Laporan Hasil Penelitian

THE EFFECT OF PAGODA LEAF EXTRACT (*CLERODENDRUM PANICULATUM L*) ON THE IL-10 LEVEL IN MAMMAE OF FEMALE RATS STRAIN (*SPRAGUE DAWLEY*) INDUCED WITH *STAPHYLOCOCCUS AUREUS* BACTERIA

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ABSTRACT

Mastitis is an infectious disease problems of found in breast feeding usually caused by *Staphylococcus aureus*. When *Staphylococcus aureus* bacteria enters the body's tissues, IL-10 immune will active helped prevent cell broken. The immune system will get worse if there is no treatment. So that it takes complementary therapies like pagoda leaves, The content of the pagoda contains tannins, alkaloids and flavonoids, where is this compound can to improve immune system. This study aims to determine the effect of pagoda leaf extract on IL10 levels in rats induced by *Staphylococcus aureus* bacteria. The type of research used in this study is true experimental with pre and posttest control design. The use of 15 Sprague Dawley strain rats divided into 3 groups: 5 negative control groups (aqua pro injections 1ml/gramBB) five tails were all given standard feed, positive control group (Amoxicilin with 9.6ml / 250gramBB) five tails were all given standard feed, and treatment group (Amoxicilin 9.6ml / 250gramBB plus pagoda leaf extract 50ml/250gramBB) five tails were all given standard feed. All groups were induced bacteria *Staphylococcus aureus* (0,2 mlx10^8ml/CFU). IL-10 levels were examined using the R & D method of the Enzyme Linked Immonosorbent Assay (ELISA) Rat system. Data analysis used ANOVA + post hoc test, with a significant value $\rho \leq 0, 05$. This study showed no difference in levels of IL10 cytokines, before induced *Staphylococcus aureus* bacteria in all groups with values ($\rho > 0.05$). Whereas after the treatment was given, the value of $\rho < 0.05$) between all groups showed that there was a difference in IL10 levels after treatment between all groups. In the negative control group, IL10 experienced a decrease (mean ± 10.4 pg / ml). While the increase in IL-10 levels was greater in the treatment group (mean ± 58.4 pg / ml), compared to the positive group (mean ± 41.8 pg / ml). The conclusion in this study is that pagoda leaf extract (*Clerodendrum Paniculatum L*) has an influence in increasing IL-10 levels as an anti-inflammatory. Pagoda leaf extract can be used as a complementary therapy

Keywords : *Staphylococcus aureus , IL-10, Ekstrak Daun Pagoda*
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ABSTRAK

