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DETECTION OF CD4+ T-LYMFOCYTE ADAPTIVE IMMUNITY CELL EXPRESSION IN MICE INFECTED WITH Salmonella typhimurium

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Abstract

Salmonella typhi is a gram-negative, intracellular facultative bacterium that can live and even reproduce in macrophages, resistant to lysosomal enzymes, which has the ability to prevent phagosome-lysosome fusion, making it difficult to kill. stimulates the immune response in the host (Abbas et alll, 2016; Tores et alll, 2000). White turmeric (Curcuma zedoaria) is one of the herbal plants used as an immunomodulator. Immodulators in white turmeric substances function to increase the function of phagocytosis in macrophages to destroy Salmonella typhimurium bacteria. The purpose of this study was to determine the difference between white turmeric extract as an immunomodulator on the number of CD4+ T lymphocytes in BALB/c mice infected with Salmonella typhimurium. This research is a true experimental research with a posttest only control group design. Mice were divided into 5 groups consisting of negative control group, positive control and test group. Analysis of CD4+ lymphocyte data showed a significant difference p=0.013, this indicated that administration of white turmeric extract (Curcuma zedoaria) had an effect on the number of CD4+ T lymphocytes in BALB/c mice infected with Salmonella typhimurium.

Keywords: Salmonella typhimurium, white turmeric extract, CD4+ T lymphocytes expression

1. PENDAHULUAN

Salmonella typhi is a Gram-negative, intracellular facultative bacterium that can live and even reproduce in macrophages, resistant to lysosomal enzymes, which has the ability to prevent phagosome-lysosome fusion, making it difficult to kill, Salmonella's main virulence in the form of lipopolysaccharide (LPS) can stimulate an immune response in host (Abbas *et all*, 2016; Tores *et all*, 2000).

Salmonella bacteria has specific hosts and pathogens in humans including Salmonella typhi and paratyphi, while those that are pathogenic in mice are Salmoneela typhimurium (Wardhani *et all*, 2019).

Salmonella typhi is the cause of intracellular bacteria in a systemic infectious disease called typhoid fever (Simanjuntak, 2009). The number of cases of typhoid fever worldwide is estimated at 21 million cases with 128,000 to 161,000 deaths each year,

most cases are in South Asia and Southeast Asia. (Word Health Organization, 2018).

Treatment of typhoid fever using antibiotics, such as chloramphenicol has been carried out since the late 1980 (Veeraraghavan et all., 2018), The increase and spread of the number of strains causes the use of various antibiotics, cases of failure in treatment using azithromycin are found followed by an increased risk of clinical and microbiological failure, as well as the emergence of cephalosporin resistance which has become a concern (Veeraraghavan et all., 2018)

White turmeric (Curcumin zedoaria) contains chemical compounds such as curcumin and flavonoids which are useful as antimicrobials (Wardhani et alll, 2019; Yanti, 2018). White turmeric (Curcuma zedoaria) is one of the herbal plants used as an immunomodulator (Primawari et all, 2014).

Immodulators in white turmeric substances function to increase the function of phagocytosis in macrophages to destroy Salmonella typhi bacteria. Compounds in this herbal plant are able to increase the number of lymphocytes, increase the toxicity of cancer killer cells (natural killer), synthesis of specific antibodies and stimulate macrophage activity. These properties will strengthen the body's defense mechanisms (Primawari et all, 2014).

Berdasarkan teori diatas, penelitian ini bertujuan untuk mengetahui pengaruh ekstrak Ekstrak kunyit putih *(Curcuma zedoaria)* terhadap limfosit T $CD4^+$ pada mencit BALB/c yang diinfeksi *Salmonella typhimurium.* Tujuan penelitian ini adalah mengetahui peran Ekstrak kunyit putih *(Curcuma zedoaria)* terhadap Limfosit T $CD4^+$ pada mencit BALB/c yang diinfeksi *Salmonella typhimurium.*

3. METODE PENELITIAN

Several stages of the research method include::

3.1 White turmeric extract *(Curcuma zedoaria)*

White turmeric extract is made from white turmeric meat that has been dried and then mashed into powder. Extraction was carried out by maceration using 96% ethanol solvent and stirring several times then macerated for 48 hours and then filtered through filter paper. The solvent extract solution was evaporated using a rotary evaporator, then white turmeric extract was made into a 100% concentration.

