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ORIGINAL RESEARCH

EFFECTIVITY OF RED SESBANIA LEAVES (*Sesbania grandiflora L. Pers*) EXTRACT TO DECREASE LEUKOCYTE LEVEL IN POST-PARTUM MICE (MUS MUSCULUS) INFECTED WITH *Streptococcus agalactiae*

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ABSTRACT

Introduction: Maternal Mortality Rate (MMR) is an indicator of public health degree. The cause of maternal mortality is a postpartum infection. *Streptococcus agalactiae* bacterium contributed to postpartum infection through vaginal or reproductive organ injury. Infection incidence was characterized by the increased level of leukocyte. The treatment of post-partum infection is done by administering antibiotics. Red sesbania leaves contain an active substance that could inhibit the microbial growth. **Objective:** This research aimed to find out the effect of red sesbania leaves extract administration as anantimicrobial agent to decrease leukocyte level in postpartum mice (*Mus musculus*) infected with *Streptococcus agalactiae*. **Method**: The method used true experimental one with post-test control group design, by dividing postpartum mice into four groups: one control group and three treatment groups at doses of 125 mg/kgBW, 250 mg/kgBW, and 500 mg/kgBW. All 0-12 hour postpartum mice were infected with *Streptococcus agalactiae* bacterium. The administration of 1 ml red sesbania leaves extract in treatment group was conducted 2 hours after the bacterial administration at doses of 125 mg/kgBW, 250 mg/kgBW, and 500 mg/kgBW. Independent variable was turi leaves extract, and dependent variable in this research : leucocyte level in postpartum mice. Test statistic used ANOVA for leucocyte levels. **Result:** The result of the analysis showed $p < 0.05$, indicating that the decrease of leukocyte level in all treatment groups (P1, P2, P3). **Conclusion**: Red sesbania leaves extract has antimicrobial activity that can reduce leukocyte level, thereby can be used as an alternative therapy to decrease maternal mortality rate due to post-partum infection.

Keywords

Red sesbania leaves (SESBANIA GRANDIFLORA L.PERS) extract,

postpartum mice,

leukocyte level,

Streptococcus agalactiae.

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INTRODUCTION

Puerperium is a period after placenta birth and ends when the reproductive organ recovers into a condition like before pregnancy [11]. This period is a critical period for mother and her baby, so that postpartum monitoring [10] is needed. One factor causing maternal mortality is postpartum infection [4]. Postpartum infection is the problem occurring most widely in developing countries [1].

Data from Indonesian Health Demographic Survey (*Survey Demografi Kesehatan Indonesia*, after that called SDKI) in 2012 reported that maternal mortality rate (MMR) is 359 per 100,000 live births. The figure is high thereby should be reduced, corresponding to the target specified in Sustainable Development Goal's (SDG's) of 2030, below 70 per 100,000 live births [6].

The presence of bacteria through birth canal or vagina and another canal during and after delivery or childbirth becomes the risk factor of postpartum infection. *Streptococcus agalactiae* is currently recognized to be the part of normal flora including that in thehuman urogenital system. The contamination of *Streptococcus agalactiae* in thebirth canal and other reproductive organs can result in infection characterizedby postpartum fever [7]. *Streptococcus agalactiae* is Group B of *Streptococcus* that can result in postpartum infection, characterizedby the increased leukocyte level [13].

Red sesbania (*Sesbania grandiflora L. Pers*) leaves contain active substances such as *Flavonoid*, *saponin* and *tannin* [5] that can inhibit microbial growth at Minimum Inhibitory Concentration (MIC) of 14% thereby can treat postpartum fever [12].

The objective of theresearch was to find out the effect of red sesbania leaves extract on the reduction of leukocyte level in postpartum mice (*Mus musculus*) infected with *Streptococcus agalactiae*.

MATERIALS AND METHODS

This study was true experimental research with post-test control group design. Independent variable was turi leaves extract, and dependent variable in this research : leucocyte level in postpartum mice.