Mastitis adalah masalah penyakit infeksi yang ditemukan pada ibu menyusui yang biasanya disebabkan oleh *Staphylococcus aureus*. Ketika bakteri *Staphylococcus aureus* masuk ke jaringan tubuh, kekebalan IL-10 akan aktif membantu mencegah sel- sel rusak. Sistem imun akan memburuk jika tidak ada penanganan. Sehingga dibutuhkan terapi komplementer seperti tanaman daun pagoda, kandungan dari tanaman daun pagoda terdiri dari *tannins, alkaloids and flavonoids*, di mana senyawa ini dapat meningkatkan sistem kekebalan tubuh. Jenis penelitian yang digunakan dalam penelitian ini adalah *true experimental* dengan rancangan *pre* dan *posttest control design*. Yang menggunakan tikus strain *Sprague Dawley* sebanyak 15 ekor yang dibagi dalam 3 kelompok yaitu kelompok kontrol negatif (*aqua pro injections)* sebanyak 5 ekor semuanya diberikan pakan standar, kelompok kontrol positif (*Amoxicilin* dengan dosis 9,6ml/250gramBB) sebanyak 5 ekor semuanya diberikan pakan standar, dan kelompok perlakuan (Amoxicilin 9,6ml/250gramBB ditambah dengan ekstrak daun pagoda (50ml/250gramBB) 5 ekor semuanya diberikan pakan standar. Semua kelompok diinduksisikan bakteri *Staphylococcus aureus* 0,2 ml/CFU. Pemeriksaan kadar IL-10 menggunakan metode *R & D system Enzyme Linked Immonosorbent Assay* (ELISA) *Rat*. Analisis data menggunakan uji ANOVA + post hoc, dengan nilai signifikan $\rho \leq 0, 05$. Penelitian ini menunjukkan tidak ada perbedaan kadar sitokin IL-10, sebelum diinduksikan bakteri *Staphylococcus aureus* pada semua kelompok dengan nilai ($\rho > 0,05$).. Sedangkan setelah diinduksikan bakteri *Staphylococcus aureus* ± 24 jam semua kelompok mengalami penurunan kadar IL10 dengan nilai ($\rho > 0,05$). Setelah diberikan treatment diperoleh nilai $\rho < 0,05$) antara semua kelompok hal ini menunjukkan ada perbedaan kadar IL-10 setelah diberikan treatment antara semua kelompok. Pada kelompok kontrol negatif kadari IL-10 mengalami penurunan (rerata ± 10,4 pg/ml). Sedangkan peningkatan kadar IL-10 lebih besar pada kelompok perlakuan (rerata ± 58.4 pg/ml), dibandingkan pada kelompok positif (rerata ± 41.8 pg/ml). Kesimpulan dalam penelitian ini adalah ekstrak daun pagoda (*Clerodendrum Paniculatum L)* memiliki pengaruh dalam meningkatkan kadar IL-10 sebagai anti-inflamasi. Ekstrak daun pagoda dapat dijadikan terapi komplementer.

Kata kunci : *Staphylococcus aureus,* IL-10, Ekstrak Daun Pagoda
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INTRODUCTION

Breast inflammation or commonly referred to as mastitis is a disease that can be accompanied by infection or not accompanied by infection, while the type of bacteria that most often causes mastitis is the *Staphylococcus aureus* bacteria. This disease usually accompanies lactation, so it is also called lactational mastitis or commonly referred to as puerperal mastitis. This situation can be fatal if it is not handled properly. Mastitis generally occurs around 1 to 6 weeks post partum. The complications that will occur in mastitis are abscess in the breast. The incidence of mastitis according to the world health organization estimates that more than 4.1 million people are diagnosed with mastitis, and an estimated 3-20% of breastfeeding mothers can experience mastitis, 1 until 10 women (10%) who experienced mastitis in the first month after delivery. The signs and symptoms of mastitis such as: Fever in the body, especially in the breast and experience inflammation of the breast accompanied by pain. The causes of mastitis include: starting from being infected by bacteria, scuffed nipples, improper breastfeeding techniques, infrequent breastfeeding frequency or short breastfeeding times, and overly fast weaning and attachment of babies to poor breasts, and babies who only sucking nipples (not including the areola) causing the nipple to squeeze between the gums or lips so that the milk flow is imperfect (WHO, 2000: Tri Anasari, Sumarni. 2014:

Amir H Lisa, et al, 2014: IDAI 2013; Anasary Tri Anasari, Sumarni. 2014 ; al, 2012; Dixon Michael J, et. al 2011: Khanal Vishnu, et al. 2015: Viduedo Silva, et al 2015: Baratawidjaja K, et al, 2014; Manzanillo Paolo, et al, 2015).

Interleukin 10 (IL-10) including type II cytokines. This type of cytokine has an organizational form and binds to receptors (IL-10) is an anti-inflammatory cytokine containing anti-inflammatory properties that can suppress the pro-inflammatory reaction Interleukin 10 has a role in limiting the immune response to pathogens, thus preventing damage to tissues and maintaining connective tissue homeostasis. Interleukin-10 levels as anti-inflammatory cytokines will decrease if there is inflammation in the body (Couper N Kevin, et al 2008; Rayitno A, et al, 2014; Garcia YF, et al, 2011; Iyer Subramanian S, et al, 2012; Kumar Ashok, et al, 2000; AmirH. Lisa, et al, 2014)

Inflammation when left without treatment will result in autoimmune or autoinflamation disorders, neurodegenerative diseases, or cancer. As for treatment so far which is still often used for inflammation is conventional treatment. Even though there are now many herbal plants that can be used as complementary therapies, one of them is the pagoda leaf plant (*Clerodendrum paniculatum L*: Hafiz Ihsanul, et al 2016). Shrivastava, et al, 2007).

