

Decreasing the Number of Leukocytes as an Effect of Standard Therapy and *Rhodomyrtus tomentosa* Leaves Extract Therapy

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Abstract: The postpartum period is a vulnerable period to infection due to low immunity. An increase in leukocytes characterizes the body's response to infection; several leukocytes close to normal values indicate that the infection period will end. The research objective was to determine the effectiveness of *Rhodomyrtus tomentosa* leaf extract on the number of leukocyte cells in mothers with postpartum infections at Tarakan City General Hospital. The research used an Analytical survey with a sample of postpartum mothers who came to the Tarakan City General Hospital (April-August 2022). Criteria for Group 1 were mothers with postpartum infections and had not been given any therapy; Group 2 were healthy postpartum mothers; Group 3 were mothers with postpartum infections who were given standard therapy; Group 4 is mothers with postpartum infections who were given standard therapy and on the doctor's advice the day before they were given Karamunting leaf extract as additional therapy. The research results show that giving *Rhodomyrtus tomentosa* leaf extract with standard therapy reduces the number of leukocytes to close to normal values, so it can be concluded that it has an anti-inflammatory effect on mothers with postpartum infections. Further clinical trial research is needed with adequate samples and more uniform conditions for each sample.

Keywords: Number of Leukocytes; postpartum infection; *Rhodomyrtus tomentosa*.

INTRODUCTION

The postpartum period begins when the placenta has come out, and there is uterine involution and cleaning of bacterial contamination from the reproductive tract¹. During the puerperium, birth canal injuries or placental implantation scars occur during childbirth, which makes it easier for bacteria to invade. The most commonly isolated bacteria were *Escherichia coli* (32.1%) from Gram-negative and *Staphylococcus aureus* (33.9%) from Gram-positive². The ascending bacteria enters the mother's blood supply through the placental intervilli and infected tissues up to the spleen, causing systemic damage through the blood vessels, causing inflammation and leukocyte infiltration^{3,4}. According to the research, the obtained data showed that from 2003 until 2008, 461 of 6077 patients in Khanevadeh Hospital suffered postpartum infection⁵.

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Postpartum infection of the genital tract occurs in childbirth or the postpartum period. Infection can occur because the vaginal flora increases during labor and after surgery, and surgical interventions also play a role in increasing vaginal microflora into the reproductive tract³. It is usually characterized by fever, flank pain, and foul-smelling vaginal discharge and is associated with neonatal sepsis⁶.

The immune system decreases postpartum, causing susceptibility to infection during recovery⁴. The inflammatory response of patients with postpartum infections is significantly increased⁷. The response of innate immunity to inflammation and tissue abnormalities results from the release of cytokines by tissue cells that trigger an inflammatory response⁸. The inflammatory response of postpartum infection induced by Gram-negative bacteria mainly depends on the recognition of bacteria by proteins⁹.

Karamunting (*Rhodomyrtus tomentosa*) is a natural ingredient that the people of Kalimantan widely consume to treat infectious diseases¹⁰. *Rhodomyrtus tomentosa* leaves contain active compounds – phenols, flavonoids, saponins, tannins, alkaloids, and triterpenoids¹¹. Previous research reported that ethanol extract of *Rhodomyrtus tomentosa* had a bacterial effect by reducing *E. coli* colonies in postpartum mice infected with *E. coli*⁹. Research by Winarsih et al.¹² stated that administration of the lowest dose of ethanol extract of *Rhodomyrtus tomentosa* leaves was able to reduce levels of TNF- α , IL-6, and IL-1 β as anti-inflammatory effects in mice model of *E.coli*-induced puerperal infection. However, there is still limited research on the effects of *Rhodomyrtus tomentosa* leaf extract on postpartum mothers. Hence, this research aims to determine the effectiveness of *Rhodomyrtus tomentosa* leaf extract on the number of leukocyte cells in mothers with postpartum infections at Tarakan City General Hospital in April-August 2022.

