldi Y, Goddess ON, Uthia R. Immunomodulatory test and, number of leukocyte cells from basil leaf extract (*Ocimum basilicum* L.) in male white mice. Sci J 2016;6:2087-5045.
8. Subowo. Immunology. Jakarta: CV Sagung Seto; 2009.
9. Mardiana L. Leaf Miraculously Cuts Disease. Jakarta: Self-Helpers; 2013.
10. Kresno SB. Immunology and Laboratory procedures. Jakarta: University of Indonesia; 2001.
11. Zabriskie JB. Essential Clinical Immunology. New York: Cambridge University Press; 2009.
12. Aldi Y, Yahdian R, Handayani D. Immunomodulatory activity of meniran ethanol extract (*Phyllanthus niruri* Linn.) against broiler chicken. J Pharm Clin Sci 2014;1:20-6.
13. Hansen PJ. Laboratory Procedures Use of Hemacytometer. Florida: University of Florida; 2000.
14. Aldi Y, Purnamasari D, Dillasamola D, Friardi I. Test immunomodulatory effects of ethanol extract skin of purple sweet potato (*Ipomoea batatas* (L.) lam) with carbon clearance method and the number of leukocytes. Res J Pharm Biol Chem Sci 2016;7:975-8585.
15. Priyanka S, Mukund H. Evaluation of immunomodulatory activity of extract from rind of *Nephelium lappaceum* fruit. Int J Pharm Pharm Sci 2017;9:38-43.
16. Venkatasthya S, Nagaraja S, Lim LC, Nahlah EI. Phytochemical analysis and immuno-modulatory effect of *Moringa oleifera* flowers. Int J Pharm Pharm Sci 2017;9:24-8.

Asean Journal of Pharmaceutical and Clinical Research

ORIGINALITY REPORT

3%	0%	0%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Surabaya University Student Paper	3%

Exclude quotes	On	Exclude matches	< 3%
Exclude bibliography	On		