From the phytochemical test results found that pagoda plants contain active compounds such as: flavonoids, tannins, alkaloids, phenolic acids, sterols, glycosides, phenolic acids, and glycosides (Joseph Jeenu, 2013). From the results of research conducted in India showed that pagoda leaves are also used as traditional medicine in Korea, for *HIV, Syphilis, typhoid, Cancer*, Jaundice, hypertension (M. Praveen, et al., 2012; Thakur, et al., 2013)

From the various facts facts above which suggest that pagoda plants on the part of the pagoda roots and flowers contain active compounds that function as anti-inflammatory agents which are so effective in curing various diseases such as like cancer, infections, boils. In certain areas the pagoda is strongly believed to be a complementary therapy in certain ınterested ın conductıng research for pagoda leaves for mastitis where the title of the penetration was the effect of giving extracts of pagoda leaves (*clerodendrum paniculatum L)* to the levels of il-10 in female mice induced by the bacterium *staphylococcus aureus* " besides that pagoda leaves are available especially in South Sulawesi, Elisa examination can be done at the Hasanuddin University Hospital in Makassar. As well as research on the benefits of pagoda leaf extract as an anti- inflammatory on mammae sprague dawley mice induced by *Staphylococcus aureus* bacteria has never been done

METHODS

The type of research used in this study is pure experimental or true experimental, which is an experiment conducted in the laboratory, with pre and post test control design. The group was divided into 3 (three) groups: negative control group, positive control group, treatment group.

This research was carried out in the UNHAS Hospital laboratory for examination for *Enzyme Linked Immunosorbent Assay* (ELISA) and culture of *Staphylococcus aureus bacteria*, Biopharmaca laboratory for drying plants, Pharmacy Laboratory of

drying plants, Pharmacy Laboratory of State Islamic University (UIN) Makassar to extract and phytochemical testing, animal laboratory UNHAS for the process of rats adaptation to the end of the treatment given.

The sample in this study were Sprague Dawley rats with a body weight of about 200- 250 grams. With use of 15 rats strain Sprague Dawley divided into 3 groups: 5 negative control groups (aqua pro injections 1ml/gramBB) were all given standard feed, positive control group (Amoxicilin with 9.6ml / 250gramBB) as many as 5 rats were all fed standard, and group feeding (Amoxicilin 9.6ml / previously the pagoda leaves were picked ± 5 kg of raw leaves, after that it is cleaned first, then sifted to obtain a smooth simplicia sample. After that the sample is ready to be extracted by maceration method, so that it is obtained ± 500 gram pagoda thick extract. The experimental animals used in this study were rats strain Sprague Dawley with weight 200-250 grams. And needed *Aqua pro injection* (for negative group), and *amoxicillin* 500 mg, (For positive groups), and the bacteria used are *Staphylococcus aureus bacteria strain* standard . To make a sample of bacteria by making a suspension in solution NaCl as much 10 ml mixed with *Staphylococcus aureus* bacteria colonies that golden yellow with turbidity level Mc Farland 2 x 10^8 CFU. Accuracy of turbidity level Mc Farland measured by the Densi check tool. So that the dosage is injected into as many rats as possible 0,2 mlx10^8 ml/CFU. Bacterial induction is carried out by intramuscular technique in the breast part precisely in the lactiferous duct in rats in all groups (Negative, positive and treatment) after the induction of bacteria is then observed ±24 hours later to see an infection reaction in the rat breast area (There are signs of infection in the breast)

The research data collection instrument was carried out by method *Enzyme Linked Immunosorbent Assay* (ELISA) to measure IL-10 levels. *Histopathology* test done at the hospital University of Hasanuddin Makassar. The tissue taken for the sample is breast tissue in all groups.

