CENTELLA ASIATICA EXTRACT INCREASED ON THE LEVEL OF INTERLEUKIN 6 (IL-6) IN MICE

I Nengah Kerta Besung¹, Nyoman Mantik Astawa², I Ketut Suatha³, Hartaningsih⁴
¹Postgraduate Program, Udayana University
²Faculty of Veterinary Medicine, Udayana University
³Faculty of Medicine, Udayana University
⁴Disease Investigation Centre Denpasar
E-mail: kertabesung@fkh.unud.ac.id

ABSTRACT

Salmonellosis is still problem in many developing countries including Indonesia. The main problem in controlling and handling the disease is that only few antibiotics are available to cure the disease. In addition, the prolonged use of such antibiotics often lead to bacterial resistant against the antibiotics. A herbal drugs such as Centella asiatica (in Indonesia is known as pegagan) contains triterphenoid saphonins which acts as immunostimulant capable of enhancing the phagocytic activity of macrophages. However, no study has been conducted to investigate the use pegagan in activating macrophage of mice infected with Salmonella typhi. A study was therefore conducted to find out the ability of Centella asiatica in enhancing on the level interleukine (IL)-6 following challenge with Salmonella typhi. It is therefore expected that herbal drug such as Centella asiatica can be used as an alternative medicine to prevent and cure salmonellosis in both animals and human.

Experimental laboratory studies were conducted using Completely Factorial Randomized Design. Mice were divided into 4 groups and they were treated respectively with destilated water (negative control), 125, 250, and 500 mg/kg bw of Centella asiatica extract. The treatment was conducted daily for 2 weeks and the mice were then inoculated with $10^5$ cells of S. typhi. The level of IL-6 response were examined 24 hours, 2 weeks, and 4 weeks after inoculation with S. typhi. The result showed that treatment of mice with Centella asiatica extract significantly (p<0.05) enhanced IL-6 level of Balb/c mice following inoculation with S. typhi. The highest IL-6 level were observed in mice treated Centella asiatica extract at the dose of 500 mg/kg BW (385.9257±125.4314 pg/ml serum). And the highest IL-6 level were observed at 2 weeks following inoculation with S. typhi (533.4262 ± 81.7184 pg / ml).

A further study is recomended to examine the cellular immune response and more detailed study on the humoral immune response of animals or human before this herbal is used as alternatif medicine to prevent and cure typhoid fever. It is also important to study the best preparation, the half life, and the side effect of Centella asiatica in human and animals.

Key words : pegagan, Centella asiatica, IL-6, antibody, S. typhi
INTRODUCTION

Salmonellosis still remains a public health problem in developing countries, in Indonesia for example. The disease is often known as typhoid fever or typhus disease. The incidence rate of the disease increases in the long dry season and the beginning of the rainy season. Incidence of the disease in children is mainly occurred at the age of 5 years or older with mild clinical manifestations. The younger the child, the more unspecific the clinical signs of the disease. In addition, the mortality rate of the disease in child is lower than the adult (Supali, 2002).

There are many constrains in order to prevent and control of salmonelosis. Incorrect antibiotic treatment on salmonellosis can cause resistance. In addition, the cost for maintenance of the infection is relatively expensive, and the restoration from the infection needs quite long time. Therefore, it needs to find alternatives for prevention of salmonellosis in the future that may tackle these constrains in more easier, cheaper and more effective ways. An alternative that can be done is to increase the body defence so that, in the same way, it can prevent the occurrence of infection. The body defence can be increased through activated fagositic cell, such as: macrophage and neutrophil. Both of these cells play an important role to eliminate infectious agents that entered the body (Tizard, 2000).

Activated macrophages also have capability to produce interleukin. This interleukin is a substance that helps interacting between the cells. There are several kinds of interleukin (IL) that are released by macrophages, such as: IL-1, IL-4, IL-6, and TNF. Basically, these interleukins have important roles in inflammation reaction and immunity management system. Specifically, interleukin can increase or even halt the cell’ growth and also increase the activity of cell chemo-taxis.

Centella asiatica (C. asiatica), or more commonly called pegagan, has been famous for traditional medicine for years. Pegagan is also used for wound healing and improving memory span. It can also increase hyperplasia cell activity and the existence of collagen in wound tissue (Sagrawat and Khan, 2007). Jayathirta and Mishra (2004) believed that C asiatica extract from 100 to 500 mg/kg bw in mice could significantly increase the total of white blood cells and macrophage phagocyte ability against carbon molecules in those mice. Furthermore, there was a significant linear relation between C. asiatica doses and the total of white blood cells, as well as the macrophage ability to phagocyte carbon molecules. While, Rao, et all (2006) found that giving pegagan to the mice could significantly affect the length of dendrite hippocampus cells. The scientific benefits of C. asiatica in humans and animals
have been evoked these days. However, research on *C. asiatica* ability to induce IL-6 related to infection had not been reported.

