THE EFFECT OF 7% TABLE SALT EXTRACT ON THE INTERLEUKIN-6 (IL-6) LEVELS TOWARD WOUND HEALING IN FEMALE WISTAR RATS INDUCED BY STAPHYLOCOCCUS AUREUS

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ABSTRACT

Objective: This study aimed to determine the effectiveness of administering a 7 % concentration of table salt extract on Interleukin-6 (IL-6) levels in female rats (Wistar strain) induced by Staphylococcus aureus.

Methods: This research used a True Experiment Design Experiment. The research was carried out at the Microbiology Laboratory of Hasanuddin University Hospital, Makassar, in April 2021. The research sample consisted of 12 rats divided into 2 groups, 6 animals for each. The treatment with a table salt concentration of 7% was given for 7 minutes and 15 minutes, then examined the IL-6 cytokine levels by Elisa Rat R & D system method. Collecting data was using observation sheets and data analysis conducted by the Kruskal Wallis and Shapiro Wilk test.

Results: Interleukin-6 (IL-6) was a pleiotropic pro-inflammatory cytokine and a regulator of the acute phase response to antigen reagent (RA). A study showed that IL-6 had a role in the inflammatory process caused by Staphylococcus aureus bacterial infection. The mean value of IL-6 levels was 72,131 pg/ml before treatment and 65,035 pg/ml after 7% table salt extract treatment.

Conclusion: Immersion of 7% concentration of table salt for 7 minutes reduced the levels of IL-6 cytokines, about 72,131 pg/ml before immersion and 65,035 pg/ml after immersion of 7% table salt extract. Therefore, 7-minutes immersion of 7% table salt was more effective to accelerate the wound healing process in mice induced by staphylococcus aureus bacteria.

Keywords: IL-6 Cytokine Levels, 7% table salt extract, wound healing, Staphylococcus aureus

I. INTRODUCTION

Salt is a chemical compound with sodium chloride as the main component and an ionic compound consisting of positive ions (cations) and negative ions (anions), thus forming neutral compounds. Salt is formed from the reaction of an acid and a base. Salt contains elements of sodium (Na) and chlorine (Cl), so it is called Sodium Chloride (NaCl), which has benefits in limiting the growth of organisms and preventing spoilage (Production, Different, and Salt 2015, Zhou et al. n.d 2016, Li 2019). Interleukin-6 (IL-6) is a pleiotropic pro-inflammatory cytokine and is a regulator of the acute phase response to antigen reagent (RA). The role of IL-6 in biological activities, including regulation of immune response, inflammation and hematopoiesis. Interleukin-6 has an essential role in the pathogenesis of RA. A study showed that IL-6 has a role in the inflammatory process caused by Staphylococcus aureus bacterial infection (Solehati et al. 2016, Susantiningsih, 2018. Klemm Coroline.,2019)
Staphylococcus aureus is a gram-positive bacterium, non-sporing, cocci-shaped, and arranged in clusters like grapes. Staphylococcus is a member of the normal flora of human skin and mucosa that can cause infection in humans and animals. Staphylococcus aureus can cause infections ranging from minor skin infections such as folliculitis and furunculosis. Staphylococcus usually does not cause infection in healthy skin. However, if allowed to enter the bloodstream or internal tissues, these bacteria can cause various infections. Staphylococcus aureus produces Staphylococcal enterotoxins (SE), which can cause food poisoning (Tracey A, Taylor, Chandrashekhar G, 2020).

The previous study found that the growth curve of Staphylococcus aureus at 0%, 10% and 20% NaCl in the first 0 to 3 hours did not show a difference in the number of cells in Staphylococcus aureus cultures with or without NaCl. A study conducted by Liana c Chan also discusses Staphylococcus aureus infection with the results of Priming's research improving control of Staphylococcus aureus bacterial infection. Another study revealed that 3% NaCl concentration inhibited Escherichia coli and Staphylococcus aureus bacteria growth at 37 °C. Besides, several other alternatives were also used to suppress Staphylococcus aureus bacteria, including Pagoda leaves can be used as a therapy for mastitis caused by Staphylococcus aureus infection. days of treatment with hexidine, povidone, and chlorhexidine resulted in 99.98% (p=0.0005), 90.73% (p=0.002) and 65.97% (p=0.004) a decrease in Staphylococcus aureus colonies (Ibrahim Fitri.,2018).

