EFFECTIVENESS 7% SODIUM CHLORIDE CONCENTRATION OF TABLE SALT ON WOUND SIZE OF FEMALE WISTAR RATS INDUCED BY STAPHYLOCOCCUS AUREUS BACTERIA

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ABSTRACT

Seawater is used as a food preservative to reduce inflammation and heal skin disorders such as eczema. In addition, the antiseptic properties of seawater can help heal abrasions, infected wounds, rashes and also increases skin elasticity. This study aimed to test the effectiveness of table salt extract to the skin tissue of female Wistar rats induced by Staphylococcus aureus to find out changes in the wound area and wound healing. This research used the True Experimental Method. The study was conducted in April 2021, involving 24 female Wistar rats, divided into 4 groups, each group consisting of 6 rats. The next stage was an incision of 1.5 cm with a depth reaching the dermis layer. All samples were induced by Staphylococcus aureus bacteria and then soaked for 7 and 15 minutes in the intervention group (7% table salt concentration) and control (DTT water). The observation sheets and histopathological tests used for collecting the data. Data were analyzed using the Kruskal-Wallis and Shapiro Wilk test. The statistical test found that the intervention group (7% concentration of table salt) had an average value of 0.020, and the control group (DTT water) had an average value of 0.035, p-value <0.05 of each. Therefore, soaking 7 minutes with a 7% concentration of table salt effectively influence the wound size of female Wistar rats induced by Staphylococcus aureus.

Keywords: 7% table salt, wound area, wound healing, Staphylococcus aureus

I. INTRODUCTION

Seawater used as a food preservative can also reduce inflammation and heal skin disorders such as skin inflammation and eczema. In addition, the antiseptic properties of seawater can help heal abrasions, infected wounds, rashes and increase skin elasticity (Chrisayu and Sulistiyono, 2016).

In Indonesia, natural treatment using seawater has become a tradition in many places, such as Southeast Sulawesi, known as the Bajo Tribe, who live in the sea to speed up the delivery of the umbilical cord. Then in some communities in Ambon and Nusa Tenggara have a unique tradition for children after circumcision by throwing themselves into the sea so that the wound becomes dry quickly (Dwi Ratih, 2015, Leikawa, 2016).

The growth of Escherichia coli bacteria may reduce by high salt levels. While NaCl can inhibit the growth of Staphylococcus aureus bacteria. Treatment failure associated with bacteria causes resistance and causes problems in society (Zhu and Dai, 2018, Cabezas-Pizarro et al., 2018, Balouiri, Sadiki, and Ibsnouda, 2016).
The wound healing process is dynamic, with the ultimate goal of restoring function and tissue integrity. The wound healing is assessed by microscope at the wound surface area and a decrease in granulation after 7th and 15th days. In the wound healing process, the wound shrinks on the 7th day. The wound healing process is a biological procedure defined to regenerate tissue that involves inflammation, proliferation, and maturation phases. Wound healing mechanisms involve complex interactions between various cell types, extracellular matrix components and cytokine mediators. In recent years, efforts to research and develop new antimicrobials have increased to prevent resistance to microbes (Kozar, Hamilton, and Koscova, 2019., Klemm C et al, 2017., Sh Ahmed A et al, 2019., Balouiri M, Sadiki M, Ibnsouda SK, 2016).

This research is a follow-up to a previous study entitled Test of the Effectiveness of 7% NaCl Table Salt to Increase Collagen in Mice Wounds. The researcher wishes to examine the effectiveness of NaCl concentration of 7% of table salt on changes in incision wounds in female rats of the Wistar strain induced by Staphylococcus aureus bacteria, assessing the NaCl content in table salt whether it has the effect of healing infected wounds quickly. In another study, the basic principles of biochemical treatment revealed the role of inorganic salts in the body, which contains 12 types of content, one of which is sodium chloride (NaCl) which can be used for biochemical treatment and has a healing effect on various diseases (Amalia A, Dwiyanti RD, Haitami). H., 2018).

Salt or sodium chloride is one of the essential needs in everyday life. Based on the literature study and analysis from various sources above, the researchers will test the effectiveness of giving folk salt extract to the skin tissue of female rats with the Wistar strain induced by Staphylococcus aureus bacteria by observing changes in the wound area and wound healing.