1. Population, Sampling and Sample

Female mice (*Mus musculus*) at 14 days gestation were used with the following inclusion as sampling with criteria: healthy and have not received any treatment, exclusion criteria: Being sick before treatment, had the anatomic disorder, had ever been used in another experiment previously, and drop out criteria: died since adaptation in the stall. Total sample were 28 female mice

The mice are divided into 4 groups, each groups contents 7 mice : Control (infected with *Streptococcus agalactiae* bacteria 5 x 10^3CFU/ml), treatment 1 (infected with *Streptococcus agalactiae* bacteria 5 x 10^3CFU/ml, and given red sesbania leaves extract at dose of 125mg/kgBW), treatment 2 (infected with *Streptococcus agalactiae* bacteria 5 x 10^3CFU/ml, and given red sesbania leaves extract at dose of 250mg/kgBW), and treatment 3 (infected with *Streptococcus agalactiae* bacteria 5 x 10^3CFU/ml, and given red sesbania leaves extract at dose of 500mg/kgBW). Bacteria infection was conducted within

0-12 hour puerperium/ as soon as after childbirth. The red sesbania leaves extract was given in treatment group 2 hours after bacterial infection, and 24 hours later it was terminated.

The treatment of mice (*Mus musculus*) has met the ethical requirement of treating tested animal as specified by Poltekes Kemenkes Malang (Malang Health Ministry's Health Polytechnic). Mice (*Mus musculus*) were selected as the subject of research as they are reared easily and relatively healthy, and appropriate to be used in many types of researches.

2. *Streptococcus agalactiae* Bacterium

Streptococcus agalactiae bacterium was obtained from Saiful Anwar Hospital's Microbiology Laboratory stock that has been identified in 2017. *Streptococcus agalactiae* was cultured on blood agar medium for 24 hours before exposure, when the bacteria growthed, measurement was conducted using spectrophotometry with expected absorbance value (optical density) of 0.1 equivalent to 10^3 CFU/ml. Dissolution was conductedat some levels: 10^{-6}, 10^{-5}, 10^{-4}, by 1 cc bacteria. It was taken from tube I (10^{-6} bacteria/ml) and then put into tube II (10^{-5}); Furthermore, homogenization was conducted using vortex method at each level dissolved, up to tube III (10^{-4}) and tube IV.So that the final result is obtained, the dose of 5 x 10^{-3} CFU/ml. Individual mice received *Streptococcus agalactiae* bacteria intravaginally by 0.2 ml out of 10^3 CFU/ml.

3. Preparation of Postpartum Infected Model Mice

All tested animals used were taken from Biotechnology Laboratory of Malang State University. The tested animals used were pregnant female mice (*Mus musculus*) at 14-day gestation, weighing 30-40 grams, selected based on inclusion criteria. As soon as the mice give birth (puerperium), the mice in control and treatment groups were infected with 0.2 ml *Streptococcus agalactiae* intravaginally at adoseof 5 x 10^3 CFU/ml. The preparation of *Streptococcus agalactiae*was obtained from Microbiology Laboratory of Brawijaya University. Two hours after *Streptococcus agalactiae* administration, the mice became postpartum infection model mice.

4. Preparation of Red Sesbania Leaves Extract using Maceration and Evaporation Methods

Red sesbania (*Sesbania grandiflora L. pers*) leaves were obtained from Mojokerto Regency, East Java Province. The extraction process was conducted in Pest and Plant Diseases Laboratory of Agricultural Faculty of Malang Brawijaya University, using maceration and evaporation method. The solvent used was ethanol 96%, with the paste-form coarse extract as the product, dissolved with aquabidest, and stored at 4^0C.

5. Preparation and Administration of Red Sesbania Leaves Extract Solution

About 53.88 mg red sesbania leaves extract was stocked; the concentrations used were: 2.5mg; 5mg; 10mg. 1 ml extract was administered to the treatment

group orally using feeding tube 2 hours after being infected. The administration of red sesbania leaves extracts was conducted once a day, at adose corresponding to the treatment groups: P1 (125mg), P2 (250mg) and P3 (500mg).

6. Tested Animal Surgery

Mice surgery was conducted 24 hours postpartum.Termination process was carried out using ketamine infection of 0.1 ml until the mice cannot move, and then they were put onto operation table at the supine position, with the womb on the top, and then the womb of mice was sterilized using alcohol spray 70% and sectioned. The mice's blood was taken from the right heart using a1-cc syringe, followed by taking liver organ to count the number of bacteria colonies.

7. Leukocyte Level Measurement

One cc blood was taken from the mice's heart and then put into blood accommodating tube wit EDTA and blood leukocyte level was measured in the laboratory with the following procedure. The blood in the tube was shakento be homogeneous. The solution in the pipette was dropped into hemocytometer and read using amicroscope with 10 x magnification of leukocyte number, conducted by pipetting the blood EDTA up to 0.5 ml limit.

