THE INFLUENCE OF 7% TABLE SALT EXTRACT ON IL-10 CYTOKINE LEVELS IN INCISION WOUNDS OF FEMALE WISTAR RATS INDUCED BY STAPHYLOCOCCUS AUREUS BACTERIA

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ABSTRACT

This study determined the effect of a 7% concentration of table salt on IL-10 cytokine levels and wound recovering of female Wistar rats induced by Staphylococcus aureus bacteria. It was a True Experiment Study with pre and post-test designs. There were 24 female Wistar rats, divided into 4 groups, and each group consisted of 6 rats. Those experimental animals soaked for 7 minutes and 15 minutes in the 7% table salt immersion (intervention group) and DTT immersion (control group). The study was carried out in April 2021. Examination of IL-10 cytokine levels was using the Elisa Rat R & D system method. Data collected by observation sheets and analyzed by the Kruskal Wallis and Shapiro Wilk test. The study showed the differences in IL-10 cytokine levels before and after treatment; the pretest value = 0.024 and post-test value = 0.018 (α <0.05), which means a significant difference in IL-10 cytokine levels. The increase in IL-10 cytokine levels was more significant in the intervention group with an immersion of 7 minutes (mean 75.85 pg/ml) than the control group with 7 minutes (mean 73.98 pg/ml). This study revealed that 7% table salt immersion for 7 minutes effectively accelerate increasing levels of IL-10 cytokines compared to other groups.

Keywords: IL-10 Cytokine, 7% Table Salt, Staphylococcus aureus

I. INTRODUCTION

Natural saltwater containing Sodium Chloride (NaCl) is known to clean and treat wounds. Soaking in saltwater has many health benefits, one of which is to treat acute and chronic skin diseases. NaCl in the saltwater immersion can positively affect people with skin diseases with indications of tissue damage to the skin. NaCl is isotonic and physiological saline that is good for cleaning, washing, and compressing wounds. Saltwater with high levels of NaCl can be an alternative for seawater (natural saltwater) because it can be prepared at home practically without seawater, especially for people who live far from the sea (Chrisayu & Sulistiyono, 2016; Kesehatan et al., n.d.; Portugal-Cohen et al., 2011).

Interleukin 10 is one of the most potent anti-inflammatory cytokines, which functions to inhibit all pro-inflammatory cytokines that play a role in the onset of pathological pain (IL-1β, TNF-, chemokines, IL-6 and IL-12) and inhibit the function of macrophages in assisting T cell activation. Thus, the final effect of IL-10 activation is the inhibition of non-specific and specific inflammatory reactions mediated by T cells (Wiener et al., 2019).
The results found a significant increase in the content of IL-10 in mice treated with Sodium Aescinate (SA) on day 3, 7, 14 and day 19 compared to the control group. This suggests that Multidrug-resistant (MDR) bacteria might cause more IL-10 production than non-MDR bacteria (Zhang et al., 2015, Peñaloza et al., 2018).

The wound recovering process includes inflammation, reepithelialization, wound contraction, and collagen metabolism. Many factors affect the slow recovery of wounds in the wound healing process, including infection, poor nutrition, suppressed immune system, drugs, diabetes, radiation, disease, smoking, and stress, which should be avoided. The wound healing process requires treatment that includes wound cleansing and debridement, application of topical antibiotic preparations and dressings (Kharaghani et al., 2016; Marchand et al., 2017; Wibowo et al., 2016).

*Staphylococcus aureus* is one of the most common infection-causing bacteria and classified as a gram-positive pathogenic bacteria that is invasive and capable of causing various diseases in animals and humans. Severe infections from *Staphylococcus aureus* can occur when the immune system is weakened due to hormonal changes, illness, injury, steroid use or other drugs that affect immunity. One indicator of infection caused by *Staphylococcus aureus* bacteria is characterized by tissue damage accompanied by abscesses or pus (Widiastuti & Pramestuti, 2018, Amalia et al., 2016).

Research that has conducted on the effect of increasing collagen in the administration of 7% NaCl table salt on the duration of wound healing in mice, with the results of the study that soaking and cleaning wounds using NaCl concentration of 7% was able to reduce infection and germs in the wound, and perfect wound closure appeared, both inside and outside the wound (Samida, 2018). Thus, this study determined the effect of a 7% concentration of table salt on IL-10 cytokine levels and wound recovering of female Wistar rats induced by *Staphylococcus aureus* bacteria.

### II. MATERIALS AND METHODS

#### Site and Design

The research was carried out in the Laboratory of the Hasanuddin University Hospital. This study used quantitative research methods with a True Experimental Research Design.