This research was approved and issued by the Ethical Commitment of the University of Hasanuddin Makassar on February 5, 2018 with No. 116 / H4.8.4.5.31 / PP36-KOMETIK / 2018.

Data is processed and analyzed using computer assistance, to see the effect of giving pagoda leaves (*Clerodendrum paniculatum L*), to levels of IL10 cytokines. IL-10 levels are seen using the method *Enzyme Linked Immunosorbent Assay* (ELISA). Three levels of IL-10 were examined (3) time, blood collection first when rats have not been treated to assess whether IL-10 levels are normal or not, then taking blood the second time after the mouse was induced by *Staphylococcus aureus* bacteria, and taking blood last time when mice were given good in the negative, positive and treatment groups.

The bivariate test uses a test *one way anova* to see differences in IL-10 levels in the negative control group before, during and after treatment, positive control before, during and after being given treatment, and treatment group before, during and after being given treatment and done. But if the data is not normally distributed then a test is carried out *Kruskal-Wallis*. other than that also done test *repeted anova* if the data is normally distributed to see differences in IL-10 levels in each group, namely the negative, positive, and treatment groups before, during and after being given treatment.

RESULTS

Table 1. Phytochemical test results of the compound of Leaf Pagoda (Clorodendum Paniculatum L).

Sample Nama	Identification of Compound Groups			
	Flavonoid	Steroid/ tripernoid	Alkaloid	Tanin
DaunPagoda (*Clorodendum Paniculatum L).*	+	--	+	+

Describsion : + (positive): there are indications of bioactive compounds

Table 2. The mean body weight of mice in each group

Weight	**Mean ± SD**	**Min-Maks**	**Nilai ρ**
Negative control group	226 ± 12	213 – 239	0, 56
Poaitive control group	220 ± 8	208 – 233	
Treatment Group	220 ± 10	208 – 231	

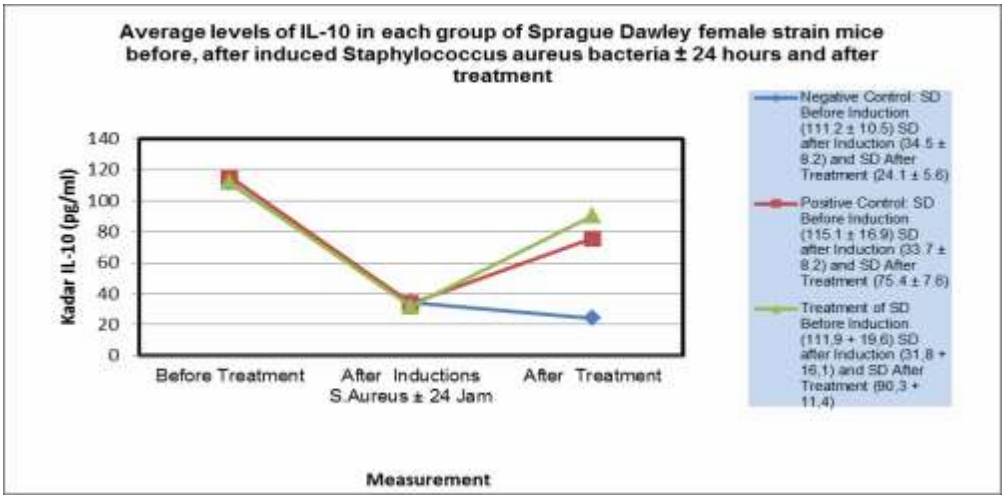
*One way ANOVA. Significant value ρ 5 0.05. There were no differences in body weight in all groups, both in the negative, positive and treatment control groups (ρ = 0.56)

Table 3:Average differences in IL-10 levels in each female rat group Sprague Dawley strain before, after induction of *Staphylococcus aureus* bacteria and after treatment.