3.2 Grouping of experimental animals

The mice used were balb/c strain male mice weighing 20-25 grams as many as 30 mice, the mice were acclimatized in the animal laboratory for 7-14 days. Mice were divided into 6 groups, that is :

Group name	Treatment
Treatment 1	Infected with Salmonella typhimurium, given the extract
Treatment 2	Infected with Salmonella typhimurium, given 0.13 mg of chloramphenicol
Treatment 3	Infected with Salmonella typhmuriumi, given white turmeric extract, given 0.13 mg of chloramphenicol
PC	Infected with Salmonella typhimurium
NC	Not given treatment

3.3 Administration of white turmeric extract (Curcuma zedoaria) and

Salmonella typhimurium infection in BALB/c mice

Giving white turmeric extract (Curcuma zedoaria) is done by sonde according to the specified dose. The treatment group of mice received standard feed and acclimatized for 7 days. On the 8th day, the mice were infected with 105 CFU of Salmonella typhimurium intraperitoneal.

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3.4 CD4$^+$ T Lymphocyte Examination

The spleen was collected by necropsy on mice with a 1 ml syringe and performed on the left side of the abdomen, the spleen was washed twice with PBS, the spleen was placed in a petri dish containing 5 mL PBS, the spleen was crushed with a sterile pounder (syringe tip), 4 The filtered spleen was put into a centrifuge tube and centrifuged at 2500 rpm for 5 minutes at 10°C, the pellet was added with 1 mL of PBS.

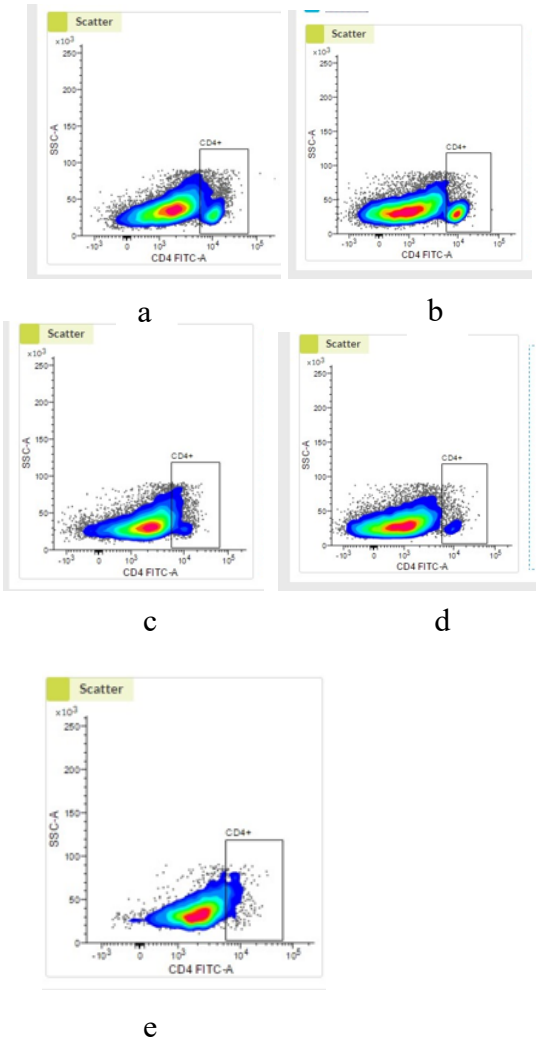
Pipette the 50 µL lymphocyte isolation mixture into a microtube and add 450 µL PBS, then centrifuge again at 2500 rpm for 5 minutes at 10°C, remove the supernatant, add 50 µL of GK15 FITC-conjugated anti-mouse CD4+ cell surface marker antibody. and incubated for 20 minutes, put in a flowcytometry cuvet, then examined with a BD FACS CaliburTM flowcytometer.

3.5 Results Analysis

The results of the CD4+ expression analysis that had been obtained were then analyzed using the SPSS for Windows application. The test used is the Men's Whitney test. In the Men's Whitney test, if the significance value is below 0.05 ($p < 0.05$) then continue using the post hoc test (Tukey) to find out which concentration groups are different or to find out the differences between treatment groups.