MATERIALS AND METHODS

The sample results from routine complete blood counts originating from peripheral blood met the inclusion criteria in the study until it met the required number of samples. The research is an analytical survey whose sample is all postpartum mothers who come to Tarakan City Public Hospital (April-August 2022). Purposive sampling was used as a sampling technique. Postpartum mothers were surveyed using the following criteria: Group 1 is a postpartum infection mother without any administration, while Group 2 is a healthy postpartum mother. Criteria of group 3 are postpartum infection mothers who are given standard therapy, and group 4 criteria are Postpartum infection mothers who were given standard therapy and based on the doctor's suggestion a day before the kara munting leaf extract was given as an additional dose (200 mg/kg BW) orally once a day.

The phlebotomy equipment is prepared cleanly and completely (tourniquet, handsoen, 70% alcohol swab, sterile 5cc syringe, EDTA Vacutainer, sterile plaster, label) then the phlebotomy is performed aseptically. The blood in the syringe is distributed in a tube with EDTA anticoagulant. The tube is coded according to the patient's identity. The EDTA blood sample must be fresh to maintain the viability and quality of the test results. The examination was conducted in the clinical pathology laboratory of Tarakan City General Hospital, and routine blood tests were performed using flow cytometry. Before the sample is checked, a quality control tool is carried out to guarantee the examination results.

All procedures of this study were by the ethical standards of the committee responsible for human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2013. The Research Ethics Subcommittee of Tarakan City Public Hospital approved this research with letter No: 026/KEH/RSUKT/2022. Data of the result was performed in Mean \pm SD. The calculation was performed using SPSS for Windows software. Data analysis used a one-way ANOVA test, post hoc Honest Significance Different (HSD), and Pearson correlation, statistically significant if p-value <0.05.

RESULTS AND DISCUSSION

Examination of the leukocyte count in postpartum women was carried out using the flow cytometry method with the results of the normality assumption test on the leukocyte count data (Table 1). The results of the research show that the data is normally distributed. Thus, a parametric statistical test was then carried out using ANOVA to determine differences in the effect of extracts and standard therapy on the number of leukocytes in women with postpartum infections.

Table 1. Normality Assumption Test

Group	<i>p-value</i>	Distribution
	Number of Leukocyte	
(1) mothers with postpartum infection without therapy	0.832	Normal
(2) Healthy Postpartum mother	0.170	Normal
(3) Mothers with postpartum infection with standard therapy	0.245	Normal
(4) Mothers with postpartum infection with standard therapy and ekstrak	0.194	Normal

The ANOVA test showed data on leukocyte count levels obtained p-value = 0.002 ($p < 0.05$), so it can be interpreted that there were significant differences in leukocyte count levels between group 1 and group 3 given standard therapy and group 4 given standard therapy and extract of Karamunting leaves. Then, we found the difference between giving standard therapy and extracting karamunting leaves, which had the most significant effect on the HSD test (Table 2).

Table 2 shows that subset 1 contains data for groups 2,3 and 4, meaning that the average leukocyte count of the four groups does not have a significant difference. In this experimental research, group 2 and 1 differed significantly, while group 3 and 4 did not differ significantly from group 2.

Table 2 shows that the number of leukocytes in Group 4, given 200 mg/kg BW of Karamunting leaf extract, was significantly the same as Group 2 (healthy postpartum mothers) and Group 3 (mothers with postpartum infection with standard therapy). In other words, administration of karamunting leaf extract at a dose of 200 mg/kgBW proved that the number of leukocytes was the same as that of healthy postpartum mothers and functioned as an anti-inflammatory.