**MATERIALS AND METHODS**

A total of 48 mice, with at least 8 weeks of age, was weighted and adapted to the environment for 2 weeks. The mice (*Mus musculus* Balb/c) were randomly divided into 4 groups, and each group consisted of 12 mice. Group I was a control group that only given 1 ml / day sterile aquades; while, group II, group III and group IV were given 125 mg / kg bw / ml, 250 mg/kg bw / ml, and 500 mg / kg bw / ml of extract *pegagan* respectively. Those treatments were given daily for 14 days.

As many as $10^5$ lethal dose 50 (LD 50) of *S. typhi* in 1 ml Phosphate Buffer saline was infected intraperitoneal to each of the mice on day 15. The blood from as many as 4 mice from each group was also collected to examine the IL-6 levels and antibody titers. Blood sampling was continued to examine 2 weeks and 4 weeks after *S. typhi* infection.

Levels of IL-6 in mice serum was performed by capture ELISA (BMS 603, Vienna, Austria). The result was then analyzed from the color intensity that generated by the ELISA DAX 800 (Automatic Diagnostic, USA) on a filter with a wavelength of 450 nm. Concentrations of IL-6 serum result were determined based on comparison of the optical density (OD) value from each serum samples with standard IL-6 OD values of IL-6 using logistic regression parameters.

**DATA ANALYSIS**

Levene’s Test at 5% significance level was used to evaluate data homogeneity. While, the group differences were analyzed by using 5% significance level in analysis of variants. Furthermore, the homogeneous data were analyzed by Least Significant Difference (LSD) at 5% significance level. All of data were analyzed by using version 15 of SPSS.

**RESULTS**

A total of 48 mice was used for sera collection in this study. The sera were tested for IL-6 on ELISA test. The result of IL-6 level can be seen in figure 1.
Figure 1. IL-6 Level from Each of Mice Group Treatments

The level of IL-6 fluctuated depending on the dose of *C. asiatica* given. The highest IL-6 level was observed in mice given by *C. asiatica* extract 2 weeks after *S. typhi* inoculation (533,426475± 81,7184 pg/ml) where the control group had the lowest level (82,1337±2,9464 pg/ml). In general, IL-6 level in all groups increased in week 2, but gradually decreased in week four. The analysis result between *C. asiatica* doses and interval observation period can be seen in Table 1.

Table 1. Variety of IL-6 Levels in Mice that Treated with *C. asiatica* Extract in Dose Variatio and Interval Observation Period.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of square</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correction model</td>
<td>839025.051</td>
<td>11</td>
<td>76275.005</td>
<td>92.025</td>
<td>0.000</td>
</tr>
<tr>
<td>Intersep</td>
<td>1584090.83</td>
<td>1</td>
<td>1584090.83</td>
<td>1911.18</td>
<td>0.000</td>
</tr>
<tr>
<td>Dose</td>
<td>685667.622</td>
<td>3</td>
<td>228555.874</td>
<td>275.749</td>
<td>0.000</td>
</tr>
<tr>
<td>Time</td>
<td>69667.422</td>
<td>2</td>
<td>34833.711</td>
<td>42.026</td>
<td>0.000</td>
</tr>
<tr>
<td>Group* Time</td>
<td>83690.008</td>
<td>6</td>
<td>13948.335</td>
<td>16.828</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>29838.740</td>
<td>36</td>
<td>828.854</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2452954.62</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correction</td>
<td>868863.791</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The table 1. shows there was a significant difference (p<0.01) between dose of *C. asiatica* extract and interval observation period. There was an interaction between dose of *C. asiatica* extract and interval observation period to the IL-6 level in the treated mice. The difference between them can be shown in Table 2.
Table 2. The Difference Doses in *C. asiatica* Extract to the IL-6 Level in Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Group</th>
<th>Mean difference</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>125 mg/kg bw</td>
<td>-2.17116</td>
<td>.854</td>
</tr>
<tr>
<td></td>
<td>250 mg/kg bw</td>
<td>-48.6191(*)</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>500 mg/kg bw</td>
<td>-289.2787(*)</td>
<td>.000</td>
</tr>
<tr>
<td>125 mg/kg bw</td>
<td>250 mg/kg bw</td>
<td>-46.4479(*)</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>500 mg/kg bw</td>
<td>-287.1075(*)</td>
<td>.000</td>
</tr>
<tr>
<td>250 mg/kg bw</td>
<td>500 mg/kg bw</td>
<td>-240.660(*)</td>
<td>.000</td>
</tr>
</tbody>
</table>

