The Effects of Aloe Vera on TNF-a Levels, the Percentage of Nk Cells and Th 17 Cells in Rat That Received Izoniazid and Rifampycin

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ABSTRACT

Background: The present study was undertaken to investigate the hepatoprotective effect of Aloe vera against side effect of antituberculosis drug. Material and methods: Twenty-five rats will be divided into five groups, namely the control group (without any treatment), the group of rats treated with antituberculosis drugs, and a group of rats were treated antituberculosis drugs and got Aloe vera extract at a dose of 40; 80; and 120 mg/kg body weight. Antituberculosis drugs are isoniazid and rifampicin at a dose of 50 mg/kg body weight. Results: Antituberculosis treated group showed significantly increase levels of TNF-a, the percentage of NK cells and the number of Th17 cells compared with the control group (p < 0.05). All doses of Aloe vera reduce levels of TNF-a compared with the antituberculosis group (p < 0.05), although it has not yet reached levels comparable to the control group (p > 0.05). Aloe vera at first and the third dose lower the number of NK cells compared to the antituberculosis group, although it has not yet reached a significant difference (p > 0.05). The first dose of Aloe vera was significantly decreased the percentage of Th17 cells compared to the antituberculosis drug group (p < 0.05), although it has not yet reached levels comparable to the control group (p > 0.05).

Conclusions: It was concluded that administration of Aloe vera can suppress the production of TNF-a and the percentage of Th17 cells as a result of antituberculosis drug administration. Thus, Aloe vera can be a useful alternative to natural materials in the successful treatment of tuberculosis through the inhibition of side effect.

Keywords: tuberculosis, liver damage, side effects, isoniazid, rifampicin.

1. INTRODUCTION

Tuberculosis is an infectious disease remains a problem worldwide, especially in developing countries. In 2010, 8.8 million cases occurred which is equivalent to 128 cases per 100,000 population. Cases of tuberculosis were reported commonly found in Asian countries (59%) and in Africa (26%). Indonesia still ranks fourth in the world for the number of cases of pulmonary tuberculosis after India, China, and South Africa. Every year there are from 0.37 to 0.54 million new cases of pulmonary tuberculosis and approximately 140,000 deaths (1).

Tuberculosis is now the world’s health problems were serious and must be addressed because of the emergence of resistance to first-line drugs and second, particularly with regard to multi-drug resistant (MDR) and extensively drug resistant (XDR) against Mycobacterium tuberculosis (2). Increased prevalence of Mycobacterium tuberculosis (MTB) which is resistant to many drugs would increase the morbidity of the disease (3). In addition, three first-line drugs are pyrazinamide, isoniazid and rifampicin have hepatotoxic effects (4). Long-term treatment with antituberculosis drug use can lead to drug-induced liver injury (DILI). The mechanism of metabolic anti-TB drugs and liver injury is not so much an explanation, but there was a significant increase in alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lipid peroxidation, intracellular calcium and activity CYP4502E1 and also a decline in activity of glutathione, glutathione peroxidase and catalase (5, 6).

The mechanism of DILI was includes cell stress, mitochondrial damage, and specific immune response (7). The liver as the organ that serves to detoxify cell will experience prolonged stress. Stress on the cell will trigger an increase in inflammatory cytokines. As a result, the liver
cells become more susceptible to apoptotic effects of TNF-α, Fas ligand and IFN-γ (8). These effects can be inhibited by inhibitors of apoptosis proteins (IAPs) or Bcl-2 (9).

Aloe vera is a plant that works with a variety of pharmacological benefits. Part of the plant that is often used is the gel inside the plant that is colorless but function in oral or topical application as immunomodulators (10-13). Previous research proves that Aloe vera extracts contain antioxidants that can function as a hepatoprotective. Aloe vera can prevent elevated levels of ALT, AST, ALP, ACP, bilirubin, total protein (albumin and globulin) in rats given isoniazid dose of 50 mg/kg in 30 days (14). Throughout the researchers know, the effects of Aloe vera on the combined antituberculosis drugs is unknown. Therefore, this study aimed to investigate the benefits of Aloe vera in preventing liver damage as a side effect of antituberculosis drug administration.

2. MATERIAL AND METHODS

Animals
A total of 25 rats will be divided into five groups, namely the control group (without any treatment), the group of rats treated by anti-tuberculosis drugs, and a group of rats were given antituberculosis drugs and got Aloe vera extract at a dose of 40; 80; and 120 mg/kg body weight. Antituberculosis drugs are isoniazid and rifampicin at a dose of 50 mg/kg body weight.

Aloe vera extraction
Aloe vera plants are cleaned and aerated to dry, with a moisture content of 5%. Plants that have been dry cut and ground to a form a powder. A total of 300 g of powder put in a flask (2000 ml) for macerated in 96% ethanol. Maceration was performed for 6 hours while shaken using a shaker with a speed of 40 RPM. Aloe vera powder marina was refluxed for 3 hours and filtered using Whatman filter paper (No. 42). Pulp filtration was refluxed again with 96% ethanol, repeated 2 times. Ethanol in the filtrate was removed by evaporated using a vacuum evaporator at 40°C, in order to obtain a crude extract. Aloe vera extract was performed 30 minutes before the administration of anti-tuberculosis drugs.