Previous research showed that soaking and cleaning wounds using 7% NaCl concentration reduced infection and germs in the wound, and perfect wound closure was seen, both inside and outside the wound. (Samida, 2018).

This study aimed to determine the effect of a 7% table salt concentration on IL-6 cytokine levels and wound healing.

II. MATERIALS AND METHODS

Location and Research Design
This research was carried out in several places. The principal place is the Microbiology Laboratory of Hasanuddin University Hospital, Makassar.

This study used experimental research that examines the effects of table salt in vivo. Therefore, the proper Experiment design was conducted by doing randomization, grouping experimental animal to be a group of 7 minutes treatment and group of 15 minutes treatment.

Population and Sample
The population in this study was experimental animals, the female rats (Wistar strain) aged 4 months and bodyweight of 200 g that met the inclusion and exclusion criteria. The number of samples in this study was 12 female rats. Sample selection was carried out by purposive sampling, a sampling directly based on the criteria.

Research methods
The instruments used in this study were observation sheets and Enzyme-Linked Immunosorbent Assay (ELISA) from the Bioassay Technology Laboratory to analyze levels of interleukin-6 (IL-6).

Data Analysis
Analysis of interleukin-6 (IL-6) levels was using Enzyme-Linked Immunosorbent Assay (ELISA) from Bioassay Technology Laboratory. To test the effectiveness of table salt soaking time was using the Kruskal Wallis statistical test.

III. RESULT

A. Laboratory Testing Phase
1. Microorganism Culture Testing
2. Chemical Analysis Test
3. Salt pH Testing
B. Research implementation stage:

At this stage, it began with an adaptation of experimental animals, female rats of the Wistar strain, for 7 days. After the adaptation process, incisions were made for all groups with a length of 1.5 cm and a depth of reaching the dermis layer after Staphylococcus aureus bacteria were induced. One hour after the incision, the wound was soaked. Wound immersion was divided into 2 groups consisting of groups of 7 minutes and 15 minutes. Each group consisted of 6 rats. This immersion was carried out on days 1-7 to determine immersion using 7% NaCl solution. The analysis result of wound area measurements and wound healing in 7 minutes and 15 minutes of immersion was seen in the intervention group.

C. Differences in Interleukin-6 (IL-6) Levels in the Pre and Post Intervention Groups

1. The Results Of The Measurement Of Interleukin 6 Levels In 7 Minutes And 15 Minutes Of Immersion In The Intervention Group

Table 1 Differences in Interleukin-6 (IL-6) Levels in the Pre and Post Intervention Groups

<table>
<thead>
<tr>
<th></th>
<th>Data</th>
<th>Mean (Pg/ml)</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre Intervention</strong></td>
<td>NaCl 7 minutes</td>
<td>72.131</td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td>NaCl 15 minutes</td>
<td>77.325</td>
<td></td>
</tr>
<tr>
<td><strong>Post Intervention</strong></td>
<td>NaCl 7 minutes</td>
<td>65.03</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>NaCl 15 minutes</td>
<td>68.496</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 show that the levels of interleukin 6 before immersion in 7% NaCl 7% for 7 minutes of 72.131 pg/ml, and after immersion of 65.03 pg/ml. At the same time, the immersion of 7% NaCl for 15 minutes decreased with the value of IL 6 before soaking 77,325 pg /ml and after soaking 68,496 pg/ml. So, 7 minutes of NaCl immersion was more effective in this case and accelerated healing in rat incision wounds.

