The Mammary Histopathology Depiction of Female Mice Induced by Staphylococcus Aureus Bacteria after Scaevola Taccada Extract Administration

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Abstract: The public is concerned about chemical-based anti-inflammatory drug usage, so it is an indication to find an anti-inflammatory drug made from herbal plants, one of them is from the Scaevola taccada (Gaertn.) Roxb. This research was conducted by finding out the histopathological depiction of female mice (Gaertn.) Roxb. induced by Staphylococcus aureus bacteria after the administration of Scaevola taccada (Gaertn.) Roxb. Samples were divided into two groups, namely the control and treatment group. The control group was given by antibiotic amoxicillin 9.59 mg/kg bb, while the treatment group was given by antibiotic amoxicillin 9.59 mg/kg bb plus Scaevola taccada extract 400 mg/kg bb, an anti-inflammatory determination is done by the formation of artificial inflammation or mastitis with the induction of staphylococcus aureus bacteria in mammary parts of rats.

1 INTRODUCTION

Inflammation is a protective response toward tissue injury caused by physical trauma, chemicals, damage, or microbiological substances. Anti-inflammatory drugs are a class of drugs that have analgesic (pain relief), antipyretic (heat-reducing), and anti-inflammatory properties. This drug is relatively safe if it is used by at the appropriate dosage. But concerns will arise if it is consumed in high doses or long term because it will cause side effects such as liver poisoning, digestive tract disorders, kidney disorders and others (Sitti Amirah Rahmawati, Safriani Rahman, 2014).

Staphylococcus aureus is a major pathogen in humans that causes various clinical infections. At present, staphylococcus aureus is also used to diagnose mastitis known as "infectious mastitis" which is being applied to describe acute case conditions. Staphylococcus aureus can express a variety of surface proteins that can play key role in the infection process because it can promote bacterial adhesion to host cells and tissues, as well as obtain important nutrients and avoid immune responses (Contreras and Rodriguez, 2017) (Yagdiran et al., 2016) (Habib et al., 2015) (Cells, 2019).

Clinical infection caused by staphylococcus aureus is likely to have a general or even serious impact. It can be seen from antimicrobial resistance increasing even clinical disease is also increasing due to staphylococcus aureus bacteria (Tong et al., 2015). Mastitis is an inflammatory disease of the breast. Staphylococcus aureus is the most dominant bacterium that is often found by inflamed breasts, in other studies saying that the mammary glands sinuses and epithelial cells were damaged due to staphylococcus aureus bacteria (Chen et al., 2014).

According to WHO 2013, many health cares of medicinal plants have been used by preventing or treating certain diseases. Because of the efficacy and availability and also affordable prices so that the demand for herbal medicines that use natural ingredients continues to increase. There are millions of people in major regions of developing countries covering from 70 to 80 percent of health care needs in the world population (Essien et al., 2017).
Scaevola taccada (Geartn.) Roxb. is trusted by the public for treating digestive, anti-tumor, and anti-inflammatory problems. The fruit can be used as juice to overcome menstrual problems and ringworm. The roots are also commonly used by people as a treatment for dysentery, syphilis, and beriberi. The Decoction of the leaves and bark can prevent tachycardia, this plant can also reduce the frequency of heartbeats, slow the pulse and stimulate the heart to contract normally, anti-inflammatory, anti-fungal, anti-bacterial and cough medicine (Mejin, 2009) (Chandran and Arunachalam, 2015) (Review and Scavola, 2017).

According to research conducted (Suthiwong, Thongsri, and Yenjai, 2016), there is strong antifungal activation found in the content of Scaevola taccada (Geartn.) Roxb. Scaevola taccada (Geartn.) Roxb. has also been used as dermatological aid in Hawaii. A mixture of root bark crushed with salt is used by curing skin diseases. In Indonesia, the roots are used as an antidote when consuming poisonous fish and crabs. Scaevola taccada is reported by having chemical constituents from alkaloids, flavonoids, lipids, terpenoids, glycosides, and saponins (Mejin, 2009) (Chandran and Arunachalam, 2015).

Previous research on chemical component groups found in diethyl ether fraction of Scaevola taccada (Gaertn.) Roxb. was Flavonoid group with a wavelength of 239.50 nm and had hydroxy, aromatic, ketone, alkyl group and supported by spot spotting after spraying with benedict reagents and Antimony (III) Chloride reagents. The research results also support traditional plants used for the treatment of several diseases and inflammatory conditions (Rachmat Kosman, 2012).