Blood sampling
After the completion of the treatment, the rats were anesthetized and surgery for blood sampling. Before surgery, the rats receive the ketamine as anesthetic. Blood serum was stored at -80°C until analysis.

Analysis of TNF-α
The levels of serum TNF-α in rats were analyzed using ELISA techniques (Rat TNF-α ELISA MAX, BioLegend, San Diego, CA, USA). The analysis carried out in accordance detailed instructions on the kit.

Analysis of NK and Th17 cells
The numbers of NK cells and Th17 in the blood were analyzed using a flow cytometer. The analysis carried out according to the instructions the standard flow cytometer.

Ethics
This study has receive the review of conduct and approved by the Health Research Ethics Committee, Health Polytechnical of Malang, Malang, East Java, Indonesia (Number 149/KEPK-POLKESM/2016).

Statistical analysis
Levels of TNF-α, the percentage of NK cells, and the percentage of Th17 cells are shown in mean ± standard deviation. Differences between treatment groups were analyzed using one way ANOVA test with SPSS 17.0 statistical package. If the ANOVA test found significance in the group will be conducted post hoc test. P value <0.05 was defined as a statistically significant difference.

3. RESULTS
Levels of TNF-α are presented in Figure 1. The levels of TNF-α increased significantly in the group received antituberculosis compared to the control group (P<0.05). All doses Aloe vera reduce levels of TNF-α than the group treated by antituberculosis (P<0.05), although...
it has not yet reached levels comparable to the control group (P > 0.05).

The percentage of NK cell counts are presented in Figure 2. Levels of NK cells was significantly increased in the antituberculosis group compared to the control group (P < 0.05). Aloe vera at first and third dose lowered the number of NK cells compared to the antituberculosis group, although it has not yet reached a significant difference (P > 0.05).

The percentage of Th17 cells are presented in Figure 3. The percentage of Th17 cells significantly increased in the antituberculosis group compared to the control group (P < 0.05). First dose of Aloe vera reduced the percentage of Th17 cells compared to the antituberculosis group (P < 0.05), although it has not yet reached levels comparable to the control group (P > 0.05).

4. DISCUSSION

In this study, administration of anti-tuberculosis shown to increase levels of TNF-a, the percentage of NK cells, and the percentage of Th17 cells significantly more than the control group. This shows that the administration of anti-tuberculosis trigger an immunological response as part of a drug toxic effects in the liver. First-line antituberculosis drugs, namely isoniazid and rifampicin are effective drug for the treatment of tuberculosis, but associated with toxic reactions in the liver (6). The main metabolic pathways was acetylated by hepatic enzyme N-Acetyltransferase 2 (NAT2). Isoniazid is acetylated be acetylsalicylic acid and then hydrolyzed to acetylsalicylic acid. Isoniazid metabolites such as acetylsalicylic acid, hydriazine are hepatotoxic. Enzyme complex drug will migrate to the surface of the cell as vesicles to serve as immunogen as targets of cytolytic T cells (15, 16).

In this study, all doses of Aloe vera reduce levels of TNF-a compared to the antituberculosis group (P < 0.05), although it has not yet reached levels comparable to the control group (P > 0.05). This indicates that Aloe vera can suppress the production of TNF-a. We speculate that the mechanism through antioxidant activity of Aloe vera which suppresses NF-kB signal. Various studies have shown that the active ingredient of Aloe vera was able to inhibit NF-kB signals (17, 18). The active ingredients of Aloe vera is thought to inhibit the production of TNF-a is acemannan. The inability the extract to returns the corresponding basal levels of TNF-a suspected to be caused by aloeide which have the opposite to acemannan (19). NF-kB is a molecule that involved in cell growth such as survival, migration and invasion (20). In this study, administration of the first dose of Aloe vera reduced the percentage of Th17 cells compared to the antituberculosis group (P < 0.05), although it has not yet reached levels comparable to the control group (P > 0.05). This finding shows that Aloe vera is able to suppress the growth of Th17 cells that arise in the context of response to anti-tuberculosis drug administration. We speculate that this mechanism by inhibiting the activation of NF-kB. Beside that, a decrease in T cell activation or proliferation, potentially due to increased apoptosis and cell death and/or an increase in the frequency of regulatory T cells that function suppression of effector T cells (21). Overall, this study expands on previous findings that the juice of Aloe vera (dose of 1 ml/kg body weight) can prevent elevated levels of hepatic biochemistry markers in rats treat by single dose of isoniazid (50 mg/kg) for 30 days (14).

5. CONCLUSION

It was concluded that administration of Aloe vera can suppress the production of TNF-a and the percentage of Th17 cells as a result of antituberculosis drug administration. Thus, Aloe vera can be a useful alternative to natural materials in the successful treatment of tuberculosis through the inhibition of side effect.

- Conflict of interest: All authors declare that no conflict of interest in the research and publication of this study.
- Authors contributions: conceived and designed the experiments: Herin Mawarti, Mukhammad Rajin, Zulfikar Asunta. performed the experiments: Herin Mawarti, Mukhammad Rajin, Zulfikar Asunta. analyzed and interpreted the data: Herin Mawarti, Mukhammad Rajin. contributed reagents, materials, analysis tools or data: Herin Mawarti, Mukhammad Rajin, Zulfikar Asunta. wrote the paper: Herin Mawarti, Mukhammad Rajin, Zulfikar Asunta

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