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Application of *Scaevola taccada* (Gaertn.) Roxb. Reduce Pro-inflammatory Cytokines Interleukin-1 β in Sprague Dawley Mice Suffering from Mastitis

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Abstract

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BACKGROUND: Mastitis is an inflammation of the breast tissue, usually caused by bacteria. Mastitis stimulates pro-inflammatory cytokines. The cytokine interleukin-1 β (IL-1 β) is a crucial mediator of the inflammatory response. This cytokine has adverse effects of hosting immunity that mediates resistance to pathogens and also exacerbates damage during chronic disease and acute tissue injury. *Scaevola taccada* (Gaertn.) Roxb. has been used as an ethnomedicine for healing sores in several provinces in Indonesia.

AIM: This study aimed to assess the profile of pro-inflammatory cytokine IL-1 β through the treatment effect of leaf extracts of *S. taccada* (Gaertn.) Roxb. as adjuvant for healing mastitis.

METHODS: This study was a true control group experiment using the pre-test-post-test control design aimed to measure the effect of hydroalcoholic compounds in leaf extracts of *S. taccada* on the systemic pro-inflammatory activity of interleukin-1 β (IL-1 β). The treated animals were 18 mice of Sprague Dawley strain induced by *Staphylococcus aureus*. These treated mice were divided into three groups in which each group consisted of six mice. The mice in the Group I (negative control) were given 1 ml aquabides/250 g body weight, those in the Group II (positive control) were delivered with 9.6 mg/ml amoxicillin/250 g body weight, and those in the Group III (experimental) were given 9.6 mg amoxicillin/250 g body weight + 400 mg/ml leaf extracts of *S. taccada*/g body weight for 5 days, respectively. Pathological examinations were carried out from the inflamed tissues to prove the healing process of the treated mice. IL-1 β levels were determined using the enzyme-linked immunosorbent assay method. Data were analyzed using ANOVA and *post hoc* tests.

RESULTS: There were statistically significant differences of IL-1 β levels after the administration of leaf extracts of *S. taccada* among all the treated mice groups at $p < 0.05$. The Group III had the lowest IL-1 β level with the mean value ± 1.45 pg/ml compared to the IL-1 β level in the Group II (positive control) with the mean value ± 3.82 pg/ml and the IL-1 β level in the Group I (negative control) with the mean value ± 5.22 pg/ml. The pathological analysis of breast tissues of the treated mice proved that leaf extracts of *S. taccada* (Gaertn Roxb.) could reduce damaged tissues, cellular infiltration, and subcutaneous edema induced by this pathogenic microorganism.

CONCLUSION: Leaf extracts of *S. taccada* had a significant function as adjuvant for healing mastitis by reducing pro-inflammatory cytokine IL-1 β .

Introduction

Scaevola taccada (Gaertn. Roxb.) is a dense, spreading shrub that grows in coastal areas, precisely in the waters of tropical regions. This plant is commonly found in Indonesia, including in coastal areas in Makassar Strait. The specific characteristics of this plant include green leaves that contain a lot of water, the flowers are white, and the fruits are green [1], [2], [3].

S. taccada (Gaertn) Roxb. contains chemical compounds such as flavonoid, lipid, terpenoid, alkaloid, and saponin. This plant is commonly used as ethnomedicine in Indonesia, particularly for healing wounds. It is also utilized for healing various diseases that include beriberi, infection of the eye, sore,

dysentery, skin disease, coughs and influenza, malaria, tuberculosis, pain due to menstruation, headache, injury, and contraception drugs. In addition, it has significant functions as antitumor, anti-inflammatory, antibacterial, antifungal, antioxidant, and antiviral effects. Local people generally process this plant as the traditional medicine by boiling method, and directly put the concoction of this plant on injured parts of the body in the form of squeezed juices [4], [5], [6], [7].

IL-1 β belongs to a member of the IL-1 family that plays significant functions as a pro-inflammatory cytokine, and it has agonist activity. IL-1 β is seldom or even unexpressed in healthy cells or tissues. However, this pro-inflammatory cytokine is rapidly expressed in cells or activated by receptors during the invasion of pathogens or damaged cells [8], [9].

IL-1 β is produced by hematopoietic cells. The secretion of IL-1 β is regulated through intracellular and extracellular multiprotein complexes that require caspase-1 to activate, cleavage, and secrete an active cytokine. When injury or inflammation takes place in various conditions, IL-1 β is activated by inducing reaction of inflammation and transmission of pain at different levels; even it causes chronic pain [10], [11].

Mastitis is an inflammation of the breast due to bacteria. This inflammation disease is caused by *Staphylococcus aureus* that stimulates the secretion of pro-inflammatory cytokine IL-1 β that induces inflammation [12], [13].