In this case, the researchers concluded by utilizing the Scaevola taccada (Gaertn.) Roxb. to reduce inflammation caused by staphylococcus aureus bacteria), so it needs to be re-tested whether there is an effect of the Scaevola taccada (Gaertn.) Roxb. on inflammation that occurs in mammary bacteria induced by female mice induced by staphylococcus aureus bacteria.

2 METHOD

This research was experimental research by using the post-test only controls group design. The study was conducted at Biopharmaca Laboratory and Biology Pharmacy Laboratory of UIN Makassar and for the adaptation of mice to the end of the treatment at Hasanuddin University animal laboratory. Subjects in this study were twelve strains Sprague Dawley mice with the body-weight of 200-250 grams, divided into control and treatment groups. The main ingredients were amoxicillin and Scaevola taccada extract. Other materials were cotton alcohol, 10% formalin buffer solution, and container pots, injection syringes, tissue, light microscopes, glass objects, microtomes, water baths, and glass covers. Mouse cages are plastic tubs covered by wire and given sawdust and place to eat and drink.

The sample of this study was divided into two groups. The control group was induced by staphylococcus aureus (0.2 ml x 108 ml/CFU), and they are given amoxicillin antibiotics at a dose of 9.59 mg/ml/ 250 gram bb of mice for five days. While the treatment group induced by staphylococcus aureus (0.2 ml x 108 ml/CFU), it was given amoxicillin antibiotics at a dose of 9.59 mg/ml/ 250 gram bb of mice for five days and Scaevola taccada extracts at dose of 400 mg/ml/kg bb of mice for five days. Then on the 6th day, the mice in euthanasia were taken and the mammary part and examined for processing and making the histopathological preparations.

The material was the Scaevola taccada plant obtained from Watang Suppa Village, Suppa District, Pinrang Regency. They are cleaned from the dirt attached by using flowing water and then cut the sample into small pieces. They are dried to contain water content below 10%. Scaevola taccada was sieved with a mesh size of 40 so that a smooth Simplicia sample was obtained. After that, the sample is ready to be extracted by maceration method. Extraction by the maceration method used 70% ethanol solvent. First, the sample is moistened with 70% ethanol until fully submerged for 15 minutes. After that, it is sufficient again to be two liters with 70% ethanol at the temperature room for 3 x 24 hours while occasionally stirring. Macerate is then filtered and the pulp is macerated again. The extract obtained then evaporated by using a rotary evaporator until it thickens, then dried with the help of a water bath. The extract obtained is then evaporated by using a rotary evaporator until it thickens, and then dried with the help of the water bath. The resulting viscous extract is inserted into porcelain vial and weighed the extract weight. Furthermore, the dose is converted to obtain a dose of 400 mg/kg bb.

Then, staphylococcus aureus cultured was planted in BHIB medium and incubated for 18-24 hours at 37°C C in an incubator. Then the bacteria were planted on NA (Nutrient Agar) medium and re-incubated for 18-24 hours at 37°C C. After bacterial
incubation, do gram staining. Biochemical tests were carried out by NA colonies for staphylococcus aureus by planting on DNase agar medium and then mannitol salt agar, then doing bacitracin and Novobiocin tests followed by catalase coagulase test. Then it was re-incubated for 18-24 hours with a temperature of 37°C. The bacteria that grew on biochemical tests were matched with the identification table of staphylococcus aureus bacteria. To make bacterial samples that were injected into mice by making suspension in a physiological NaCl solution as much as 10 ml mixed with golden yellow S. aureus bacterial colony with turbidity level of Mc Farlan 2 x 10^8 CFU. The accuracy of the Mc Farland turbidity level is measured by the Densi check tool.

Flavonoid compounds are found in almost all parts of plants including the outer bark, leaves, fruit, and roots. Flavonoids are also natural compounds that have potential as antioxidants that can counteract free radicals that play a role in the generation of degenerative diseases through the mechanism of damage to the immune system, lipid oxidation, and protein (Aminah, Tomayahu, and Abidin, 2017).

Scaevola taccada plant used in this study is only part of the leaf. The Scaevola taccada leaf can be used as an anti-inflammatory because it contains several compounds. One of them is the flavonoid compound.

The subjects were 12 female Sprague Dawley mice that were adapted by seven days at Hasanuddin University animal laboratory, which met the inclusion criteria. The results of the histopathological study of female mammary mice induced by bacterium Staphylococcus aureus after Scaevola taccada (Gaertn.) Roxb. extract administration by comparing with the control group were only given antibiotics.