Group	IL-10 levels (pg / ml)			**Value ρ**
	Before Induction	**After Induction**	**After *Treatment***	
	***Mean* ± SD**	***Mean* ± SD**	***Mean* ± SD**	
Negative Control (n =6)	111,2 ± 10,5	34,5 ± 8,2	24,1 ± 5,6	0.00[a]
Positif Control (Antibiotic *Amoxicilin* 9,6 ml/250grBB) (n =6)	115,1 ± 16,9	33,7 ± 8,2	75,4 ± 7,6	0.001[a]
Treatment (Antibiotic *Amoxicilin* 9,6ml/250grBB + pagoda leaf extract50 ml/grBB (n =6)	111,9 + 19,6	31,8 + 16,1	90,3 + 11,4	0,003
Nilai ρ	0, 58[b]	0.68[b]	0.000	

The results of the statistical test of one way ANOVA before induced *Staphylococcus aureus* bacteria in each group obtained the value ρ = 0.58 greater than the value of α = 0.05. This means that there is no significant difference in the levels of IL-10 cytokines before induced *Staphylococcus aureus* bacteria between the negative, positive and treatment control groups. Whereas IL-10 levels after induced *Staphylococcus aureus* bacteria ± 24 hours in each group obtained values ρ = 0.68 greater than the value of α = 0.05.

Picture 1: Trend of IL-10 levels in each group of female rats with Sprague Dawly strain before, after induced Staphylococcus.Aureus and after treatment..



Description: IL-10 levels in each group before induced *Staphylococcus aureus bacteria* (homogeneous), and after induced *Staphylococcus aureus* bacteria all groups experienced a decrease in IL-10 levels. Whereas after giving the treatment the positive control group and the treatment increased, the group while the negative control group decreased.

Table 4: Analysis of differences in IL-10 levels before, after induced *Staphylococcus aureus bacteria* and after treatment in each group

Measurement	Mean ± SD	Difference Mean	Nilai ρ*	Nilai P
Before Treatment				
Negative Control Positive Control	111,2 ± 10,5 115,1 ± 16,9	-3.8	1,00	0,58
Negative Control Treatment	111,2 ± 10,5 120,4 ± 16,7	-9.1	0,92	
Positive Control Treatment	115,1 ± 16,9 120,4 ± 16,7	-5.3	1,00	
After Inductions bakteria *s. aureus*				
Negative Control	34,5 ± 8,2	0,8	1,00	
Positive Control Negative Control Treatment	33,7 ± 8,2 34,5 ± 8,2 30,8 ± 6,6	3,7	1,00	0, 68
Positive Control Treatment	33,7 ± 8,2 30,8 ± 6,6	2,9	1,00	
After *Treatment*				
Negative Control Positive Control	24,1 ± 5,6 75,4 ± 7,6	-51.3	0.00	0,00
Negative Control Treatment	24,1 ± 5,6 90,3 + 11,4	-66.2	0.00	
Positive Control Treatment	75,4 ± 7,6 90,3 + 11,4	-14.9	0.56	

Table 5: Analysis Of Differences In Il-10 Levels Between Groups Of Female Sprague Dawley Strain Rats Before, After Induced By *Staphylococcus aureus* Bacteria And After Treatment.

Measurement	Mean ± SD	Difference Mean	Value ρ*	Value P
Negative Control				
Before After induction *s.aureus*	111,2 ± 10,5 34,5 ± 8,2	76,7	0,000	0.000
Before After *treatment*	111,2 ± 10,5 24,1 ± 5,6	87,1	0,000	
After induction *s.aureus* After *treatment*	34,5 ± 8,2 24,1 ± 5,6	10,4	0,049	
Positive Control				
Before After induction *s.aureus*	115,1 ± 16,9 33,7 ± 8,2	81,4	0,000	
Before After *treatment*	115,1 ± 16,9 75,4 ± 7,6	39,6	0,008	0, 001
After induction *s.aureus* After *treatment*	33,7 ± 8,2 75,4 ± 7,6	-41,8	0,000	
Treatment				
Before After induction *s.aureus*	111,9 + 19,6 31,8 + 16,1	80.0	003	0,002
Before After *treatment*	111,9 + 19,6 90,3 + 11,4	21.6	.064	
After induction *s.aureus* After *treatment*	31,8 + 16,1 90,3 + 11,4	-58.4	.001	

