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The effect of red Turi leaf extract (*Sesbania glandiflora L.Pers*) on the reduced levels of TNF-α, IL-1β and the number of bacterial colonies on the puerperium *Mus Musculus* ovarian inoculated by *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is one of the bacteria that causes postpartum infection, where this bacterium releases peptidoglycans and lipoteichoic acid (LTA) that start the inflammation response, which is then responded by macrophages by releasing pro-inflammation cytokines such as Tumor Necrosis Factor-Alpha (TNF-α) and Interleukin 1-Beta (IL-1β). Red Turi leaf extract, which is known to contain saponins, flavonoids, and tannins which work by suppressing the growth of bacteria and forcing bacterial cells to undergo lysis, causing the reduction of immune system activation which results in the reduction of secretion of pro-inflammation cytokines such as TNF-α and IL-1β. This research aims to analyze the effect of red Turi leaf extract in reducing TNF-α, IL-1β, and bacterial colonies on the puerperium *Mus Musculus* Ovarian Inoculated by *Staphylococcus aureus.* Results in this study proved that red Turi leaf extract of doses of 125, 250, and 500 mg/kgBW/day was able to reduce TNF-α, IL-1β, and the number of bacterial colonies on the puerperium *Mus Musculus* Ovarian Inoculated by *Staphylococcus aureus.* The optimum doses to reduce the number of bacterial colonies were doses of 250 and 500 mg/kgBW/day. Thus, the infection caused by intravaginal inoculation *Staphylococcus aureus* for 24 hours on the puerperium *Mus Musculus* Ovary was alleviated by treatment with red Turi leaf extract by reducing TNF-α, IL-1β, and the number of bacterial colonies on the puerperium *Mus Musculus* Ovarian Inoculated by *Staphylococcus aureus.*

Keywords: Red Turi leaf extract; *Staphylococcus aureus;* TNF-α; IL-1β; Bacterial colonies

1. Introduction

The maternal mortality rate in Indonesia is still considered high in the world and in 2008 among countries of the Association of Southeast Asian Nations (ASEAN) and the South-East Asia Region (SEARO). Data from the Directorate of Maternal Health (DKI) states that in 2013 one of the causes of maternal death in Indonesia is caused by infection, at 7.3%. Seen from this data, maternal death caused by infections are still quite high, one of which is caused by pregnancy infections. Even though occurrences of pospartum infections have decreased, this matter is still considered worrisome because this may increase the morbidity of mothers during postpartum, and thus it becomes important to continue to make the effort to reduce occurrences of postpartum infections [1].

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A pregnancy infection is the entry of pathogenic organisms through wounds of the uterus or womb and vagina caused by the imbalance of normal flora in the genitalia tract. Based on results of tests of bacteria types on episiotomy were found to have the *Staphylococcus aureus* bacteria, which becomes one of the causes for pospartum infections [2,3]. Bacteria that are present in the cervix and vagina will obtain access through amnion fluids during or after delivery process and will then invade dead uterus tissue [4]. During delivery process, vaginal bacteria infects the decidua and the place of implantation of the placenta first, then spreads to the myometrium, parametrium, and nearby organs (per continuitatem), and will grow more quickly in the presence of dead tissue [5]. Infection and abscesses in ovaries caused by *Staphylococcus aureus* bacteria occur simultaneously after infection of the Fallopian tubes (per continuitatem); in addition, the pathogenesis of infection and formation of abscesses caused by *Staphylococcus aureus* bacteria in the ovary may also occur through lymphatic blood flows [6].

Several kinds of toxins released by *Staphylococcus aureus* among others include exotoxins, exfoliative toxins, and superantigens. Exotoxins produced by *Staphylococcus aureus* are often linked with toxic shock syndrome [7]. Inflammation response begins with the release of exotoxins and cell antigen components, and then the inflammation reaction will be responded by macrophages by secreting pro-inflammation cytokines. Tumor Necrosis Factor-Alpha (TNF-α) and interleukin 1-Beta (IL-1β) are the pro-inflammation cytokines that play a role as indicators of occurring inflammation, and the two cytokines (TNF-α and IL-1β) are the primary response in the case of acute inflammation [8].