This study aimed to analyze the profile of IL-1 β before and after the administration of leaf extracts of *S. taccada* (Gaertn) Roxb. to the mastitis model mice of Sprague Dawley strain.

Materials and Methods

Materials

Fresh leaves of *S. taccada* (Gaertn) Roxb. were collected from the local coastal areas in Makassar Strait, located in Subdistrict Suppa, Pinrang District, South Sulawesi Province, Indonesia.

Extraction process

Fresh leaves of *S. taccada* (Gaertn) Roxb. were washed well with water, and then they were cut into small pieces. After that, the fresh leaves dried using herbs dryer at 45°C for 4 days. Subsequently, the dried leaves of *S. taccada* (Gaertn) Roxb. were grinded and filtered with the 40 mesh size to gain refined powders, and then they were repeatedly macerated using 70% ethanol for 3 days. Afterward, the macerated leaves evaporated using a rotary Rotavapor until formed congealed extracts, and then they were weighted with 400 mg/gr body weight [2], [6].

Phytochemical screening

The congealed leaf extracts of *S. taccada* (Gaertn) Roxb. were screened using phytochemical screening to identify their bioactive compounds that contained flavonoid, alkaloid, steroid, saponin, and tannin [14], [15].

Bacterial culture

The bacterial strain used in this study was *S. aureus*. This bacterial species is a common strain that induces mastitis. Strains of *S. aureus* were derived

from the Laboratory of Microbiology at the General Hospital of Hasanuddin University in Makassar, and those bacteria were cultured on Mannitol Salt Agar media based on the standard requirement of *S. aureus* McFarland of 10⁵–10⁸ ml/cells.

Test method on the experimental animals

The method used in this study was a true control group experiment using the pre-test-post-test control design. The experimental animals were mice of Sprague Dawley strain. The numbers of treated animals were 18 mice with 200–250 g body weight. The treated mice were divided into three groups, and each group consisted of six mice. The mice of Group I (negative control) were given 1 ml aquabides/250 g body weight, Group II (positive control) were given 9.6 mg amoxicillin /250 g body weight, and Group III (treatment) were given 9.6 mg amoxicillin/250 g body weight + 400 mg leaf extracts of *S. taccada*/gr body weight, respectively.

IL-1 β levels were measured in three replications for all the treated mice groups. The measurement I was conducted before induction of *S. aureus*. The measurement II was performed in \pm 24 h later after induction of *S. aureus* with the dose of 0.1 ml/10⁵–10⁸ cells that affected breast tissues of lactiferous ducts in the treated mice. The measurement III was done 5 days later after treatment for each treated group.

Pathological examinations of breast tissues were then conducted for each treated group to prove the healing process. IL-1 β levels were determined using the enzyme-linked immunosorbent assay.

Statistical analysis

Data presented in the form of mean value \pm standard deviation at confidence interval 95%. ANOVA test was used to determine the statistically significant differences among all the treated mice groups after the measurement of IL-1 β levels.

Results

Results of phytochemical screening were qualitatively done in leaf extracts of *S. taccada* (Gaertn) Roxb. by giving 70% ethanol, and their extracts contained bioactive compounds that include alkaloid, flavonoid, terpenoid, saponin, and tannin.

As indicated in Table 1, the pro-inflammatory cytokine of IL-1 β in all the three treated mice groups for the measurements I, II, and III showed statistically significant differences within the critical limit value of $p < 0.05$. Therefore, there were substantial differences

Table 1: The profile of cytokine pro-inflammatory IL-1 β in through the administration of leaf extracts of *Scaevola taccada* (Gaertn.) Roxb. in mastitis

Group	Treatment	Measurement of IL-1 β levels			p-value
		I (Before induction by <i>S. aureus</i>)	II (\pm 24 h later after induction by <i>S. aureus</i>)	III (After treatment for 5 days)	
Group I (negative control)	1 ml aquabides/250 g body weight	Mean \pm SD 0.53 \pm 0.37	Mean \pm SD 5.79 \pm 0.67	Mean \pm SD 11.02 \pm 2.54	0.00*
Group II (positive control)	9.6 mg amoxicillin/250 g body weight	Mean \pm SD 0.54 \pm 0.28	Mean \pm SD 6.54 \pm 1.01	Mean \pm SD 3.82 \pm 0.30	0.00*
Group III (Treatment)	9.6 mg amoxicillin/250 g body weight + 400 mg leaf extracts of <i>Scaevola taccada</i> (Gaertn.) Roxb./kg body weight	Mean \pm SD 0.63 \pm 0.23	Mean \pm SD 6.10 \pm 1.14	Mean \pm SD 1.45 \pm 0.59	0.001*

*Repeated ANOVA. All the treated mice groups showed statistically significant value within the critical limit value of $p < 0.05$. *S. aureus*: *Staphylococcus aureus*, IL-1 β : Interleukin-1 β .

