



Effect of *Thymus vulgaris* Extract on Level of Serum,IL12 and Bacterial Colonies in Liver in Mice Infected by *Salmonella Typhimurium*

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Abstract : The *Thymus vulgaris* extracts are effective as antibacterial and immunomodulating agents. Thymol is the main monoterpene phenol, isomeric with carvacrol, found in the thyme extract. On the other hand, the intracellular infection of salmonella stimulates macrophage to produce interleukin IL12 which plays an essential role to stimulate naïve CD4 T cells to differentiate Th1 cells for the enhancement of IFN- γ secretion. IFN- γ plays an important role in the activation of macrophage, as it destroys the phagocytized bacteria. The aim of this study is to determine the effect of *Thymus vulgaris* extracts on serum IL-12 level and to analyze the ability of *Thymus vulgaris* extracts to decrease bacterial colonies in the liver of mice infected with *S. typhimurium*. This study used 20 male mice strain Balb/c. The mice were randomly divided into 5 groups which consisted of 4 mice each; the positive control (mice infected with *S.Typhimurium*), negative control (mice without infection), and treatment groups of D1, D2, D3 (mice administrated with extract of *Thymus vulgaris* 250, 500, 750 mg/kg BW and infected with *S. typhimurium*). *Thymus vulgaris* extract causes the increase of serum IL12 and decreases *S. typhimurium* colonies in the liver of mouse samples infected by *S.typhimurium* bacteria.

Keywords : *Thymus vulgaris*, IL-12,*S.Typhimurium*.

Introduction

Salmonella Typhi is a Gram-negative bacilli which causes typhoid fever in human. This bacterium may survive in phagosome to escape the immune system. Some complications of typhoid fever are perforation of ileum, bacteremia, and endovascular infection^{1, 2}. An infection of mice with *Salmonella typhimurium* (*S. typhimurium*) has consequences to systemic infection and a typhoid fever disease similar to that seen in humans after infected with *S. typhi*^{3,4}. *Salmonella* is a very successful enteric pathogen because it has developed strategies to cope with most of the immune defenses employed by the host during the different phases of the disease⁵. *S. typhimurium* results in systemic infection and a disease which shows similar mechanism as *S. typhi*⁶.

The genus *Thymus*, member of the *Lamiaceae* family, contains about 400 species of perennial aromatic, evergreen or semi-evergreen herbaceous plants with many subspecies, varieties, subvarieties and forms⁷. In folk

medicine, some *Thymus* spp. are used for their antihelminthic, expectorant, antiseptic, antispasmodic, antimicrobial, antifungal, antioxidative, antiviral, carminative, sedative, and diaphoretic effects. They are usually administered by infusion or are used externally in baths to cure rheumatic and skin diseases^{8,9}. *Thymus vulgaris* essential oil (TEO) is a mixture of monoterpenes. The main compounds of this oil are the natural terpenoid thymol and its phenol isomer carvacrol (CVL)^{10,11}. Several studies have been performed with plant extracts^{10, 12, 13}, which have antioxidative, antimicrobial, antitussive, expectorant, antispasmodic, and antibacterial effects^{14,15}. Terpenoids, flavonoid aglycones, flavonoids glycosides, and phenolic acids were also found in *Thymus* spp.¹⁶.

The extract of *Thymusvulgaris* which may contains high levels of flavonoids which have antioxidants and antibacterial that can be useful to improve immune response through increasing IL12 and decreasing bacterial colonies in the liver is analyzed in this study. This is to determine the effect of *Thymus vulgaris* extract to increase cytokines which play a key role for macrophage activation in phagocytosis of intracellular microbes such as Salmonella infection.

Materials and Method

The study required 15 days to accomplish. The 20 male mice were randomly divided into 5 groups each consisting of 4 mice, including the positive control (infected with 10^8 cfu*S. typhimurium*), negative control (without infection) and treatment groups of D1, D2, D3 (mice administrated with extract of *Thymus vulgaris* 250, 500, 750 mg/kg BW and infected with *S. typhimurium*) and analyzed by measuring the bacterial colonies in liver, IL-12 blood levels by ELISA after obtaining Ethical clearance approval by the studies Faculty of Medicine Brawijaya University.

Thymus vulgaris Extract Preparation.

Thymus vulgaris was dried, ground and sieved to produce a fine powder. The powder was then weighed each 100 grams to be extracted. The extraction process used the macerated method. Each extraction process required 100 grams of *Thymus vulgaris* powder which was extracted using ethanol 96%. The extract was left to settle overnight at Pharmacology Laboratory, Brawijaya University. Every mouse received 0.5cc of concentration.

Inoculum Preparation

After administered with *Thymus vulgaris* extract for seven days, the mice were orally infected with 300 μ L *S. Typhimurium* bacteria (concentration 2×10^8 cells / mL) two times with 2-day interval with the duration of seven days. On the day 15, all mice were sacrificed and the whole blood was taken.