2. Comparison of IL-6 levels in NaCl immersion for 7 minutes and 15 minutes

Figure 1 Comparison of IL-6 levels in NaCl immersion for 7 minutes and 15 minutes
Figure 1 shows a difference in IL-6 levels before and after soaking in 7% salt for 7 minutes before soaking 72,131 pg/ml and after soaking 60,035 pg/ml. Then, the immersion for 15 minutes was 77,325 pg/ml before immersion and 68,496 pg/ml after immersion. From these data, it can be seen that IL 6 levels decreased more in 7 minutes immersion compared to 15 minutes immersion. So, 7 minutes of immersion was more effective in this case, being able to accelerate wound healing in rats.

3. The Effectiveness Of NACL On Wound Healing

The first week of the highest fibroblasts was in the intervention group 7 minutes. Then the lowest was in the control fibroblasts of 15 minutes immersion. In the 2nd week, each intervention and control group experienced an increase in fibroblasts and the slowest increase in the control group of 15 minutes immersion, and the highest increase was in the 7 minutes immersion group. In the 3rd week, the intervention group (7% table salt) and the control group (DTT water) continued to increase. Again, the highest number of fibroblasts was found in the intervention group, particularly 7% salt for 7 minutes immersion (Table 2).

Table 2 Changes in the number of fibroblasts

<table>
<thead>
<tr>
<th>Fibroblast: 11</th>
<th>Fibroblast:10</th>
<th>Fibroblast: 14</th>
<th>Fibroblast:13</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. control (7 minutes)</td>
<td>a. control (15 minutes)</td>
<td>b. NaCl 7% (7 minutes)</td>
<td>d. NaCl 7% (15 minutes)</td>
</tr>
</tbody>
</table>

Histopathology Subject day 7

<table>
<thead>
<tr>
<th>Fibroblast: 31</th>
<th>Fibroblast: 20</th>
<th>Fibroblast: 45</th>
<th>Fibroblast: 37</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. control (7 minutes)</td>
<td>b. control (15 minutes)</td>
<td>NaCl 7% (7 minutes)</td>
<td>d. NaCl 7% (15 minutes)</td>
</tr>
</tbody>
</table>

Histopathology Subject day 14

<table>
<thead>
<tr>
<th>Fibroblast: 53</th>
<th>Fibroblast: 50</th>
<th>Fibroblast: 70</th>
<th>Fibroblast: 60</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. control (7 minutes)</td>
<td>b. control (15 minutes)</td>
<td>NaCl 7% (7 minutes)</td>
<td>d. NaCl 7% (15 minutes)</td>
</tr>
</tbody>
</table>

Histopathology Subject day 21

IV. DISCUSSION

1. Salt Definition

Salt is a chemical compound whose main component is sodium chloride and an ionic compound consisting of positive ions (cations) and negative ions (anions), thus forming neutral compounds (without charge). Salt is formed from the reaction of an acid and a base. Salt contains elements of Sodium and Chlor (NaCl), so it is called Sodium Chloride (NaCl), which has benefits in limiting the growth of organisms and preventing spoilage (Production, Different, and Salt 2015, Zhou et al. n.d 2016, Li 2019).

Salt acts as a complement to the daily household needed being a food ingredient used as a preservative and electrolyte source for the human body. High salt content results in high osmotic pressure and low water activity. In
addition, salt has bactericidal (killing power) and bacteriostatic (inhibiting power) properties, which inhibit the growth of bacteria, especially *Staphylococcus aureus*. In addition, salt can also accelerate wound healing because salt is also antiseptic or inhibits or slows the growth of microorganisms, even killing germs found in wounds (Li, 2017, Kammoun et al., 2019).

2. **Interleukin 6 (IL-6)**

Interleukin-6 (IL-6) is a pleiotropic pro-inflammatory cytokine and is a regulator of the acute phase response to antigen reagent (RA). The role of IL-6 in biological activities, including regulation of immune response, inflammation and hematopoiesis. Interleukin-6 has a vital role in the pathogenesis of RA. A study showed that IL-6 has a role in the inflammatory process caused by *Staphylococcus aureus* bacterial infection (Solehati et al. 2016, Susantiningsih, 2018, Klemm Coroline.,2019).