The second stage is to suck at acetic acid 1%, shaken to be homogeneous. The solution in the pipette was used to count leukocyte with a hemocytometer. Leucocyte level measurement was taken 24 hours post partum, and showed mean of leucocyte level in control group was high (> 10.6 103/µL), it showed that the mice infected by Streptococcus agalactiae. After giving turi leaves extract at 24 jam postpartum, the leucocyte levels were lower in treatment groups. Decrease of leucocyte levels were showed in mice after giving turi extract. Test statistic used ANOVA for leucocyte levels.

RESULTS

Leukocyte level was analysed :

Characteristics of Postpartum infected model mice

Table of Characteristics of Postpartum Infected Model Mice

Sample	Group			
	Control	P1 (S.A + sesbania 125 mg/kg BW)	P2 (S.A + sesbania 250 mg/kg BW)	P3 (S.A + sesbania 500 mg/kg BW)
1	14.50	9.50	8.90	6.20
2	16.40	10.90	5.90	7.60
3	11.40	10.50	5.60	6.00
4	12.70	10.50	10.10	7.90
5	12.00	10.80	6.30	6.20
6	13.70	10.60	10.30	8.80
Mean ± SD	13.45 ± ± 1.83	10.47 ± 0.50	7.85 ± 2.16	7.10 ± 1.17

DISCUSSION

Streptococcus agalactiae can induce the occurrence of postpartum infections

At 24 hours after infection, the average result of blood leukocyte of control group mice was higher than treatment group with *Sesbania Grandiflora L.PERS* extract. Research Baker and Barret (1973) cited by Mhalu (2016) found the microbes are more than 30% in the vagina of normal adult women. Contamination of streptococcus agalaciae in both birth lesions and other reproductive organs during the

puerperium can lead to infections characterised by puerperal fever [7]. In the study Rosati et al. (1998), the cytokine response to Streptococcus agalactiae, with a dose of 5 x 103 CFU / ml administered intraperitoneally, within two h of infection, bacteria colonised all organs of mice including peritoneum, liver, spleen, blood, kidney except brain [9]. Leucocyte count is a good indicator of the body's response to infection. Normal adult leukocyte levels range from 5000 to 10,000 / µL [13].

Streptococcus agalactiae bacteria, which are normal flora, will rapidly migrate from the vagina to the uterus, where there is a placental implantation wound, thus contaminating the injury and may become pathogenic, and lead to postpartum infections. Under conditions of infection, there will be an immune response of the body, in this case involving inflammatory mediators as the body's defence mechanism against infection [7].

Effect of *Sesbania Grandiflora L.PERS* Extract on decreasing levels of blood leukocytes

In the Yusniawati (2015) study, the active substance in *Sesbania Grandiflora L.PERS* has the effect of inhibiting bacterial (bacteriostatic) growth at a minimal inhibitory level (KHM) of 14%. *Sesbania Grandiflora L.PERS* have active substances namely flavonoids, saponins and tannins that can function inhibit microbial growth [12].

Flavonoids cause damage to the permeability of bacterial cell walls. Also, it is also able to inhibit bacterial motility [3]. Nuria et al. (2009) state that the mechanism of action saponin as antibacterial is to disrupt the stability of bacterial cell membranes that cause bacterial cell lysis, and the decrease in surface tension resulting in increased permeability of bacterial cells [7]. The process will lead to cell membrane damage and lead to the release of important components of the bacterial cell, i.e. proteins, nucleic acids, and nucleotides [3]. Tannins may inhibit growth in the formation of the Streptococcus Agalactiae membrane structure [2].

With the provision of *Sesbania Grandiflora L.PERS* extract, it will cause bacterial growth restriction by the active ingredients contained in it at a minimum inhibitory level (KHM) of 14% [12]. The administration of the extract may also affect macrophage activity to decrease the levels of proinflammatory cytokines by reducing the transcription factor (NF-kB), thereby resulting in reduced blood leukocyte levels [2]. In this study, showed the average leukocyte level in the control group was higher than the treatment group

CONCLUSIONS

The results of this study showed Streptococcus agalactiae dose 5 x 103 CFU / ml could induce postpartum infections through bacterial contamination of the wound.Both in the birth canal and uterus and *Sesbania Grandiflora L.PERS* extract has a function as an immunomodulator and antimicrobial because it can affect immune response and inhibit the growth of bacteria so the occurrence of puerperal sepsis can be prevented.

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