Calculate bacteria colonies in liver:

The liver will be taken using a septic technique then performed weighing, after that the network liver is obtained and crushed with a mortar and with 4.5 ml of sterile physiological saline, then the test tube is filled with NaCl 4.5 ml, after that the 0.5 ml of mortar is inserted on the first tube and homogenization using a vortex, after that 0.5 ml of the first tube is then inserted into the second tube and so do until the tube dilution fourth that has been done 1;10 at each dilution, In the last tube solution taken, than samples of each tube 0.1 ml was inoculated on MaCOKey agar (BSA) order then, calculate the number of colonies on each plate dilution containing 30-300 CFU. calculate CFU\gram of tissue by the formula:

$$\frac{\text{The number of CFU} \times \text{Dilution} \times 10}{\text{Tissue weight (grams)}}$$

Statistical Analysis

Each data was subjected to statistical analysis. The data were analyzed using normality test of the Kolmogorov - Smirnov test. The IL-12 data have p-value of 0.200 ($p > 0.05$), so it can be concluded that the data IL-12 has a normal distribution. Thus it can be tested by ANOVA, because the assumption of normality of data distribution have been appropriate. Because of the results of ANOVA test there was no significant

difference between IL-12 in each treatment group, then no need to proceed with the test of multiple comparisons.

Results

Based on the analysis, the results show that the different doses of *Thymus vulgaris* extract affect differently on IL-12 level. The existence of the effect of *Thymus vulgaris* extract started to appear where the IL-12 in mice induced *Salmonella typhimurium* bacteria becomes higher, after the treatment was given in the form of *Thymus vulgaris* extract started at a dose of 250 mg/kg BW (D1), compared to IL-12 in the positive control group. Then the IL-12 increased more when the mice were given higher doses of 500 mg/kg BW (D2), until the highest doses of 750 mg/kg BW (D3). Thus, based on the descriptive assessment seen from the IL-12 mean, it can be said that the administration of treatment in the form of *Thymus vulgaris* extract at a dose of 250 mg/kgETV (D1), 500 mg/kgETV (D2), and 750 mg/kgETV (D3) showed different influences, where the higher dose of *Thymus vulgaris* extract will further increase the IL-12 level. The overall differences in IL-12 in each treatment above can also be depicted in the graphic form as follows.

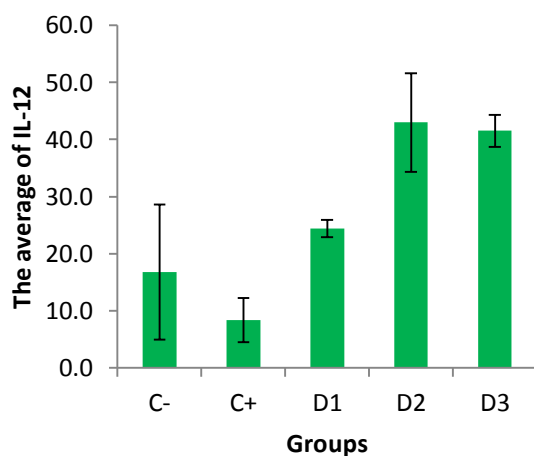


Figure 1. Graph of IL-12 at each dose of *Thymus vulgaris* extract

According to the analysis, the findings show that the different doses of *Thymus vulgaris* extract affect differently on the colony of *Salmonella typhimurium*. The existence of the effect of *Thymus vulgaris* extract started to appear where the colony of *Salmonella typhimurium* in mice induced with *Salmonella typhimurium* bacteria in the group D1 becomes lowest than positive control group, after the treatment was given in the form of *Thymus vulgaris* extract started at a dose of 250 mg/kg BW (D1). Then the colony of *Salmonella typhimurium* becomes zero when the mice were given higher doses of 500 mg/kg BW (D2), and doses of 750 mg/kg BW (D3).

The overall differences in the colony of *Salmonella typhimurium* in each treatment above can also be depicted in the graphic form as follows.

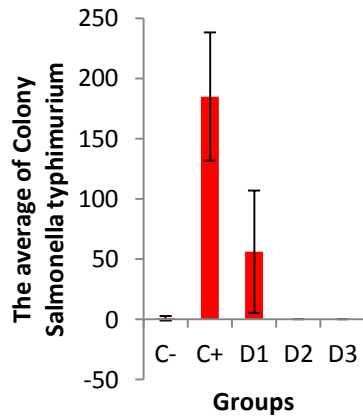


Figure2. Graph of the colony of *Salmonella typhimurium* at each dose of *Thymus vulgaris* extract

