Specific Immunomodulatory Effect of Water Extract of *Stachytarpheta jamaicensis* herbs

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Abstract. Immunomodulators can alter immune system and work specifically and non-specific. Traditionally *Stachytarpheta jamaicensis* herbs is used to modulate immune system. This study aims to determine the humoral and cellular immune system activity of water extract of *S. jamaicensis* herb through hemagglutination antibody titer test and delayed type hypersensitivity tests in mice. The extract was made by boiling in distilled water. The doses of the extract were 50, 100 and 200 mg/kg bw and zymosan 10 mg/kg bw ip were used as a comparison. The parameter measured in the hemagglutination antibody titer test was the hemagglutination antibody titer value and lymphocyte count, while the parameters in the delayed type hypersensitivity test are the thickness of the hind footpad, percent inhibition of delayed type hypersensitivity response and lymphocyte count. Test results showed extracts at dose of 50 mg/kg bw gave hemagglutination antibody titer value lower than control group, but the extract at doses of 100 and 200 mg/kg bw produced antibody titer values higher than control group (p<0.05). The extracts at all doses could inhibit the increasing of hind footpad thickness compared to control group (p <0.05). The result also showed that there was increasing lymphocyte count compared to control group. It can be concluded that water extracts can affect humoral and cellular immune systems.

1. Introduction

The specific immune system has the ability to recognize specific antigens easily. Same as nonspecific immune system, the specific immune system is divided into two types, cellular and humoral. In specific cellular immune systems, T cells will play a role in activating B cells, monocytes into macrophages and delayed type hypersensitivity responses.(1,2)

In Indonesia, *Stachytarpheta jamaicensis* L. is used to treat several diseases such as infections, urinary tract, sore throat, cough, rheumatism, irregular menstruation, and hepatitis A.(3) Various pharmacological effect of *S. jamaicensis* has been evaluated. It can be used as antimicrobial, antifungal, antioxidant, anti-inflammatory, antinociceptive, antidiarrhea, antihypertensive, ant dyslipidemia, hepatoprotective, wound healing, and anthelmintic.(4) Based on previous research using carbon clearance method, water extract of *S. jamaicensis* at doses of 62.5 mg/kg BW had phagocytosis index lower than zymosan but higher than methylprednisolone, so it has potency as immunosuppressant.(3) Therefore, this research was conducted to evaluate the specific immunomodulatory effect of water extract of *S. jamaicensis* herb using hemagglutination antibody titer and delayed type hypersensitivity tests.
2. Material and Methods

This study was a preclinical experimental and conducted accordance to approval of the institutional ethics committee No. 6002.4/KEP-UNJANI/I/2019.

2.1. Sample collection and determination
S. jamaicensis herb were collected from Purworejo, Middle of Java, Indonesia and its determination was done in Herbarium Bandungnese, School of Life Sciences and Technology, Bandung Institute of Technology.

2.2. Processing, characterization and extraction
The herbs were washed with clean running water and air-dried. Dried herbs were mashed with a grinding machine into powder and stored in dry container. The dried herbs then characterized according to WHO guideline of Quality control methods for herbal materials. The examined characterizations were determination of water content, ash and extractable matter. Extract was made by boiled water. A total of 100 g of dry powder herbs was added with 1200 mL of distilled water and heated to 95°C for 30 minutes, then filtered. Filtrate and residue were separated and the filtrate was concentrated and dried at 40°C (yield ±9.8% w/w).

2.3. Phytochemical screening
Phytochemical screening of secondary metabolites from dried herbs and extract (such as alkaloids, flavonoids, saponins, terpenoids and steroids) was carried out according to the WHO guideline.

2.4. Specific immunomodulatory assay
In this study, specific immunomodulatory assay was done using hemagglutination antibody titer test and delayed type hypersensitivity tests.

2.4.1. Experimental animals.
Male Swiss Webster mice (20-25 g) were used as the experimental animals were obtained from Bioscience and Biotechnology Research Center, Institut Teknologi Bandung.

2.4.2. Preparation of 1% Sheep Red Blood Cell (1% SRBC).
Sheep blood was collected freshly and centrifuged at 3000 rpm to obtain red blood cell sediment. Phosphate buffer saline (PBS) pH 7.2 was added and then the suspension was homogenized and centrifuged at 1500 rpm. Washing process was carried out until the top layer was clear, then the top layer was discarded. The lower layer of SRBC suspension was added with PBS solution with the same volume to obtain 50% SDMD suspension. To obtain 1%SRBC, 0.2 ml 50% SRBC was added with PBS up to 10 ml.

2.4.3. Preparation of test solution.
The extract was prepared at doses of 50, 100 and 200 mg/kg bw. Zymosan at dose of 10 mg/kg bw was prepared and used as comparator.