Treatment of infections of *Staphylococcus aureus* bacteria is conducted by antibiotics, but recently treatment has become ineffective and the length of treatment has become longer, this is because the bacteria has become resistant toward several types of antibiotics [9,10]. Thus, it becomes important to treat postpartum infections by utilizing natural plants that possess antimicrobial benefits.

Turi is a plant that has been known to have antimicrobial properties [11]. There are two kinds of Turi plants based on their flowers, which are white- and red-flowered Turis; the plant often used in medicine is the red-flowered plant because of greater contained saponin, flavonoid, tannin, glycoside, peroxidase, vitamins A and B, egatin, zantoegatin, basorin, resin, calcium oxalate, sulfur, iron, and sugar content compared to the white ones [12]. Researches use red Turi leaves because the active substances in the leaves are more complete than in other parts of the plant [13]. A research by Trilupi (2016) showed that the saponin, flavonoid, and tannin content in red Turi leaves had an antimicrobial effect on Streptococcus agalactiae bacteria [14]. The flavonoid content can also become an immunostimulator by increasing phagocytosis activities by triggering IFN-γ production [15], and saponins have anti-inflammation properties, shown by its proven ability to heal edema on the infected back legs of mice [16]. Thus, it is suspected that red Turi leaves, in addition to having an antibacterial effect on *Staphylococcus aureus,* also has anti-inflammation properties and can become an immunomodulator that affects the secretion of pro-inflammation cytokines.

2. Material and methods

This research used the mice (*Mus Musculus)* were 14-day old healthy pregnant mice, that had not had further treatment. Acclimatization was done over 3 days. The mice (*Mus Musculus)* were 14-day old healthy pregnant mice, that had not had further treatment. Acclimatization was done over 3 days. The 25 replicated research mice were divided into 5 groups, which are a negative control group (without treatment), a positive control group (*Staphylococcus aureus)*, and treatment groups of P1 (*Staphylococcus aureus* and red Turi leaf extract at a dose of 125 mg/kg BW/day), P2 (*Staphylococcus aureus* and leaf extract at a dose of 250 mg/kg BW/day), and P3 (*Staphylococcus aureus* and leaf extract at a dose of 500 mg/kg BW/day).

The postpartum infected model mice were created by intravaginal inoculation of *Staphylococcus aureus* of 5 x 10^7 CFU/ml, by 0.2 ml during 0-12 hours postpartum. Red Turi Leaf extract at a dose of 125 mg/kg BW/day, 250 mg/kg BW/day and 500 mg/kg BW/day. Extraction of red Turi leaves were conducted at the Materia Medica Laboratory in Batu, Malang, resulting in a paste. Dilution with distilled water was conducted before treatment. Treatment was given 2 hours after inoculation with *Staphylococcus aureus* at the various doses for each treatment group. Treatment was given orally through feeding tubes.

TNF-αand IL-1β content were measured with mouse ELISA kits for TNF-α and IL-1β. The entire procedure for measuring TNF-α and IL-1β content followed the standards of Bioassay Technology Laboratory. Measurement of the number of bacterial colonies in the ovary used the Pour Plate Slide method.

TNF-α an IL-1β content was analyzed with one-way ANOVA, while the number of bacterial colonies in the ovaries was analyzed with the Kruskal-Wallis test. Analysis utilized SPSS 22 for Windows.

3. Results and discussion

Research results showed that red Turi leaf extract was able to reduce TNF-α IL-1β and The Number of Bacterial Colonies a on The Puerperium *Mus Musculus* Ovarian Inoculated by *Staphylococcus aureus.* This was affected by the substances contained in red Turi leaf extract which include saponins, flavonoids, and tannins.