II. MATERIALS AND METHODS

Location and Research Design

This research was conducted in 4 places, namely the Biopharmaceutical Laboratory, Faculty of Pharmacy, Hasanuddin University; Oceanography Laboratory, Faculty of Marine, Hasanuddin University; Research and Science Development Laboratory, Faculty of Mathematics and Natural Sciences, Hasanuddin University; and Anatomical Pathology Laboratory, Hasanuddin University Hospital. The type of research used was quasi-experimental.

Population and Sample

The population in this study were experimental animals, the female Wistar strain rats. The selection of rats was based on the size and no infection or adhesion before treatment, also rats aged 4 months, because in adulthood have an immunological response that seen quickly. Each intervention groups consisted of 6 samples, with the total sample used in this study was 24 samples.

Method of Collecting Data

The instruments used in this study were observation sheets and histopathology to assess changes in the area of the incision and wound healing.

Data Analysis

Data processing was carried out using a computerized method with the SPSS (Statistical Product and Service Solution) - version 25 program. The results of the analysis were presented in the form of narratives and tables. The data analysis technique used the Kruskal-Wallis test. The Kruskal-Wallis test assessed changes in the wound area and wound healing after being given a 7% concentration NaCl of table salt and DTT water. If value < 0.05, a significant difference detected between table salt immersion and DTT on changes in wound area.

III. RESULTS

1. Laboratory Analysis Stage
   1. Microorganism Culture Testing
   2. Chemical Analysis Test
   3. Salt pH Testing
Based on Table 1, the study analyzed the difference in the duration of soaking wounds with a concentration of 7% NaCl. The results found that for immersion in 7% NaCl concentration with a soaking time of 7 minutes, the wound appeared dry on the day 7th. However, for the 15 minutes of 7% NaCl immersion, 7 minutes of DTT water, and 15 minutes of DTT water showed the differences. Unfortunately, the wound had not recovered in those groups; the wound still appears in the middle on the 7th day.

Table 1 Results of Observation of Wound Closures in the Intervention and Control Group

<table>
<thead>
<tr>
<th>No</th>
<th>Sampel</th>
<th>Immersion time</th>
<th>Changes in wound area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Salt 7% 7 minute</td>
<td>The incision does not bleed, does not swell.</td>
<td>The wound is starting to close left and right</td>
</tr>
<tr>
<td>2</td>
<td>Salt 7% 15 minute</td>
<td>The incision does not bleed, does not swell.</td>
<td>The wound hasn't healed, it looks like a wound in the middle</td>
</tr>
<tr>
<td>3</td>
<td>DTT 7 minute</td>
<td>The incision does not bleed, does not swell.</td>
<td>The wound hasn't healed, it looks like a wound in the middle</td>
</tr>
<tr>
<td>4</td>
<td>DTT 15 minute</td>
<td>The incision does not bleed, does not swell.</td>
<td>The wound hasn't healed, it looks like a wound in the middle</td>
</tr>
</tbody>
</table>

2. The implementation stage of research

At this stage, it began with the adaptation of experimental animals, female Wistar rats, 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, then induced by Staphylococcus aureus. One hour after the incision, the wound was soaked. Wound soaking intervention was divided into 4 groups, 7 minutes and 15 minutes of intervention groups and 7 minutes and 15 minutes of DTT water groups, 6 rats of each group. This immersion was carried out on days 1-7 to determine the effect of immersion using 7% NaCl solution. The analysis of wound area was conducted by measurements and wound healing in 7 minutes and 15 minutes of immersion in the intervention group.
3. **Bivariate Analysis**

1. Changes in wound area

**Table 2 Kruskal-Wallis test results**

<table>
<thead>
<tr>
<th></th>
<th>Test Statistics</th>
<th>DTT</th>
<th>Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kruskal-Wallis</strong></td>
<td>.018</td>
<td>.098</td>
<td></td>
</tr>
<tr>
<td><strong>Df</strong></td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Asymp. Sig.</strong></td>
<td>.035</td>
<td>.020</td>
<td></td>
</tr>
</tbody>
</table>

Based on Table 2, it was revealed the Asymp Sig. 7% salt immersion value of 0.020 and DTT of 0.035. Each value of Asymp was obtained. Sig < 0.05 concluded a significant difference between salt and DTT immersion on the change of incision wound in female Wistar rats induced by *Staphylococcus aureus* bacteria.

