RESEARCH ARTICLE

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

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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

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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% $FeCl_3$ was used. For detection of saponin, 0.1 M H_2SO_4 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% $BaCl_2$ and 9.95 mL of 1% H_2SO_4 while the standard solution was equivalent to a bacterial density of $150x10^6$ 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 8^{th} 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 10^7 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.

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