#### Population and Sample

The population in this study were experimental animals of female Wistar rats with inclusion and exclusion criteria. They must be considered the same size and age, healthy, and had a rapid immunological response. Totally 24 rats involved and then placed in the Biopharmaceutical Laboratory, Faculty of Pharmacy, Hasanuddin University, Makassar.

#### Data Collection

The data were collected by direct data collection using observation sheets.

#### Data Analysis

The Elisa Rat R & D system method was used to analyze interleukin-10 (IL-10) levels using. In addition, the Kruskal Wallis statistical test was applied to find out the effectiveness of salt soaking time.

### III. RESULTS

Table 1. Analysis of differences in the increase in IL-10 levels in the 7% concentration of people's salt immersion group and DTT water (pre & post)

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Kruskal-Wallis</em></td>
<td>4.367</td>
<td>1.060</td>
</tr>
<tr>
<td><em>df</em></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><em>Asymp. Sig.</em></td>
<td>0.024</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Kruskal-Wallis***
Based on Table 1, it is known that each Asymp. sig values from pre and post immersion were < 0.05. Thus, it concluded a significant difference between 7% of table salt immersion and DTT water.

Table 2 Comparative analysis of the increase in IL-10 levels with immersion time between 7 minutes and 15 minutes in the intervention and control groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (Pg/ml)</th>
<th>Min - Max</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre Intervention</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DTT 7 Minute</td>
<td>71.295</td>
<td>49.61 - 98.03</td>
<td>19.40</td>
</tr>
<tr>
<td>DTT 15 Minute</td>
<td>70.722</td>
<td>49.45 – 94.39</td>
<td>15.60</td>
</tr>
<tr>
<td>Salt 7 Minute</td>
<td>70.726</td>
<td>52.74 – 75.49</td>
<td>11.48</td>
</tr>
<tr>
<td>Salt 15 Minute</td>
<td>70.604</td>
<td>67.61 - 98.83</td>
<td>9.35</td>
</tr>
<tr>
<td><strong>Post Intervention</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DTT 7 Minute</td>
<td>73.982</td>
<td>67.04 – 87.39</td>
<td>7.76</td>
</tr>
<tr>
<td>DTT 15 Minute</td>
<td>71.640</td>
<td>55.86 – 97.09</td>
<td>16.88</td>
</tr>
<tr>
<td>Salt 7 Minute</td>
<td>75.841</td>
<td>56.28 – 131.86</td>
<td>29.03</td>
</tr>
<tr>
<td>Salt 15 Minute</td>
<td>74.689</td>
<td>62.93 – 93.66</td>
<td>10.14</td>
</tr>
</tbody>
</table>

Based on Table 2, it can be seen that the mean value of DTT water of 7 minutes immersion; before treatment was 71.29 pg/ml and after treatment was 73.928 pg/ml. The DTT water of 15 minutes immersion; before treatment was 70.722 pg/ml and after treatment was 71.640 pg/ml. Immersion by 7% table salt for 7 minutes; before treatment was 70.726 pg/ml and after treatment was 75.841 pg/ml. Then, immersion by 7% table salt for 15 minutes; before treatment was 70.604 pg/ml and after was 74.689 pg/ml. From the mean value, it can be seen that the intervention and control groups experienced an increase in IL-10 levels after immersion.

![Figure 1 Comparison of the increase in IL-10 levels in 7% salt (NaCl) immersion and DTT](image)

Figure 1 showed the difference in IL-10 levels before and after immersion in 7% table salt at 7 minutes and 15 minutes. The most significant improvement in IL-10 levels after immersion was found in salt immersion with a concentration of 7% for 7 minutes.

**The effectiveness of 7% salt on wound healing**

Table 3 Changes in the number of fibroblasts

| Fibroblast: 11 | Fibroblast: 10 | Fibroblast: 14 | Fibroblast: 13 |
In the first week, the highest fibroblast of the intervention group of 7% table salt was 7 minutes intervention, and the lowest was in the 15 minutes intervention. In the 2nd week, each intervention and control group experienced an increase in the number of fibroblasts. The slowest increase occurred in the control group of 15 minutes, while the highest increase was in the 7 minutes of the 7% table salt group. At week 3, the intervention group and control group continued to increase, while the highest number of fibroblasts was found in the 7% table salt intervention group at 7 minutes of immersion.

IV. DISCUSSION

A. Salt bath against wound recovery and *Staphylococcus aureus* bacteria

Salt is one of the most important auxiliary materials for humans, one of which is that salt has a role in inhibiting the growth of bacteria, especially *Staphylococcus aureus*. Salt has bactericidal (killing power) and bacteriostatic (inhibitory) properties. The osmotic action of salt solution on foodstuffs is due to the food acting as a semipermeable membrane lowering the water content so that salt acts to inhibit bacteriological and enzymatic activities. The research was conducted on collagen in the administration of 7% NaCl table salt on the duration of wound healing in mice. The study found that soaking and cleaning wounds using 7% NaCl concentration was able to reduce infections and germs in the wound, and perfect wound closure appeared both inside and outside the wound (Fabian Alvarez Primo et al., 2020., R Raguvaran, 2017., Samida, 2018).