Discussions

Effect of Extract *Thymus vulgaris* on Levels of IL12

This study showed that the extract of *Thymus vulgaris* affected the increased levels of IL-12 in groups D1, D2 and D3. *Thymus vulgaris* extract at a dose of 750 mg/kgBW could increase the IL-12 levels higher than the provision of *Thymus vulgaris* extract at a lower dose of 250 mg/kgBW (D1). Moreover, the dose of 500 mg/kg BW (D2) was more effective to increase IL-12 levels than the dose of 250 mg/kgBW. The lower dose 250 mg/kg BW (D1) could increase the IL-12 levels compared to IL-12 in the positive control group. Then the IL-12 increased more when the mice were given higher doses of 500 mg/kg BW (D2). Extract at a dose of 500 mg/kgBW could increase the average IL-12 levels better than a 750 mg/kgBW and 250 mg/kg BW doses. *Thymus vulgaris* extract at a dose of 250 mg/kgETV (D1), 500 mg/kgETV (D2), and 750 mg/kgETV (D3) showed different influences, where the higher dose of *Thymus vulgaris* extract provided will further increase the IL-12. The dominant active compound contained in the extract is Thyme contains many active ingredients include thymol, carvacol and flavonoids. Major chemicals of thyme are essential oil (borneol, carvacol, linalool and thymol) bitter principle, tannin, saponins and triterpenic acids have the potential as super antioxidants, supposed to act as an immunomodulator in modulating the increase of IFN- γ and IL-12 secretion. IFN- γ secretion by macrophages also stimulates the formation of free radicals to destroy components of *M. tuberculosis* bacteria which are the DNA and the bacteria's cell walls that play a role in combating the *M. tuberculosis* bacteria¹⁴ in other studies showed that extract of *Thymus vulgaris* can induce an increasing value of TLR5 and CD14 expression by mice PBMCs, with optimal dose: 250 mg/kg B.wt, Since the level of cytokines plays an important role during the protective response in a biological system, it is interesting to investigate the effect of *Thymus vulgaris* on cytokines¹⁷. IL-12 has multiple biological activities, and it is a key factor that drives Th1 responses and IFN- γ production. Early application or production of IL-12 during infection may activate Macrophages and augment a host's cell-mediated immunity while shaping the ultimate antigen-specific immune responses. As a result, IL-12 may play a key role in protection against bacterial and viral infections, and IL-12 immunotherapy could be important in the treatment of diseases where a Th1 response is desirable. Meanwhile, cytokines including IL-12 is short in vivo half-life, and in the development of advanced drug delivery systems^{18, 19}

Other studies showed that effect of *Thymus vulgaris* on DCs co-cultured with T cells in order to determine their impact on DCs function and T-cell responses. Both compounds at 10 mg/ml significantly reduced T cell proliferation in MLR. To determine whether thymol and carvacrol modulated the production of cytokines by T cells²⁰. Also the effect of *Thymus vulgaris* extracts on MLR, they both increased CD40 expression on DCs. CD40 expression is important for DC maturation and function. It is possible that activation of DCs by these extracts has led to release of inhibitory cytokines such as IL-10. This cytokine can inhibit the proliferative responses as well as the production of IFN- γ in immunomodulatory²¹. And shown that *Thymus vulgaris* extracts increase the number of polymorphonuclears, total lymphocytes, T CD4+, CD8+ and NK cells. In this present study showed that *Thymus vulgaris* improve immune response through the increases serum IL-12 production in mice infected with *S. Typhimurium*.

Effect of *Thymus vulgaris* extract on amount of bacterial colonies in liver

Salmonella typhimurium is a facultative intracellular pathogen which causes typhoid fever in humans (the only known natural hosts and reservoir of infection). Typhoid is a systemic disease that its bacteremic phase of disease is characterized by the dissemination of organisms. The most common sites of secondary infection are the liver and spleen by penetrating the bacteria from intestine to organs such as spleen and liver and make colonies²³.

This result showed the difference in dose of *Thymus vulgaris* extract influence or different effects on the colony of *Salmonella typhimurium*. The different doses of *Thymus vulgaris* extract have different effects on the colony of *Salmonella typhimurium*. The existence of the effect of *Thymus vulgaris* extract starts to appear where the colony of *Salmonella typhimurium* in mice induced with *Salmonella typhimurium* bacteria in the group D1 becomes lowest than positive control group, after the treatment was given in the form of *Thymus vulgaris* extract starting at a dose of 250 mg/kg BW. Then the colony of *Salmonella typhimurium* becomes zero when it was given higher doses of 500 mg/kg BW (D2) and 750 mg/kg BW (D3).

Base on the result above, it was concluded that *Thymus vulgaris* have an ability to improve the immune system by increasing the level IL-12 and can decrease the bacterial colonies in the liver.

Conclusion

The *Thymus vulgaris* extract at 500 mg/kg dose has a significant effect to increase the levels IL12 and the activity of ETV significantly decreases the *S. typhimurium* colonies in liver of mice model.

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