The highest IL-6 level was at dose 500 mg/kg bw of *C. asiatica* extract and it also had a significant difference (p<0.05) compared to dose 250 mg/kg bw or 125 mg/kg bw. The *C. asiatica* extract at dose 250 mg/kg bw had significant difference (p<0.05) compared to dose 125 mg/kg bw. Nevertheless, no significant difference (p>0.05) was observed between dose 125 mg/kg bw of *C. asiatica* extract and control group.

A significant difference (p<0.05) was also found in observation period of IL-6 level in mice that given *C. asiatica* extract. Details of the difference can be seen in Table 3.

Table 3. Interval Observation Period IL-6 in Mice that Given *C. asiatica* Extract

<table>
<thead>
<tr>
<th>Observation period</th>
<th>Observation period</th>
<th>Mean difference</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day-1</td>
<td>2nd week</td>
<td>-91.9657(*)</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>4th week</td>
<td>-32.269(*)</td>
<td>.003</td>
</tr>
<tr>
<td>Week-2</td>
<td>4th week</td>
<td>59.6963(*)</td>
<td>.000</td>
</tr>
</tbody>
</table>

Level of IL-6 in mice that given *C. asiatica* extract and observed in week 2 showed higher significant different (P<0.01) compared to the mice observed in day 1 or week 4. In 4th week, there was a higher significance (P<0.01) different compared to day 1

**DISCUSSION**

IL-6 levels in mice given the highest seen at doses of *pegagan* 500 mg / kg bw was observed in the second week (533.4262±81.7184 pg). Statistical analysis showed that
administration of *pegagan* can enhanced levels of IL-6 significant difference (p<0.05), with highest levels found in *pegagan* dosage of 500 mg/kg bw (385.9257±125.4315 pg), which is significant difference (p<0.05) than doses of 250 mg/kg bw (145.2661±24.4383 pg), 125 mg/kg bw (98.81813±9.5618 pg) or control (96.6470± 15.2474 pg). Levels of IL-6 at doses of 250 mg / kg was significant difference (p<0.05 ) compared with a dose of 125 mg / kg, but between the dose of 125 mg with controls showed no significant difference (p>0.05).

The same research results obtained by Hongzong (2009), medecassoside content on *pegagan* may increase levels of IL-6 in mice suffering from arthritis. In mice that are experiencing arthritis will experience increased levels of IL-6, then will be decreased along with wound healing. Kwon et al. (2008) found that ethanol extract of *C. asiatica* ultrasofication increased levels of TNFα and IL-6 is secreted by T cells Further found that with the addition of extract of *C. asiatica* causes activation of NK cells increased by 10%.

These results prove that *pegagan* can enhanced levels of IL-6 in the body. Increased levels of IL-6 is due to the stimulation caused by germs *C. asiatica* and *S. typhi*. *C. asiatica* stimulate macrophages to increase their activities, thus becoming more responsive to antigens that enter the body. Furthermore, the germ *S. typhi* also provide signals captured by the macrophage to migration and phagocytosis. Macrophages that perform phagocytosis will issue a cell mediators such as IL-1 and IL-6, which stimulates other macrophage cells to respond and approached the source of stimulation. Expenditures of IL-6 in this chain will increase the number of IL-6 in the circulation of the body.

Levels of IL-6 has begun to rise on the first day of bacterial infection. The highest levels found in week two, and finally declined in the fourth week. Production of IL-6 caused by the activation of macrophages and the presence of antigen stimulation. Activation of macrophages without the stimulation of infection will not result in increased levels of IL-6. In this study, antigen stimulation in the form of the infection of *S. typhi* given only once on the first day. Thus, IL-6 production will be increased beginning the first day until the second week and gradually began to decline.

**CONCLUSIONS**

* C. *asiatica* extract increased the levels of IL-6 in mice Balb/c infected with *S. typhi* significantly (p<0.05). The highest level were found at a dose of 500 mg / kg body weight two weeks after infection by *S. typhi* (533.4262 ± 81.7184 pg / ml).
REFERENCES


Baratawidjaja KG. 2006 Imunologi Vaskuler dalam Imunologi Dasar ed. 7. Jakarta. BP.FKUI. hal: 384-428


