Immunomodulatory Effect of *Momordica charantia* L. Fruit Ethanol Extract on Phagocytic Activity and Capacity of Mice Peritoneal Macrophages

Parawansah¹,*, Tomy Nurtamin², Sufiah Asri Mulyawati³, Nuralifah⁴, Wa Ode Arlina Misnaeni⁴

¹Department of Pharmacology, Faculty of Medicine, Universitas Halu Oleo, Kampus Hijau Bumi Tridarma Anduonohu, Kendari 93232, Indonesia
²Department of Physiology, Faculty of Medicine, Universitas Halu Oleo, Kampus Hijau Bumi Tridarma Anduonohu, Kendari 93232, Indonesia
³Department of Microbiology, Faculty of Medicine, Universitas Halu Oleo, Kampus Hijau Bumi Tridarma Anduonohu, Kendari 93232, Indonesia
⁴Department of Pharmacology, Faculty of Pharmacy, Universitas Halu Oleo, Kampus Hijau Bumi Tridarma Anduonohu, Kendari 93232, Indonesia

*Corresponding author. E-mail: parawansah_biom@yahoo.co.id

Received date: Oct 21, 2017; Revised date: Mar 19, 2018; Accepted date: Apr 9, 2018

Abstract

BACKGROUND: The purpose of this research is to understand the secondary metabolites of *Momordica charantia* L. extract, as well as to disclose the potential of *M. charantia* extract in the phagocytic activity and capacity of peritoneum macrophages.

METHODS: Examination of immunomodulatory effect was done by giving *M. charantia* ethanol extract on 5 treatment groups, given intra-peritoneally to mice daily. *Echinacea* extract as positive control and double distilled water as negative control were also given. On the 8th day, mice were infected with *Staphylococcus epidermidis*. After 30 minutes, peritoneum fluid was obtained to observe the activity and capacity of macrophage cells.

RESULTS: The results showed significant phagocytic activity ($p<0.05$) at a concentration of 1,200 ppm compare to the other groups. Meanwhile the macrophage cell capacity was found statistically insignificant ($p>0.05$). The highest phagocytic activity was the group treated with 1,200 ppm (62%), significantly higher than other groups.

CONCLUSION: The secondary metabolite content of *M. charantia* is alkaloids, flavonoids, tannins, saponins, and triterpenoids. The 1,200 ppm *M. charantia* ethanol extract is potential in inducing phagocytic activity and capacity. These results indicate that the *M. charantia* can be suggested as a natural immunomodulator.

KEYWORDS: pare fruit, *Momordica charantia* L., phagocytosis, macrophage, immunomodulator

Indones Biomed J. 2018; 10(2): 144-7

Introduction

The body's natural immune system is normally able to overcome infection when it is not being suppressed or disordered. The immune system is helpful in supporting body homeostasis. If the immune system is disturbed, then the body has to be treated with materials that can modulate the immune system, namely immunomodulator. Immunomodulators are substances or compounds, which stimulate, suppress or modulate components of the immune system of the body.(1) The immunomodulator works after the incoming antigen in the nonspecific immune system. In the nonspecific immune system, there are phagocyte (macrophages) that play a major role in the treatment of phagocytosis.(2) Immunomodulators have a function of maintaining the immune system, so that the body can withstand the attacks of harmful microorganisms such as bacteria, viruses, fungi and protozoa.(3) Usually, immunomodulators are used clinically in patients with...
impaired immunity to cope with cases of malignancy, HIV/ AIDS, malnutrition and allergies.(4)

A potential plant as immunomodulator, *Momordica charantia* L. or in Indonesian known as Pare, is not well developed. Eventhough, *M. charantia* has lots of useful chemical contents such as glycoside, saponin, alkaloid, fat oil, triterpenoid, protein and steroid.(5) Parse of charantia and muricata varieties are empirically used as diabetes drugs, rabies, antivenom, anthelmintic, jaundice, hemorrhoids, leprosy, rheumatism, and malaria.(6)

Based on previous research, *M. charantia* is efficacious as antidiabetes, antitumor, antiulcerogenic, antimutagenic, antioxidant, immunomodulator and uric acid.(7) It has been reported that protein content (α- and β-momorcharin) in *M. charantia* has an inhibitory effect on the HIV virus.(5)

Due to the potency of *M. charantia* as immunomodulator, research on immunomodulatory effects of *M. charantia* by measuring phagocytic activity and capacity of mice peritoneal macrophage was conducted.

**Methods**

This study was approved by Health Research Ethics Commission Universitas Halu Oleo, No. 2275/UN29.20/PPM/2017.

**Extraction**

*M. charantia* fruit powder was macerated with 96% ethanol every 24 hours for 3 days at room temperature. The filtrate was collected and concentrated with a rotary vacuum evaporator at a temperature of 55°C to obtain a viscous extract.