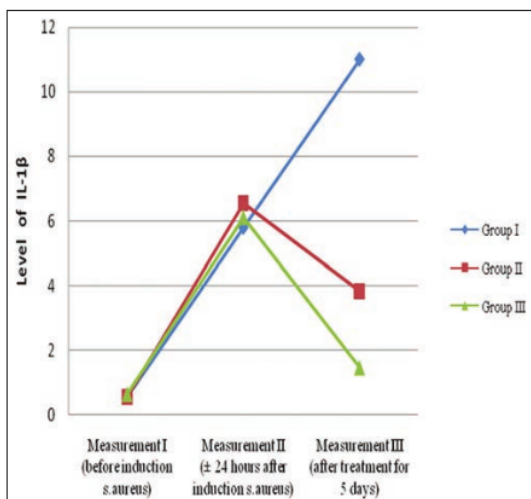


Figure 1: The graph of the cytokine pro-inflammatory IL-1 β levels in mastitis through the effect of leaf extracts of *Scaevola Taccada* (Gaertn.) Roxb

in IL-1 β levels in all the treated groups for the measurements I, II, and III.

In the Group I, the IL-1 β levels increased for the measurement I until III with the mean value \pm 10.48 pg/ml at $p = 0.00$. Therefore, there were statistically significant differences in IL-1 β levels during measurement.

In Group II, the IL-1 β levels increased for the measurement I until III with the mean value \pm 6.0 pg/ml. Then, the IL-1 β levels reduced after the treatment of 9.6 mg amoxicillin/250 g body weight with the mean value \pm 2.72 pg/ml at $p = 0.00$. This indicated that there were statistically significant differences in IL-1 β levels during measurement.

In Group III, the IL-1 β levels increased for the measurement I until III with the mean value \pm 5.47 pg/ml. Then, the IL-1 β levels reduced after the treatment of 9.6 mg amoxicillin/250 g body weight + 400 ml leaf extracts of *S. taccada* (Gaertn.) Roxb. with the mean value \pm 4.57 pg/ml at $p = 0.01$. This showed that there were statistically significant differences in IL-1 β levels during measurement (Figures 1 and 2).

Description of the figures: Figure 1: Microscopic examination of the breast tissues in normal mice. (a) blood vessels, (b) epithelial cells, (3) lactiferous

ducts. Figure 2: Microscopic examination of the breast tissues in treated mice for the group I (negative control) in 5 days later after treatment. (a) blood vessels, (b) epithelial cells, (3) lactiferous ducts. (a) Inflamed cells (PMNs or polymorphonuclear leukocytes) were shown around epithelial cells and connective tissues, (b) blood vessels. There were \pm 250 inflamed cells in breast tissues. Figure 3: Microscopic examination of the breast tissues in treated mice for group II (positive control) in 5 days later after treatment. (a) Inflamed cells (PMNs or polymorphonuclear leukocytes) were shown around epithelial cells and connective tissues, but their numbers were reduced at a later time. There were \pm 70 inflamed cells in breast tissues. Figure 4: Microscopic examination of the breast tissues in treated mice for the group III (positive control) in 5 days later after treatment. (a) The inflamed cells (PMNs or polymorphonuclear leukocytes) were shown around lactiferous ducts, (b) the inflamed cells (PMNs or polymorphonuclear leukocytes) were shown around connective tissues and epithelial cells, but their numbers were reduced at a later time. There were \pm 30 inflamed cells in breast tissues.

Discussion and Conclusion

Induction of *S. aureus* on breast tissues of the treated mice delivered significant evidence to assess the activity of cytokine IL- β profile as a pro-inflammatory agent in mastitis conditions.

IL-1 β is a pro-inflammatory cytokine, and it is activated by the invasion of pathogens or damaged cells [8], [9]. During inflammation process, cytokine IL-1 β is rapidly increased and induces reaction of inflammation or transmission of pain at various levels, even it persists that causes chronic pain [10], [11].

Exposure of *S. aureus* can regulate the expression of TLR12 in mRNA and proteins, increases the production of IL-1 β dan TNF- α , and stimulates the expression of NF- κ B [16].