Saltwater mixed with warm water will form particles with different electrical charges, namely positively charged sodium ions (cations) and negatively charged sodium ions (anions). When the wound occurred, those ions are causing vasoconstriction, and blood clotting, which leads the wound to dry quickly. In addition, water can also kill bacteria that attack wounds, namely *Staphylococcus* and streptococcal bacteria (Amara Suneetha, Whalen Margaret, Whalen Venkataswarup 2016).

Table salt can protect against bacterial infection and the growth of sepsis through increasing acute inflammatory responses. Also, saltwater can kill bacteria that attack wounds, especially *Staphylococcus aureus* bacteria (Cantore et al., 2020., Resmy & Divya, 2019., Angkasa et al., 2017).

*Staphylococcus aureus* is one of the most common infection-causing bacteria. This bacterium is classified as a gram-positive pathogenic bacterium that is invasive and capable of causing various diseases in both animals and humans. Severe infections from *Staphylococcus aureus* can occur when the immune system is weakened due to hormonal changes, illness, injury, steroid use or other drugs that affect immunity. As a result, the indicator is infected with *Staphylococcus aureus* bacteria characterized by tissue damage accompanied by purulent abscesses (Widiastuti & Pramestuti, 2018., Amalia et al., 2016).
B. Interleukin 10 (IL-10)

This study examines the differences in the duration of wound immersion with a time of 7 minutes and 15 minutes and its effect on interleukin levels in wound healing. The study results found that the 7-minute soaking group with a salt concentration of 7% experienced quick wound recovery and was characterized by an increase in IL-10 cytokine levels. The wound healing process is dynamic of restoring function and tissue integrity, and this complex process is divided into three phases of wound healing: the inflammatory phase, the proliferative phase, and the maturation phase (Seung-Kyu-Han, 2015).

Interleukin-10 is an anti-inflammatory cytokine that can decrease the action or production of one or more pro-inflammatory cytokine proteins produced by nerves, neurons, glial cells, endothelial cells, fibroblast cells, muscles, immune cells, or other cell types. The anti-hypernociceptive cytokine IL-10 is produced by various cell types such as lymphocytes, monocytes, macrophages, and mast cells. Interleukin-10 is a potent anti-inflammatory cytokine family member, suppresses all pro-inflammatory cytokines that play a role in pathological pain (IL-1β, TNF-α, and IL-6). Research that has been conducted on pagoda leaf extract effects in increasing IL-10 levels as an anti-inflammatory. After induced by Staphylococcus aureus bacteria, all groups experienced a decrease in IL-10 levels. After treatment, there was an increase in all groups. This shows that there are differences in IL-10 levels after treatment in all groups. In the negative control group, IL-10 levels decreased, while in the treatment group, IL-10 levels experienced a more significant increase than in the positive group (Wiener et al., 2019., Satria et al., 2019).

A previous study aimed to evaluate the potential effect of sodium aescinate (SA, sodium salt of aescin) on wound healing in streptozotocin-induced diabetic rats suggested that SA exerts anti-inflammatory and antioxidant effects, making it a potential molecule for applications that accelerate wound healing in diabetics. It was also associated with inhibition of inflammatory cytokines with study results Effect of Topical Application of SA on IL-10 Levels. As described, it resulted in a significant increase in IL-10 content in SA-treated mice on days 3, 7, 14 and day 19 compared with the control group. A quantitative/experimental study shows that IL-10 can reduce the inflammatory response to Staphylococcus epidermidis CNS catheter infection revealed that IL-10 plays an important role in regulating the inflammatory response to CNS catheter infections but cannot control the bacterial load. Therefore, IL-10 can be a valuable therapeutic target for immune modulation in CNS catheter infections. However, it should be used in conjunction with antibiotic therapy for bacterial eradication (Zhang Zonglin et al., Robinson Keven M., 2015).

V. CONCLUSION

Administration of 7% concentration of table salt extract was effectively increasing levels of IL-10 cytokine on the wound recovery time of female Wistar rats induced by Staphylococcus aureus bacteria.

Immersion of 7% concentration of table salt extract for 7 minutes was more effective than 15 minutes of 7% table salt immersion on increasing levels of IL-10 cytokines in the wound recovery time of female Wistar rats induced by Staphylococcus aureus.

SUGGESTION

Further development research is expected to develop this research both in terms of method and salt concentration.

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