Table 2. The effect of the dose of Karamunting Leaf Extract on the Number of Leukocytes with the One Way Anova and HSD test

Group	N	Mean \pm SD		Notasi	p-value
		Subset 1	Subset 2		
(4) Mothers with postpartum infection with standard therapy and ekstrak	5	11334.29 \pm 3044.24		a	0.002
(3) Mothers with postpartum infection with standard therapy	5	13371.43 \pm 4577.39		a	
(2) Healthy Postpartum mother	5	14371.43 \pm 1560.72		a	
(1) mothers with postpartum infection without therapy	5		27737.14 \pm 14433.16	b	

* Repeated Measure ANOVA Test, with values are mean \pm SD n=5, p-value < 0.05 is considered significant

In previous studies testing, the ethanol extract of karamunting leaves turned out to affect reducing TNF α cytokine levels in mice models of postpartum infection induced by *E.coli*¹². There are several mechanisms for the content of antibacterial compounds in *Rhodomyrtus tomentosa*, including flavonoids containing phenolic compounds that can inhibit the growth of *E.coli*¹³. Tannins are anti-inflammatory, suppressing oxidation production by neutrophils, monocytes, and macrophages¹⁴. Thus, the ethanol extract of karamunting leaves can reduce TNF α cytokines so that, in other words, it functions as an anti-inflammatory with its metabolite compounds. Karamunting extract is given orally, reacts with mucus digestive enzymes, is absorbed quickly by the small intestine, and then is distributed through blood circulation, which then homes to lymphoid tissue. The presence of an antibacterial role in karamunting leaf extract causes many microbes to lyse so that macrophages eat the remains/debris that has been partially damaged¹⁵; macrophages, in this case after administration of the extract to postpartum mothers, experience a decrease in activation due to a decrease in the number of phagocytosed bacteria¹⁶, decreased Macrophage activation triggers a decrease in transcription factor activation (NF κ b), which results in a decrease in the proinflammatory cytokine TNF α . Karamunting leaf extract on lymphoid tissue stimulates lymphocyte proliferation, which can induce CD4+ T cells to turn into Th1 and Th2. T helper 2 will produce IL 4 and IL 10, stimulating B cell proliferation through B cell activation. IL 4 also plays an important role in producing IgG immunoglobulin, which binds to macrophages in the spleen for microbial destruction¹⁷.

Microbes enter through an epithelial barrier and are captured by antigen-presenting cells (APCs) in the tissue, or the microbes enter the lymph vessels or blood vessels. The microbe and its antigens are carried to the peripheral lymphoid organs, lymph nodes, and

spleen. The spleen is an important organ in the immune response and maintaining blood element homeostasis. A phagocytic filter removes bacteria from the bloodstream¹⁸.

By processing bacteria through macrophages as APCs to T cells, they will differentiate to the Th2 subset, increase B lymphocyte proliferation, and produce immunoglobulin A (IgA) and immunoglobulin G (IgG). IgA can bind to pathogens efficiently to prevent attachment of pathogenic bacteria. IgA-bound pathogens are efficiently recognized by phagocytes or bound by complement proteins. IgG targets pathogens to be eaten by phagocytic cells¹⁷. IgG links microbes and phagocytic cells to facilitate phagocytosis, activate oxidative pathways within phagocytes, and make it much more effective in killing pathogenic *E. coli* bacteria. It is hoped that the increase in the production of IgA and IgG antibodies in the immune system will eliminate the number of bacteria that cause infections in women with postpartum infections. The number of leukocytes in women with postpartum infection who were given standard therapy with extract of *Rhodomyrtus tomentosa* leaves showed a decrease or approached the normal value so that it had an anti-inflammatory effect in women with postpartum infection.

The limitations of this research are the small number of samples and variations in the severity of postpartum infections that cannot be controlled. Clinical trial research is needed with adequate samples and more uniform conditions for each sample.

CONCLUSION

The results of the research showed that administration of extract of *Rhodomyrtus tomentosa* leaves with standard therapy reduced the number of leukocytes close to its normal value, so it could be concluded that it had an anti-inflammatory effect in women with postpartum infections. Further clinical trial research is needed with adequate samples and more uniform conditions for each sample.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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