Based on the results of the treatment for all the experimental mice, the Group III (treatment) could rapidly reduce IL-1 β levels; even their levels almost achieved IL-1 β levels before induction by *S. aureus*. Reduction of these IL-1 β levels associated with the functions of bioactive

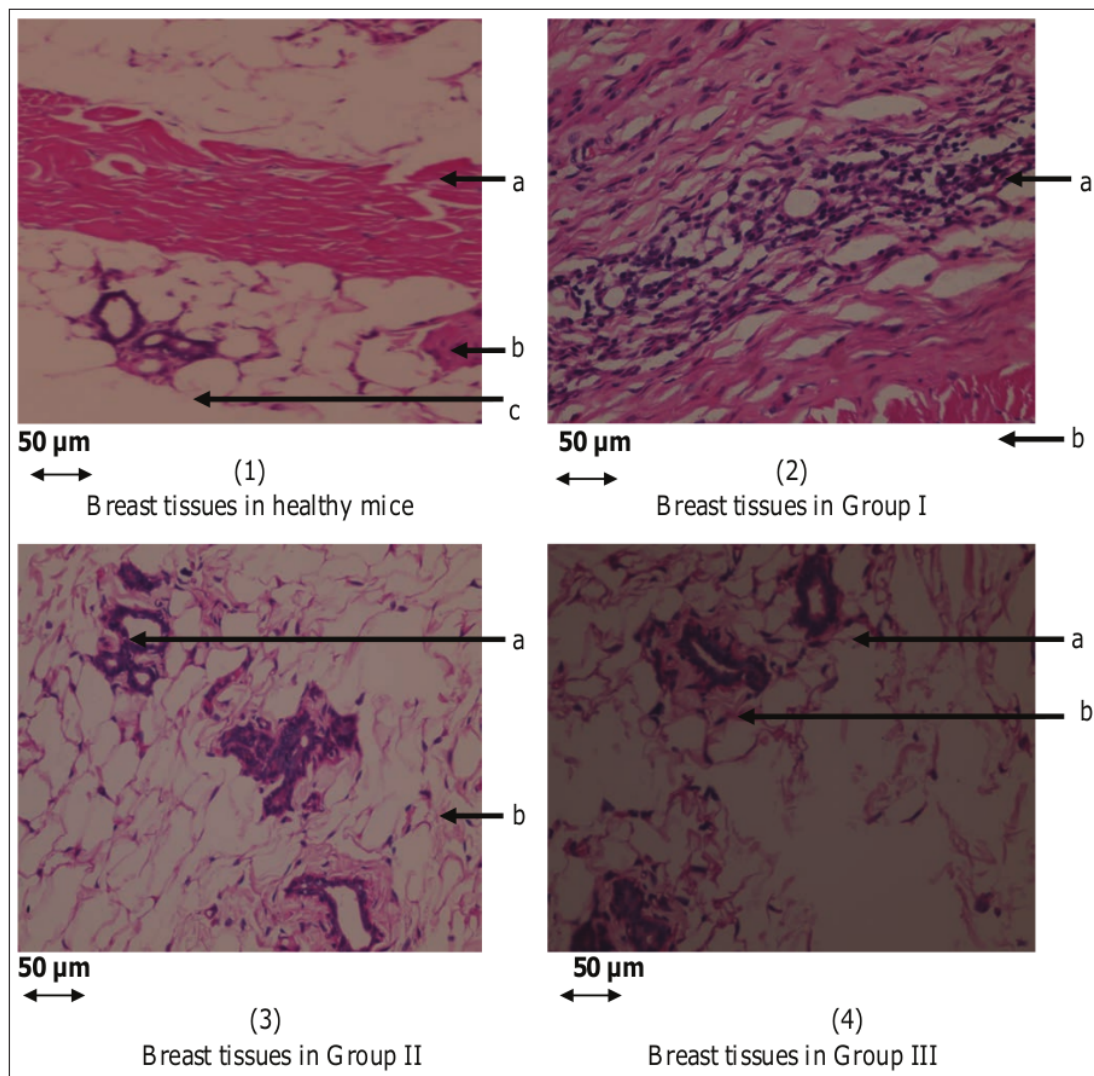


Figure 2: Results of the histopathological examinations of breast tissues

compounds in leaf extracts of *S. taccada* (Gaertn.) Roxb. that contain flavonoid, saponin, tannin, terpenoid, and alkaloid that inhibit the activity of COX enzyme and lipoxygenase that reduce biosynthesis of prostaglandins, accumulation of leukocytes, and degranulation of neutrophils that directly secrete arachidonic acids, as well as inhibit secretion of histamine [17].

Results of this study were in line with the study of Umrah that leaf extracts of *S. taccada* (Gaertn.) Roxb. increased IL-10 levels as an anti-inflammatory effect [18].

Pathological analysis of breast tissues in the treated mice revealed that leaf extracts of *S. taccada* (Gaertn.) Roxb. could reduce damaged tissues, cellular infiltration, and subcutaneous edema due to induction of pathogens.

Conclusion

This study proved that leaf extracts of *S. taccada* (Gaertn.) Roxb. had a significant function as an adjuvant for healing mastitis by reducing pro-inflammatory cytokine IL-1 β .

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