According to figure 3, it can be seen in the group that only has not been given antibiotics. There are many inflammatory cells in milk glands and connective tissue, while after being given amoxicillin, the inflammation antibiotics appear to be reduced but still appear in some cells. However, in Figure 4, inflammation cells can be seen before antibiotic amoxicillin administration and Scaevola taccada extracts, there are many inflammation cells in milk glands and connective tissue after antibiotic

3 RESULT AND DISCUSSION

The identification results of alkaloid compounds, flavonoids, steroids/triterpenoid, saponins and tannins groups in sea leaves (Scaevola taccada (Gaertn.) Roxb). extracts.

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treatment and extra Scaevola taccada inflammation cells are reduced by only visible inflammation cells in the connective tissue section.

Table 1 shows that the average number of inflammation in the control group was 54,000 while in the treatment group was 91,600 with a mean difference of 37.6. It meant that the treatment group, namely the group was given by the Scaevola taccada extract (Gaertn.) Roxb. was more influential in reducing inflammation cells than the control group that was only given amoxicillin.

Table 1: Histopathological examination results of mice induced by Staphylococcus Aureus who were given by treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean±SD</th>
<th>Mean Deviation</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Control (Amoxicillin)</td>
<td>54.000 ± 11.916</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Treatment (Amoxicillin+</td>
<td>91.600 ± 6.804</td>
<td>37.6</td>
<td>0.000</td>
</tr>
<tr>
<td>Scaevola Taccada)</td>
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Inflammation is a localized protective response caused by tissue damage or injury that functions to destroy the agent and causes injury. It is characterized by pain, heat, redness, swelling, and loss of function. Inflammation is divided into two namely acute inflammation that lasts quickly or briefly, and chronic inflammation that occurs repeatedly (Williamto and Wijayahadi, 2016).

The inflammation mechanism in the body which is commonly called inflammation is an immune response that maintains tissue homeostasis in the body and allows the body to survive during the process of injury or infection. Inflammation is also normal and healthy body processes that are usually produced by several disorders or various diseases from the body's localized response to the general response. So it can be concluded that inflammation is the body's first reaction when injury and infection occur in a person's body. Inflammation can be treated with various types of anti-inflammatory agents that are effective and safe to help treat or reduce inflammation which is called agents (Ganesh et al., 2014).

The occurrence of mastitis begins with an increase in pressure in the duct (ASI channel) due to stasis of ASI. If the milk is not removed immediately, there is excessive alveoli tension and causes the epithelial cells that produce milk to become flat and depressed so that the connective tissue permeability increases. Some components (mainly immune proteins and sodium) from plasma enter the breast milk and subsequently into the...
tissues around cells so that it triggers an immune response. Untreated ASI stasis results in an inflammatory response and tissue damage making it easier for breast infections (IDAI, 2013, Tristandi & Nariyah 2019).

There are several ways the entry of germs into the breast is through the lactiferous ducts into the secretion lobes, through cracked nipples to the lymph glands around the duct (periductal) or through hematogenous (blood vessel) spread. The most frequent organisms are Staphylococcus aureus, Escherichia coli, and Streptococcus. (IDAI, 2013).

Mastitis treatment and prevention depend on antibiotics usage. So, the abuse of antibiotics can lead to antibiotic resistance. So it is necessary to develop treatment strategies with natural ingredients that have been widely used by various countries in the world, which can reduce the risk of drug usage which is made by chemicals (Cheng et al., 2019).

In another study stated that the treatment of mastitis both infectious and non-infectious with antibiotics can provide rapid resolution. Erythromycin is considered the drug of choice because it has high efficacy, low cost, and has a low risk of inducing bacterial resistance. Handling of mastitis using antibiotic therapy with breast cleansing is more effective for relieving symptoms quickly compared to only giving antibiotic therapy (Kamal et al, 2012, Jahanfar et al, 2013).

Antibiotics have often used the treatment of mastitis. The World Health Organization also says the use of antibiotics is too excessive. But the correct use of antibiotics is also a wise treatment of mastitis. Ask for non-pharmacological assistance in mastitis such as giving a hot compress to the breast to help relieve swelling and pain. Allow breast milk to reduce swelling (Yu Z et al., 2018).