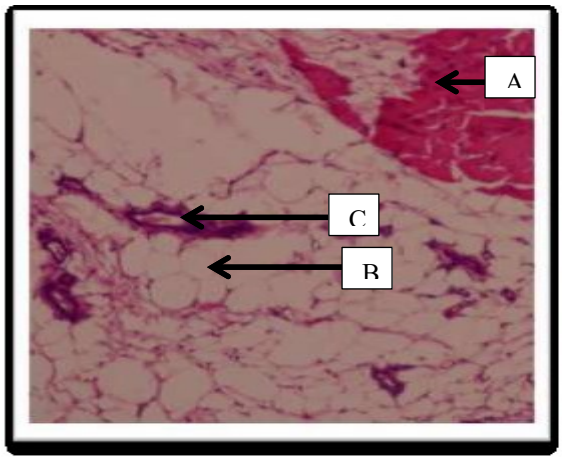
*Repetead ANOVA + post hoc in the treatment group before and after being given treatment obtained the value ρ = 0.64 greater than the value of α = 0.05. This shows no difference in levels of IL-10 cytokines before and after treatment in the treatment group. Whereas in the negative and positive control groups obtained a value of ρ <0.05, both before, after induction s. aureus ± 24 hours and after treatment.

Table 6: Analysis of Velocity in the positive control group and treatment group

Group	Persentase (%)	Difference Persentase (%)
Positive Control (Antibiotik *Amoxicilin* 9,6 mg/250grBB) (n =6)	133.4	138
Treatment (Antibiotik *Amoxicilin* 9,6 ml/250grBB + ekstrac Pagoda Leaft 50 ml/grBB (n =6)	271.5	

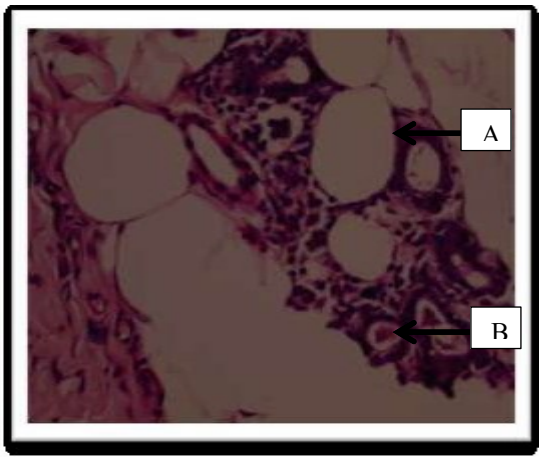
Showing the results of velocity test in the positive control group obtained 133.4% antibiotics can increase levels of IL-10 cytokines, whereas in the treatment group obtained 271.5% antibiotics and pagoda leaf extract can increase levels of IL-10 cytokines, with the difference between positive control groups and the treatment group was 138%. This shows that pagoda leaf extract can increase the levels of IL-10 cytokines by around 138%.