2.4.4. Hemagglutination antibody (HA) titre test.
The animals were divided into 5 groups (each 4 mice). At day 0, each animal was induced with 0.1 mL 1% SRBC intraperitoneal. Test solution was given for seven days. On last day, lymphocyte was measured and blood samples for hemagglutination was collected from retro orbital puncture. Blood samples was centrifuged at 3000 rpm and antibody level was carried out by determined by the haemagglutination technique. In microtitration plate, 25 μl serum were dripped, then PBS and 1% SRBC were added with the same volume and diluted into series 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1: 256, 1/512, 1/1024, 1/2048. The value of antibody titre was determined based on the last dilution where the antibody was still detected through visually visible hemagglutination. This value is then transformed into equation [2 log (titer) + 1].(7–10)
2.4.5. *Delayed type hypersensitivity test.* The animals were divided into 5 groups (each 4 mice). At day 0, each animal was induced with 0.1 mL 1% SRBC in the right hind footpad. Test solution was given for seven days. On last day, lymphocyte was measured. Then the animals were induced again with 0.1 mL 1%SRBC. The thickness of the footpad was measured 24 h after the challenge.(8,10)

2.5. *Analytical data*

The data collected were analyzed using SPSS with t-test in the 95% confidence range.

3. Results and Discussion

Characterization of simplicia are very important to ensure the quality and indicate that the it has met the applicable requirements. The result of characterization of the simplicia can be seen in Table 1. The result of phytochemical screening of secondary metabolites from dried herbs and extract can be seen in Table 2.

| Parameter             | Result (%)  |
|-----------------------|-------------|
| Water content         | 4.467±0.307 |
| Total ash             | 8.995±0.463 |
| Water soluble ash     | 5.103±0.050 |
| Acid insoluble ash    | 2.779±0.685 |
| Water extractable     | 13.346±0.485|
| Ethanolic extractable | 15.493±0.975|

Table 1. Result of the characterization of the simplicia

| Compound            | Result         | simplicia | extract |
|---------------------|----------------|-----------|---------|
| Flavonoid           | +              | +         |         |
| Polyphenol          | +              | +         |         |
| Tannins             | +              | +         |         |
| Quinones            | -              | -         |         |
| Alkaloid            | -              | -         |         |
| Saponin             | +              | +         |         |
| Steroid and Terpenoid| +             | +         |         |

+: Positive contains test compounds; -: Negatives contain test compounds

Specific immunomodulatory effect of water extract of *S. jamaicensis* was conducted with HA titre and delayed hypersensitivity test. In both tests, the induction was done using 1% SRBC as an antigen. The SRBC were suspended in a buffer solution with a pH 7 (PBS) to keep the negatively charged, so it can bind to antibodies. In HA titre test, there will be formed a bond because of the cross-link between red blood cells and antibodies (especially IgM), causing a visible deposit or clot. The result of HA titre can be seen in Table 3 and lymphocyte count can be seen in Table 4.

| Group              | HA titre     |
|--------------------|--------------|
| Control            | 2.75±0.57    |
| Zymosan            | 3.20±0.98    |
| Extract at dose of 50 mg/kg bw | 2.61±0.68   |
| Extract at dose of 100 mg/kg bw | 2.92±1.04   |
| Extract at dose of 200 mg/kg bw | 3.66±0.91   |

*n=4, *p<0.05 compared to control group using t-test*

HA titre of the extract at dose of 50 mg/kg bw was lower than other group, but HA titre of the extract at doses of 100 and 200 mg/kg bw were higher than control group. This result is in line with the lymphocyte count. The increase in primary antibody titer indicates stimulation of B lymphocytes,
T lymphocytes and macrophages. The increase in secondary antibody titer indicates stimulation of memory B cells in the process of antibody formation.(10)

Table 4. Effect of water extract of *Stachytarpheta jamaicensis* on lymphocyte count on hemagglutination antibody titre test

| Group                          | Lymphocyte (10^9/L) |
|--------------------------------|---------------------|
| Control                        | 9.00±2.86           |
| Zymosan                        | 10.93±2.61          |
| Extract at dose of 50 mg/kg bw | 12.75±3.39          |
| Extract at dose of 100 mg/kg bw| 10.62±2.41          |
| Extract at dose of 200 mg/kg bw| 12.6±2.92*          |

n=4, *p<0.05 compared to control group using t-test

The delayed-type hypersensitivity (DTH) test determines the activity of the cellular immune response, especially T lymphocytes and macrophages. The DTH response requires special recognition of the antigen administered to T lymphocytes which then proliferate and release cytokines. The release of cytokines from activated T lymphocytes will increase macrophage activity and enzyme concentrations to accelerate the elimination process. SRBC antigen induces response in which Th1 cells secrete a number of cytokines that activate macrophages and other nonspecific inflammatory mediators. The delay in response time reflects the time required for cytokines to induce macrophage activation.(10,11) The result of DTH responses can be seen in Table 5 and lymphocyte count can be seen in Table 6.