**Phytochemical Screening**

Phytochemical screening was performed using thin layer chromatography (TLC) method with Merck KGaA Analytical Chromatography silica gel plate GF254 (Cat Number #1.05554.0001) (EMD Millipore Corporation, Darmstadt, Germany).(8) The ethanol extract of *M. charantia* was spotted at a distance of 0.7 cm from the bottom edge of the plate with the capillary tube. The plate was then dried and eluted with chloroform: methanol (9:1). For detection of alkaloids, the Dragendorff reagent was used. For detection of flavonoids, ammonia was used. For detection of tanin, 1% FeCl₃ was used. For detection of saponin, 0.1 M H₂SO₄ was used. For detection of triterpenoids, Lieberman-Buchard reagent was used.

**Preparation of Bacterial Suspension**

Nutrient Broth (NB) was prepared, then 1 inoculating loop of *Staphylococcus epidermidis* was added and homogenized. Bacteria turbidity was measured according to Mc Farland standard No. 0.5 using a UV-Vis 20 D spectrophotometer at λ 625 nm. Mc Farland's standard solution was prepared with a composition of 0.05 mL of 1% BaCl₂ and 9.95 mL of 1% H₂SO₄ while the standard solution was equivalent to a bacterial density of 150x10⁶ CFU/mL. Subsequently suspension was incubated for 24 hours at 37°C.

**Mice Peritoneal Macrophage Acquisition**

The test animals used in this study were male balb/c mice aged 8-10 weeks weigh 20-30 grams. Mice were adapted for 7 days in the animal laboratory.(9) The mice were randomly divided into 7 groups following the Federer formula rule: 1 positive control, 1 negative control and 5 treatment groups. Each group consist of 5 mice, so the total number of mice was 35. The treatment groups received daily intake of extract suspension intra-peritoneally. For positive control group (C1), mice were treated with *Echinacea* extract.(10) For negative control group (C2), mice were treated with double distilled water. For treatment groups, mice were treated with the extract at 400 part per million (ppm) (T1), 600 ppm (T2), 800 ppm (T3), 1,000 ppm (T4), and 1,200 ppm (T5). On the 8th day, each mouse was infected with 0.5 mL *S. epidermidis* suspension intra-peritoneally. After 30 minutes, the mice was injected with 0.5 mL sterile phosphate buffered saline (PBS), pH = 7.8, then anesthetized with ether. Mice were placed on surgical board, then macrophage cell peritoneal fluid was obtained with 1 mL syringe.

**Immunomodulatory Effect Test**

The obtained peritoneal fluid was dripped on the glass for smear. After it fixing, the dried peritoneal fluid was incubated in methanol for five minutes. Then Giemsa dye was applied on the fluid for 20 minutes.(11) The stained fluid was rinsed with distilled water and dried. Observation was made under a light microscope with 10x100 magnification.(12) The number of macrophages was equalized with the hemocytometer, to a macrophage population of 10⁷ macrophage/mL.

**Results**

**Phytochemical Screening**

The secondary metabolites contained in the ethanol *M. charantia* extract are alkaloids, flavanoids, tanins, saponins and triterpenoids.
**Testing of Immunomodulatory Effects**

The *M. charantia* extract-induced phagocytic activity and capacity of peritoneum macrophage derived from male mice against the *S. epidemidis* are listed in Table 1.

**Tabel 1. Phagocytic activity and capacity of peritoneum macrophages.**

| No. | Groups | Phagocytosis Activity (%) | Phagocytosis Capacity |
|-----|--------|---------------------------|-----------------------|
| 1   | C1     | 60.25 ± 1.25831           | 183.00 ± 3.46410      |
| 2   | C2     | 44.50 ± 4.43471           | 179.25 ± 6.70199      |
| 3   | T1     | 54.00 ± 2.44949           | 174.50 ± 6.24500      |
| 4   | T2     | 54.75 ± 4.64579           | 174.25 ± 6.44851      |
| 5   | T3     | 54.00 ± 1.82574           | 172.75 ± 7.80491      |
| 6   | T4     | 56.50 ± 5.44671           | 173.00 ± 4.24264      |
| 7   | T5     | 62.00 ± 6.73300           | 176.25 ± 5.56028      |

**Discussion**

Phagocytic activity is the percentage of macrophage that actively perform phagocytic process among 100 macrophages. Macrophages are said to be active if they have large size and rough cytoplasm. Meanwhile, macrophages are said to be inactive if they have a small size, almost round, and the cytoplasm is not rough. The percent value of cell activity is obtained by calculating using the formula:

\[
\text{% activity} = \frac{\text{number of active macrophage}}{\text{total number of macrophage}} \times 100\%
\]

Meanwhile, phagocytic capacity is the number of bacteria that is phagocytosed divided by the number of active macrophage. The capacity value can be obtained by calculating the formula below:

\[
\text{capacity} = \frac{\text{the number of phagocytosed bacteria}}{\text{number of active macrophage}}
\]

Current study showed the averages of phagocytic activity were increased along with the increase of extract concentration, suggesting that the *M. charantia* extract increased phagocytic activity in the concentration manner. The highest phagocytic activity was the group treated with 1,200 ppm, significantly higher than other groups (*p*<0.05).