Picture 2 : Histopathology test results



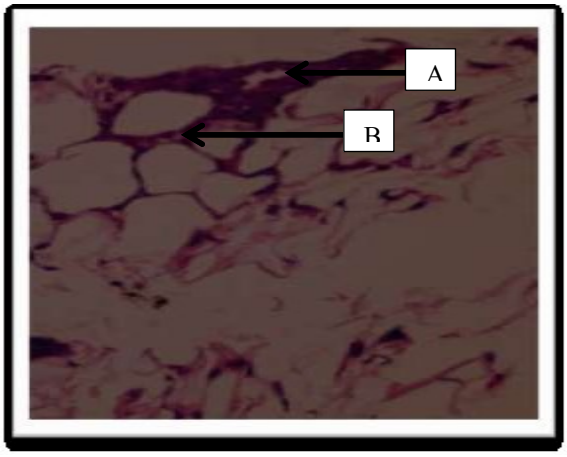
Picture 1.Histopatologi normal

(A) Tampak adanya pembuluh darah,
(B) Tampak adanya sel epitel yang normal
(C) Tampak adanya duktus laktiferus.



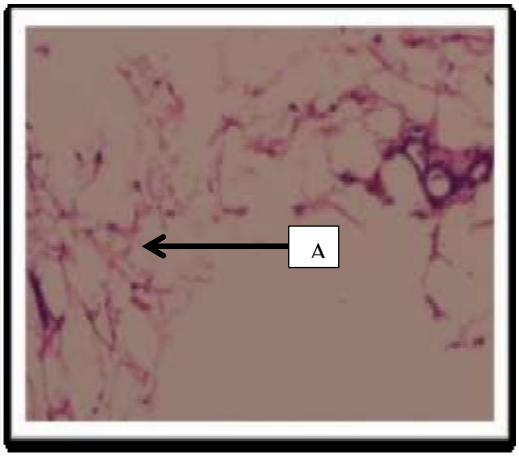
Picture 2.Histopatologi Negative

(A)Tampak adanya sel-sel radang PMN disekitar jaringan ikat
(B)Tampak adanya sel radang disekitar lapisan sel epitel



Picture 3.Histopatologi Positive group

(A)Tampak adanya sel-sel radang PMN yang mengelilingi kelenjar susu
(B) Tampak adanya sel radang disekitar jaringan ikat.



Picture 4.Histopatologi Treatmen

(A)Tampak adanya sel-sel radang PMN disekitar jaringan ikat

(Figure I) Microscopic picture of the breast of the normal control group showed that the lactiferous duct was coated with normal epithelial cells and surrounded by connective tissue and blood vessels (Figure II) Microscopic images of the mice of the negative control group, namely the group induced by *Staphylococcus aureus* bacteria without treatment, it was shown that there were inflammatory (± 100) Polymorphonuclear (PMN) cells around the connective tissue which were not found in the normal control group. It appears that thickening of the epithelial cell layer surrounding the lactiferous duct (Figure III) Microscopic picture of the breast of the mouse in the positive control group is the group induced by *Staphylococcus aureus* and given 9.6ml / 250grBB of amoxilcillin antibiotics per day orally for 5 (five) days showing the cell - inflammatory cells (± 70) Polymorphonuclear (PMN) around the connective tissue and surrounding epithelial cells and reduced lactiferous ducts (Figure IV) Microscopic images in the mice breast of the treatment control group ie the group induced by *Staphylococcus aureus* and given antibiotics amoxilcillin at a dose of 9, 6 ml / 250grBB + pagoda leaf extract with a dose of 50 ml / grBB per day orally plus pagoda leaf extract 50 ml / g BB for 5 (five) days and showed inflammatory cells (± 34) Polymorphonuclear (PMN) around the connective tissue , the mammary gland and epithelial cells are reduced.

DISCUSSION

The results of the one way ANOVA test showed no significant difference in rat body weight for all groups, both in the negative, positive and treatment control groups. Whereas the results of the one way ANOVA test showed that there was no significant difference in levels of cytokine IL-10 before induction of *Staphylococcus aureus* bacteria, both in the negative, positive and treatment controls. This indicates that before inducing the *Staphylococcus aureus* bacteria the condition of mice in each group was not in a healthy condition. After the rats were ascertained in a healthy condition, it was continued by inducing *Staphylococcus aureus* bacteria (0.2 mlx108ml / CFU). All groups were induced by bacteria so that the rats were exposed to bacteria.