Scaevola taccada (Gearth.) Roxb. is a species of plant that lives in coastal shrubs scattered throughout the coastal regions of the Pacific Ocean and Hindia (Ando et al., 2014). In general, Scaevola taccada (Gearth.) Roxb. has been used by the community as traditional medicine, among others, it is used for the treatment of digestive problems, anti-tumor, anti-inflammatory, menstrual complaints, ringworm, dysentery, syphilis, beriberi and others (Chandran and Arunachalam, 2015).

Phytochemical screening is conducted to determine the content of any secondary metabolites contained in these plants. From the results of phytochemical screening, it can be concluded that what compounds can provide anti-inflammatory activity. As screening results of phytochemicals that have been carried out at UIN Makasar pharmaceutical biology laboratory toward Scaevola taccada (Gearth.) Roxb. plant, there are flavonoid compounds, alkaloids, saponins, terpenoids, and tannins. It is in line with previous research that the content of Scaevola taccada (Gearth.) Roxb. is an alkaloid, flavonoid, Scaevola, and saponin (Rachmat Kosman, 2012) (Chandran and Arunachalam, 2015).

Traditional medicinal plants have enormous potential as the development of antimicrobial drugs. Many studies have described the antibacterial properties of traditional medicinal plant extracts isolated from traditional medicinal plants (Ilanko and Cock, 2019).

The Scaevola taccada plant is a large shrub, reaching 3-4 meters long which is typical in coastal areas where it grows very close to a splash of open seawater, usually on sandy soil or graveled soils. The leaves widen upwards containing water measuring 20 cm long and surrounded by bunches of edges. The color is yellowish-green and shiny, the edges are curved and the leaf surface is waxy. It looks attractive with a yellowish-green color. The fruits and flowers are white. Scaevola taccada blooms throughout the year and the flowers have a pleasant shape so they are called pleasure flowers or beak flowers. The fruit floats in the sea and is spread by ocean waves, this shrub becomes a new pioneering plant on the sand edge of tropical waters (Whiffin, et al. 2010, Chandran, et al. 2013).
antimutagenic and anti-carcinogenic properties contained therein to modulate the function of the cellular enzyme kuci (Panche, Diwan, and Chandra, 2016).

Products produced from arachidonic acid metabolism are one of the inflammation causes. Through phospholipase, cells have been activated by mechanical, chemical, or physical stimuli which as a way to release arachidonic acid. Arachidonic acid is also an unsaturated fatty acid with 20 carbon atoms. There are two main pathways in the metabolic process of arachidonic acid, namely cyclooxygenase by synthesizing prostaglandins as well as thromboxane and lipooxygenase which synthesize leukotriene and lipoxin (Arfan, 2016).

In particular, the flavonoid content is also able to stop the formation and release substances that cause inflammation due to allergic reactions. The compounds included in the flavonoid group have different effects on inflammation. Anti-inflammatory mechanism carried out by flavonoids can go through several pathways, namely inhibiting the activity of COX enzyme and lipoxygenase directly which causes inhibition of prostaglandin and leukotriene biosynthesis which is the final product of COX and lipooxygenase pathways (Nijveldt RJ, Nood EV, Hoorn DEV, Boelens PG, Norren KV, 2001) (Panche, Diwan and Chandra, 2016).

Previous research that said that the anti-inflammatory effect of Scaevola taccada (Gaertn.) Roxb. extract was evaluated using various methods by using mice experimental animals. The results support traditional Scaevola taccada (Gaertn.) Roxb. plant usage in several inflammatory conditions (Mejin, 2009) (Chandran and Arunachalam, 2013) (Rahmat Kosman, 2012) (Rahmawati et al., 2014) (Umrah, 2018).

Complementary therapy of saponin compounds can modulate the immune system that mediated by cell systems to increase antibody production. Saponin does not only has a stimulating effect on certain components of immunity but also it affects several non-specific immune reactions such as inflammation, as well as tannin compounds that function as antibacterial (Iqbal et al., 2007).

Thus the presence of complementary therapy of Scaevola taccada (Gaertn.) Roxb. extract can help heal inflammation caused by staphylococcus aureus bacteria at a dose of 400 mg/kg bb.

4 CONCLUSIONS

Based on the results and discussion, it can be concluded that the administration of amoxicillin and Scaevola taccada (Gaertn.) Roxb. extracts are better able to reduce inflammation cells in female mammary mammals induced by staphylococcus aureus at dose of 400 mg/kg bb compared to groups that are only given amoxicillin with mean deviation of 37.6 /kg.

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