*M. charantia* is an effective immunomodulator due to the contained secondary metabolites, such as alkaloids, flavonoids, tannins, saponins, and triterpenoids. The mechanisms of alkaloids and flavonoids in *M. charantia* are similar with the mechanisms of alkaloids and flavonoids in other plants. The flavonoid compounds have the ability to repair the immune system, meanwhile alkaloid compounds have the immunomodulatory ability. Previous research has also shown that flavonoid compounds can stimulate lymphocyte proliferation, increase T-cell count and increase interleukin-2 activity. Flavonoids also work against lymphokines produced by T cells that will stimulate phagocytes to respond to phagocytosis, one of which is macrophages.(13) The tannin compounds can also affect the activity of human physiology such as stimulating phagocyte, antitumor, and antibacterial cells.(14) Flavonoid compounds besides having immunostimulatory effects also have immunosuppressant effects. The presence of this immunosuppressant effect increase the phagocytic capacity in the treatment group.

**Conclusion**

The secondary metabolite content of *M. charantia* is alkaloids, flavonoids, tannins, saponins, and triterpenoids. The 1,200 ppm *M. charantia* ethanol extract is potential in inducing phagocytic activity and capacity. These results indicate that the *M. charantia* can be suggested as a natural immunomodulator.

**Acknowledgment**

Thanks to the Directorate of Research and Community Service, Indonesian Ministry of Research, Technology and Higher Education, which has facilitated the implementation of the research activities.

**References**

1. Revilla G, Yanwirasti, Indrama E. Imunomodulasi senyawa flavonoid kencur (Kaempferia galanga Linn.) terhadap kemampuan mikrobisidal sel netrofil secara in vitro. Majalah Kedokteran Andalas. 2008; 32: 29-36.
2. Wagner H, Jurcic K. Assay for Immunomodulation and Effects on Mediators of Inflammation Vol. 6. London: Academic Press; 1991.
3. Ghaisas MM, Shaikh SA, Deshpande AD. Evaluation of the immunomodulatory activity of ethanolic extract of the stem bark of Bauhinia variegata Linn. Int J Green Pharm. 2009; 3: 70-4.

4. Chen X, Hu ZP, Yang XX, Huang M, Gao Y, Tang W, et al. Monitoring of immune responses to a herbal immunomodulator in patients with advanced colorectal cancer. Int Immunopharmacol. 2006; 6: 499-508.

5. Anilakumar KR, Garlapati PK, Nallamuthu I. Nutritional, pharmacological and medicinal properties of Momordica charantia. Int J Food Sci Nutr. 2015; 4: 75-83.

6. Paul A, Sarmistha SR. Medicinal uses and molecular identification of two Momordica charantia varieties – a review. eJBio. 2010; 6: 43-51.

7. Parawansah, Nuralifah, Alam G, Natzir R. Inhibition of xanthine oxidase activity by ethanolic extract of Piperomia pellucid L., Acalypha indica L., and Momordica charantia L. Indones Biomed J. 2016; 8: 161-6.

8. Harborne JB. Metode Fitokimia. Bandung: ITB Press; 1987.

9. Susanti R, Yuniastuti A, Iswari RS. Aktivitas reactive oxygen species makrofag akibat stimulasi gel lidah buaya pada infeksi Salmonella typhimurium. Jurnal MIPA. 2012; 35: 1-10.

10. Stevenson LM, Matthias A, Banbury L, Penman KG, Bone KM, Leach DL, et al. Modulation of macrophage immune responses by Echinacea. Molecules. 2005; 10: 1279-85.

11. Kusmardi, Kumala S, Wulandari D. Pengaruh pemberian ekstrak etanol daun johar (Cassia siamea Lamk.) terhadap peningkatan aktivitas dan kapasitas fagositosis sel makrofag. Makara Kesehatan. 2006; 10: 89-93.

12. Chairul. Phagocytosis effectivity test of phenylbutenoid compounds isolated from bangle (Zingiber cassumunum Roxb.) rhizome. Biodiversitas. 2009; 10: 40-3.

13. Nugroho YN. Efek pemberian kombinasi buah sirih (Piper Betle L.) fruit, daun miyana (Plectranthus scutellarioides (L.) Br.) leaf, madu dan kuning telur terhadap peningkatan aktivitas dan kapasitas fagositosis sel makrofag. Media Litbang Kesehatan. 2012; 22: 1-5.

14. Haslam E. Natural polyphenols (vegetable tanins) as drugs: possible modes of action. J Nat Prod. 1996; 59: 205-15.