Table 5. Effect of water extract of *Stachytarpheta jamaicensis* on delayed type hypersensitivity responses after 24 hour

| Group                          | Paw thickness (%) | Inhibition of DTH response (%) |
|--------------------------------|-------------------|-------------------------------|
| Control                        | 153.33±10.28      | -                             |
| Zymosan                        | 33.33±23.57*      | 78.26                         |
| Extract at dose of 50 mg/kg bw | 41.67±41.94*      | 72.83                         |
| Extract at dose of 100 mg/kg bw| 90.00±70.71*      | 41.30                         |
| Extract at dose of 200 mg/kg bw| 87.50±25.00*      | 42.93                         |

n=4, *p<0.05 compared to control group using t-test

Table 6. Effect of water extract of *Stachytarpheta jamaicensis* on lymphocyte count on hemagglutination antibody titre test

| Group                          | Lymphocyte (10^9/L) |
|--------------------------------|---------------------|
| Control                        | 7.28±1.50           |
| Zymosan                        | 8.45±1.20           |
| Extract at dose of 50 mg/kg bw | 8.66±0.73           |
| Extract at dose of 100 mg/kg bw| 10.80±3.07          |
| Extract at dose of 200 mg/kg bw| 8.01±1.10           |

n=4, *p<0.05 compared to control group using t-test

The data showed that the extract at doses of 50 mg/kg bw gave inhibition DTH response higher than other doses. It implied that the extract affects immune response mediated by T lymphocyte. These results are also in line with the effect of *Stachytarpheta cayennensis* on DTH response. The result also showed that the extract supressed DTH response.(12) It probably related with the effect as anti-inflammatory, especially DTH response is common in chronic inflammatory condition such as rheumatoid arthritis, but further study about the effect in chronic inflammatory is needed.
4. Conclusion
Water extract of *S. jamaicensis* L. can increase the value of antibody titre and inhibit the thickness of the hind footpad in delayed type hypersensitivity test. It related with the lymphocyte count. It can be concluded that water extracts can affect humoral and cellular immune systems.

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References
[1] Chaplin D. Overview of the immune response. J Allergy Clin Immunol. 2010;125(2):S3-23.
[2] Nicholson LB. The immune system. Essays Biochem. 2016;60(3):275–301.
[3] Vikasari SN, Soemardji AA, Sutjiatmo AB, Suryani. Immunomodulatory effect of water extract of *Stachytarpheta jamaicensis* (L.) Vahl. J Appl Pharm Sci. 2015;5(2):062–6.
[4] Liew PM, Yong YK. Stachytarpheta jamaicensis (L.) Vahl: From Traditional Usage to Pharmacological Evidence. Evidence-based Complement Altern Med. 2016;2016.
[5] World Health Organization. Quality control methods for herbal materials. [Internet]. World Health Organization; 2011. Available from: https://apps.who.int/iris/handle/10665/44479
[6] Bekierkunst A, Yarkoni E, Flechner I, Morecki S, Vilkas E, Lederer E. Immune response to sheep red blood cells in mice pretreated with mycobacterial fractions. Infect Immun. 1971;4(3):256–63.
[7] Shukla S, Mehta A. Frontiers in Life Science immunomodulatory activity of various extracts of Stevia rebaudiana leaves in experimental animal model. 2015;3769. Available from: https://doi.org/10.1080/21553769.2014.961615
[8] Gabhe SY, Tatke PA, Khan TA. Evaluation of the immunomodulatory activity of the methanol methanol extract of Ficus benghalensis roots in rats rats. 2006;38(4):271–5.
[9] Zootecnia F De. Proposed method for agglutinating antibody titer analysis and its use as indicator of acquired immunity in pacu, Piaractus mesopotamicus. 2014;74(1):238–42.
[10] Faradilla M, Iwo M. Efck Imunomodulator Polisakarida Rimpang Temu Putih Immunomodulatory Effect of Polysaccharide from White Turmeric \ Curcuma zedoaria \ ( Christm. ) Roscoe \] Rhizome. 2014;12(2):273–8.
[11] Jacysyn JF, Abrahamsohn IA, Macedo MS. Modulation of delayed-type hypersensitivity during the time course of immune response to a protein antigen. Immunology. 2001;102(3):373–9.
[12] Okoye TC, Akah PA, Ezike AC, Uzor PF, Odoh UE, Igboeme SO, et al. Immunomodulatory effects of *Stachytarpheta cayennensis* leaf extract and its synergistic effect with artesunate. BMC Complement Altern Med. 2014;14(1):1–8.