4. RESULTS AND DISCUSSION

4.4 Examination of the Number of CD4$^+$ T Lymphocytes Using the Flowcytometry Method



Gambar 5. The results of examining the number of CD4+ T lymphocytes using the flowcytometry method from the cell population selected by the gate lymphocyte population then the expression of CD4+ T cells will be reported in the form of a percentage, at (a) KP 1, (b) KP 2, (c) KP 3, (d) positive control (e) negative control

Table 1. $CD4^+$ expression using the flowcytometry method

No	T1	T2	T3	K-	K+
1	17,18	15,73	10,80	18,7	11,99
2	19,49	15,78	12,33	18,2	17,28
3	11,11	19,11	8,14	7,6	3,88
4	7.8	18.18	9,04	16,4	5,15
5	52.05	18.00	15,05	34,3	3,05
6	10,72	11,80	5,52	12.32	10,72
Mean	19,71	16,43	10,14	17,92	8,67

Information:
T1 = KP1
T2 = KP2
T3 = KP3
K- = Kontrol negatif
K+ = Konrol Positif

The data was then subjected to statistical analysis using the Kruskal Wallis method. In the Kruskal Wallis test, the significance value was ($p<0,05$). In the research data, the significance value is 0.013, this shows that Ho was rejected and H1 was accepted which showed there were differences in each group, so it can be concluded that administration of white turmeric extract (Curcuma zedoaria) affected the number of $CD4^+$ T lymphocytes in BALB/c mice infected with Salmonella typhimurium

The research data showed that there was a significant difference in the number of $CD4^+$ T lymphocytes $p= 0,013$ ($p < 0,05$). This shows that there is a significant difference in the number of CD4+ T lymphocytes between KN and P2, KP is significant to P1 and P2. While P2 is significant to the P3 group. The significant difference showed that the number of CD4+ lymphocytes was significantly different between treatments.

In the KP group it was lower than the KN, P1, P2 and P3. The decrease in the KP group occurred because Salmonella typhimurium infection suppressed the antigen presenting of dendritic cells to CD4 T cells. Salmonella typhimurium-mediated expression of flagellin impairs the activation and proliferation of naïve flagellin-specific $CD4^+$ T cells in Peyer's patch, which is accompanied by increased splenic dissemination of bacteria thereby reducing antigen availability, thereby attenuating $CD4^+$ T cell response. (Atif *et all*, 2014).

In P1 there was an increase in the number of CD4+ T lymphocytes, the increase occurred due to the administration of chloramphenicol, splenocytes stimulated with anti-CD3 in the presence of chloramphenicol had more $CD4^+$ T cells (Yuan & Shi, 2008).

At P2 there was an increase when compared to KN and KP, the increase occurred because white turmeric extract contained compounds, one of which was flavonoids. Flavonoids can increase lymphocyte proliferation which affects the activation of CD4+ and Th 1 (T-helper) T cells so that macrophages are activated and increase phagocyte activity to kill bacteria or pathogenic microorganisms. (Lim *et all*, 2019).

In the P3 group the combination of white turmeric extract and chloramphenicol showed results that had an effect on increasing CD4+ T lymphocytes but lower than P1 and P2. This decrease occurred because white turmeric (Curcuma zedoaria) is one of the herbal plants used as an immunomodulator. Compounds in the white turmeric plant can increase the number of lymphocytes

Regulation of multiple regulators of the main target natural product is important to increase the collective potency to drug level. In two combinations the primary target NP is modulated and each constituent targets one or two of the four redundant processes to collectively achieve a therapeutic effect (Qin *et all*, 2012). The combination of white turmeric extract and chloramphenicol increases CD4+ T lymphocytes but weak inhibition of several targets in related pathways may be more efficient than strong inhibition of a single target

Intra-cellular bioavailability of natural produk is enhanced by inhibiting/ downregulating and upregulating/activating cell entry transporters. The anti-contraactive action involves regulation of pathways activated by NPs which then reduce the therapeutic effect of NPs. Drug efficacy is reported to be reduced by tissue resistance, redundancy and compensatory actions resulting in neutralization (Qin *et all*, 2012)

5. KESIMPULAN

Based on the data above, it can be concluded that:

1. Administration of white turmeric extract (Curcuma zedoaria) has an immunodulatory effect on the number of CD4+ T lymphocytes and TNF-α levels in BALB/c mice infected with Salmonella typhimurium
2. Chloramphenicol as an antibacterial against the number of CD4+ T lymphocytes and TNF-α levels in BALB/c mice infected with Salmonella typhimurium

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