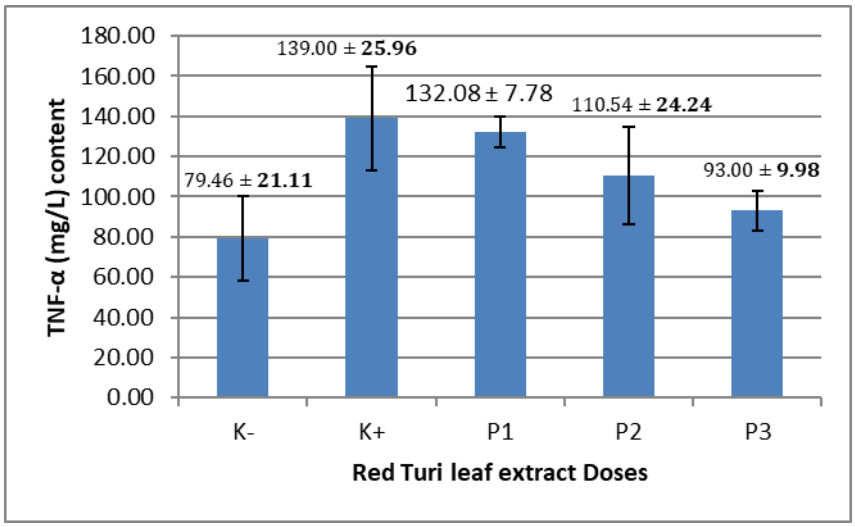

Figure 1 Histogram of average TNF-α content

Figure 1 showed that three groups given treatments of red Turi leaf extract (P1, P2, and P3) showed reductions in TNF-α content. The lowest average TNF-α content was for the group given red Turi leaf extract of a dose of 500 mg/kb BW/day, and the group given treatment of red Turi leaf extract of a dose of 125 mg/kg BW/day (P1) had the highest average content of TNF-α.

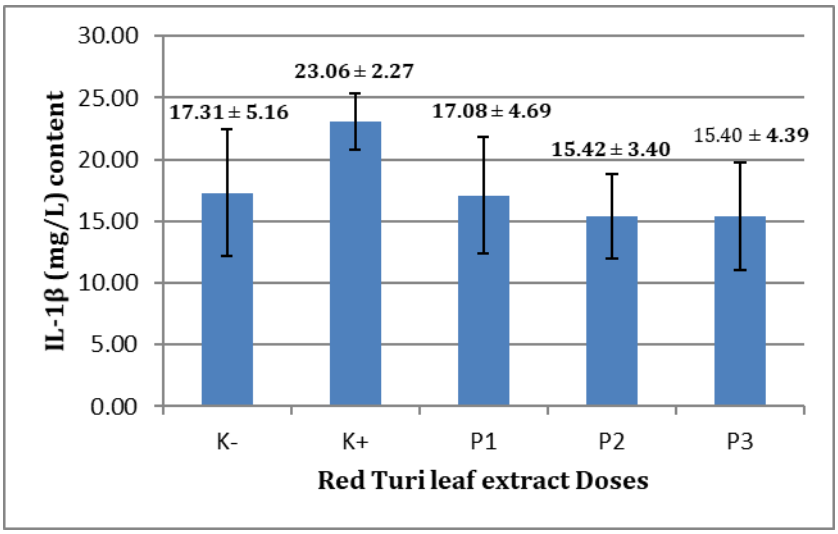

Figure 2 Histogram of average IL-1β content

Figure 1 showed that the three groups given treatments of red Turi leaf extract (P1, P2, and P3) showed reductions in IL-1β content. The group given treatment of red Turi leaf extract of a dose of 500 mg/kg BW/ (P3) had the lowest average IL-1β content, and the group given treatment of red Turi leaf extract of a dose of 125 mg/kg BW/day (P1) had the highest average IL-1β content

Table 1 Differences of effects of red Turi leaf extract on the number of *Staphylococcus aureus* bacterial colonies (CFU/ml)