Interleukin-6 is formed by many cells and affects many targets. The primary sources of IL-6 are macrophages and lymphocytes in the area of inflammation. Interleukin-6 can also be produced by bone cells under the influence of osteotropic hormones (PTH, 1,25-dihydroxy vitamin D3) and Interleukin-1 (Winda Kusumaningrum, 2016). Increased levels of IL-6 can occur in several conditions such as sepsis, autoimmune disease, lymphoma, AIDS, alcoholic liver disease, tumor development, Alzheimer's disease, infection or post-transplant rejection process (Winda Kusumaningrum, 2016, Susantiningsih, 2018). Interleukin-6 is formed by many cells and affects many targets. The primary sources of IL-6 are macrophages and lymphocytes in the area of inflammation. Interleukin-6 can also be produced by bone cells under the influence of osteotropic hormones (PTH, 1,25-dihydroxy vitamin D3) and Interleukin-1 (Winda Kusumaningrum, 2016). Increased levels of IL-6 can occur in several conditions such as sepsis, autoimmune disease, lymphoma, AIDS, alcoholic liver disease, tumor development, Alzheimer's disease, infection or post-transplant rejection process (Winda Kusumaningrum, 2016, Susantiningsih, 2018). Interleukin-6 is a key mediator of the acute phase response. Increased levels of IL-6 can occur in several conditions such as sepsis, autoimmune diseases, lymphoma, AIDS, alcoholic liver disease, tumor development, Alzheimer's disease, infection or post-transplant rejection processes (Susantiningsih,2018).

Interleukin-6 (IL-6) plays an essential role in the T cell response to alloantigens and the formation of cytotoxic T cells. Helper T cells (T helper cells) are a sub-group of lymphocytes (white blood cell or leukocyte type) resulting from the activation of CD4+ T cells that play an essential role in the immune system. In addition, TH cells participate in activating and directing other immune cells and are essential in the immune system. T cells are formed in the bone marrow, but their proliferation and differentiation occur in the thymus gland.

1. **Interleukin-7 (IL-7)** plays a role in the proliferation of T and B lymphocyte progenitor cells.

2. **Interleukin-8 (IL-8)** functions to increase PMN adhesion to vascular endothelium. The role of cytokines (Klem, 2017).

In previous studies, IL-6 was produced rapidly in response to infection/inflammation to signal an acute phase response to the host to form an immunity. The continuous irregular synthesis of IL-6 will cause pathological effects on chronic inflammation to autoimmunity. Peritoneal fluid was used using the ELISA method to analyze the number of bacteria in mice. The median results of the levels of IL-6, IL-10, the number of bacteria in sequence were as follows: in negative control (NC) 370.530 pg/mL; 67.044pg/mL; 7.4x103cfu/m in positive control (PC) 234.556pg/mL; 42.839pg/mL; 6.8x103cfu/mL; at T1 164,019pg/mL; 17,240pg/mL; 1.1x104cfu/mL; at T2 and the conclusion of the study that VA ethanol extract is effective as a prophylactic compound that prevents bacterial invasion, especially at a dose of 40mg/200g BW rats at that dose there was a decrease in IL-6 levels and the number of bacteria (Jhonson Blair Z.,2020., Kristanti Lidwina Tri Kristanti.,2019., Tanaka Toshia.,2014).

**V. CONCLUSION**

Treatment of a 7% concentration of the table salt extract effectively reduced Interleukin-6 (IL-6) level in the wound healing time of female rats of Wistar strain induced by *Staphylococcus aureus* bacteria. In addition, the 7% table salt extract for 7 minutes was more effective than salt soaking for 15 minutes in reducing Interleukin-6 (IL-6) levels in the wound healing time of female rats of Wistar strain induced by *Staphylococcus aureus* bacteria.

**SUGGESTION**
Further development research is expected to develop this research both in terms of method and addition of salt concentration and duration of immersion.

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