In the negative control group, 1 ml / 200gram BB mice aqua pro injection was given. In this group, levels of IL-10 cytokines were decreased. This is due to an immune response in the body called pro inflammation. IL-10 cytokines are no longer able to suppress proinflammatory consequences, there is an inflammatory response or infection.This is in line with research conducted by John M. Leech who got interleukin-10 levels decreased after 24 hours after induced *Staphylococcus aureus* bacteria, so that mice will be exposed. Even up to 7 days the level of Interleukin 10 has decreased dramatically, if not given treatment such as antibiotics. (Jawetz, 2005: Ziegler Christina, 2011: Li Huimin, et al, 2017: jawetz, 2005: Ziegler Christina, 2011, John M. Leech, 2017: Manzanillo Paolo, et al, 2015: Ziegeler Christina, et al, 2011) (John M. Leech,2017: Manzanillo Paolo,*et al*, 2015: Ziegeler Christina,*et al*, 2011).

In the positive control group, rats were induced by *Staphylococcus aureus* bacteria (0.2 ml x 108 ml / CFU) and given Amoxicilin treatment with a dose of 9.6 mg / ml / 250 gBB mice for 5 days. The results showed that there were differences in levels of IL-10 cytokines before bacterial induction and after antibiotic administration. The result is clear that there are differences between groups before, during and after being given Amoxicilin treatment with a dose of 9.6mg / ml / 250gramBB (S Jahanfar, et al. 2013, WHO, 2000; Guideline and Procedure Manual, 2012, IDAI, 2013: Guidelines, Mastitis And Breastfeeding, 2015: Katari Kamal, et al, 2012: The Women, 2012). Researchers thought that in the positive

group, with a single therapy Amoxicilin antibiotics were better, (can increase levels of cytokines IL-10) compared to just using aqua pro injection. In the treatment group induced by *Staphylococcus aureus* bacteria (0.2 mlx108 ml / CFU), antibiotics were given Amoxicilin at a dose of 9 mg / ml / 250gramBB + pagoda leaf extract at a dose of 50 mg / KgBB mice for 5 days). The result is clear that there are differences between groups before, during and after being given Amoxicilin and pagoda leaves. It was shown that the group given Amoxicilin + pagoda leaf extract had increased levels of IL-10 cytokines 2-fold compared to the positive control group and negative controls. The results of the histopathology test in the treatment group seen inflammatory cells.

This is in line with the results of research where Pagoda leaves contain active compounds such as tannins and flavonoids. Compounds in flavonoids can inhibit the production of pro-inflammatory cytokines, such as tumor-necrosis factor (TNF-α), inflammatory protein macrophages-1, and activate and increase interleukin-10 (IL-10), where IL-10 is activated by monocytes. Flavonoids are polyphenolic compounds that occur ubiquitously on plants. They are found to have antimicrobial, antiviral, anti-ulcerogenic, cytotoxic, anti-neoplastic, mutagenic, antioxidant, antihepatotoxic, antihypertensive, hypolipidemic, antiplatelet and anti- inflammatory activities. Flavonoids also have biochemical effects, which inhibit a number of enzymes such as aldose reductase, xan-Mu oxidase, phosphodiesterase, Ca2ATPase, lipoxygenase, cycloxygenase. Flavonoids have anti-inflammatory activity in both the proliferative and exudative phases of inflammation. (Rathee Rermender 2009: Hafiz Ihsanul, *et al* 2016). Shrivastava, *et al*, 2007:Kumar; 2000 & John M. Leech, et.al, 2017 : (Shrivastava, N Patel, *et al*. T.2007. Musa Weni, *Et al*, 2009: Hoensch HP et al.2018)

CONCLUSION

Based on the description above, researchers assume that pagoda leaf extract can be used as a complementary therapy in the treatment of mastitis because it can increase levels of cytokines Interleukin-10 which acts as an anti-inflammatory so that it can suppress the inflammatory response / infection that occurs when exposed to microorganisms.

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