Dose	Avg.	Probability					Note
		K-	K+	P1	P2	P3	
K-	0		0.008	0.008	1.000	1.000	a
K+	1700	0.008		0.008	0.008	0.008	c
P1	370	0.008	0.008		0.008	0.008	b
P2	0	1.000	0.008	0.008		1.000	a
P3	0	1.000	0.008	0.008	1.000		a

Based on Table 1. showed that there was a significant difference in the number of bacterial colonies in postpartum mice among the negative control group, positive control group, and groups given treatments of red Turi leaf extracts with doses of 125 mg/kg BW/day (P1), 250 mg/kg BW/day (P2), and 500 mg/kg BW/day (P3).

Staphylococcus aureus are bacteria that has a role in causing postpartum infections, that can induce the release of TNF-α and IL-1β because the cell walls of the bacteria contain peptidoglycans and lipoteichoic acid. The excess increase in TNF-α content will increase excess secretion of NO, which may cause septic shock and damage to various organs to occur [3,17,18]. IL-1β is an acute inflammation mediator cytokine that is similar TNF-α, having a high potential to cause tissue damage in the host, and thus known to have a detrimental effect [8].

In this research, the substances in red Turi leaf extract, which are saponins, flavonoids, and tannins could reduce the pro-inflammation cytokines of IL-1β and TNF-α. This means the red Turi leaf extract can be said to be an immunomodulator with its phytochemical contents, which are saponins, flavonoids and tannins; in addition to being able to reduce pro-inflammation cytokine content, these are also shown to be able to increase the content of anti-inflammation cytokines in postpartum mice inoculated with *Staphylococcus aureus.*

Yuswantina *et al.* (2012) stated that from research results on the substances contained in the red Turi leaf extract, which are saponins, flavonoids, and tannins that have antibacterial properties, are significantly able to reduce the number of bacterial colonies and have a greater suppressing power with a greater concentration [19].

Saponins as antibacterial substances work by disrupting the stability of cell membranes of bacteria, damaging them and forcing out vital components of bacteria as the cells undergo lysis [20]. Ahmad *et al.* (2014) mention that saponins are effective as anti-inflammation agents that reduce the expression of NF-kb P65 and P50, which will reduce the number of pro-inflammation cytokines such as TNF-α, IL-1β, and IL-6 [21].

Flavonoids are effective in hindering viral, bacterial, and fungal development by damaging the permeability of bacterial cell walls, microsomes, and lysosomes by interacting with bacterial DNA and hindering the motility of bacteria besaid that flavonoids to become immunostimulators by increasing phagocytosis by triggering the production of IFN-γ, thus hindering the production of TNF-α [15,22,23].

Tannins as antibacterial agents work by damaging bacterial cell walls and hindering the growth and development of bacterial cells, allowing for their death [24,25].

The most optimal dose that can reduce TNF-α content is 500 mg/kg BW/day (P3). This is because the chemical substances in red Turi leaf extract as saponins, flavonoids, and tannins that function as antibacterial agents can hinder bacterial growth and even lead to their lysis. In addition, the saponins, flavonoids and tannins in the red Turi leaf extract can hinder the activation of NF-kB, thus decreasing the secretion of pro-inflammation cytokines, one of them TNF-α.

4. Conclusion

The treatment of red Turi leaf extract was proven to be able to reduce TNF-α and IL-1β content as well as the number of bacterial colonies in the ovaries of puerperium *Mus Musculus* inoculated with *Staphylococcus aureus.*

Compliance with ethical standards

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Disclosure of conflict of interest

We warrant that the article is the Authors' original work and ensure no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is not under review at any other publication.

Statement of ethical approval

The treatment on the puerperium Mus musculus's ovarian Inoculated by Staphylococcus aureus has met the ethical feasibility requirements of experimental animals at the Faculty of Medicine, Universitas Brawijaya Malang no. 259/EC/KEPK-S2/07/2017.

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