![Comparison of wound area between immersion using salt and DTT](image)

**Figure 1** Comparison of wound area between immersion using salt and DTT

Based on the figure 1, the average value of the change in wound area can be seen between 7% salt and DTT immersion. The change in wound area was most effective in 7% salt immersion of 7 minutes immersion, followed by 7% salt immersion for 15 minutes. Then, the effectiveness also seen in DTT immersion for 7 minutes and the last change was in wound treated by DTT immersion for 15 minutes. So, 7% salt immersion was more effective than DTT immersion, where the duration of 7% salt immersion was more effective at 7 minutes immersion than 15 minutes of immersion.

2. The effectiveness of NaCl in wound healing

The first week of the highest fibroblast was in the intervention group of 7 minutes, and the lowest was in the control fibroblast of 15 minutes. At week 2, each intervention and control group experienced an increased the fibroblasts, and the lowest increase was in the control group for 15 minutes, and the highest increase was in the salt group for 7 minutes. However, at week 3, all groups were continued to increase. The highest number of fibroblasts in the intervention class was 7% salt immersion (Tabel 3).

**Table 3 Changes in the number of fibroblast**

<table>
<thead>
<tr>
<th>Fibroblast 11</th>
<th>Fibroblast 10</th>
<th>Fibroblast 14</th>
<th>Fibroblast 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (7 minute)</td>
<td>Control (15 minute)</td>
<td>NaCl 7% (7 minute)</td>
<td>NaCl 7% (15 minute)</td>
</tr>
</tbody>
</table>

Histopathology Subject 7th day
A. The effectiveness of 7% NaCl (Table Salt) on changes in cuts and wound healing in each group

Seawater has many elements including 55% Chlorine (Cl\(^-\)), 30.7% sodium (Na\(^+\)), 3.6% Magnesium (Mg), 7.7% Sulfates ((SO\(_4\))^\(-2\)), 1.2% Calcium (Ca\(^{2+}\)), and 1.1% Potassium (K\(^+\)). However, the most dominant substance in seawater is sodium chloride (NaCl) which is about 90% (Chrisayu dan Sulistiyo, 2016).

In this study, the same thing was found, as seen from macroscopic observations, which found that the treatment group experienced better wound recovery than the control group. It is seen with no visible signs of infection such as redness, discharge of pus and fibrosis. Immersion of 7% NaCl concentration affects physiological acid-base hemostasis in body tissues, releasing hydrogen ions to ordinary compounds. A wound is completely recovered if the wound has returned to its normal structure, tissue anatomy, tissue function, and appearance within an appropriate period (sitti samidah, 2019).

There is a phase of tissue structure repair in the wound healing process characterized by angiogenesis, granulation tissue, and collagen deposits. Neovascularization or angiogenesis is required to transfer nutrients to the wound area and help maintain granulation tissue. The last phase in the proliferative phase is developing acute granulation tissue and forming new blood vessels. This phase facilitates the entry of macrophages and fibroblasts into the wound area. Macrophages continue to function to supply growth stimulating factors for angiogenesis and fibroplasia. The secretion of platelet-derived growth factor and transforming growth factor together with the extracellular matrix stimulates the differentiation of fibroblasts to form the ground substance, namely myofibroblasts. Myofibroblasts are modified fibroblasts that closely resemble smooth muscle cells in tissue structure. The wound surface will be reduced due to the contraction of myofibroblasts (Han SK, 2015). Research has shown that fibroblasts play a role in wound healing.

By immersing NaCl at a concentration of 7% one hour after the wound for 7 and 15 minutes, it did not form any crusts, namely body fluids or blood mixed with microorganisms because the incisions soaked in saline solution would form particles with different electrical charges, namely sodium ions which positively charged and the chlorine ion negatively charged. Furthermore, the negatively charged chlorine ions will regulate the pressure of the cells around the wound. Therefore, the pressure is set so that the liquid will not escape from the cell. This causes the wound to dry quickly. In addition, NaCl is also bacteriostatic. (Moh. Projo Angkasa, 2017). Thus, the researchers assumed that 7% NaCl concentration immersion helped the tissue repair process by suppressing the fibrosis process.

This study found that macroscopic wound observation in the treatment group experienced better-wound closure than the control group because there were no signs of infection such as inflammation, discharge of pus and fibrosis in the treatment group. Immersion of 7% NaCl affects physiological acid-base hemostasis in body tissues, releasing hydrogen ions to ordinary compounds, hydrochloric acid (HCl), which ionizes in water to form hydrogen ions (H\(^+\)), and chloride ions (Cl\(^-\)) can form ions. Hydrogen (H\(^+\)) and bicarbonate ions (HCO\(_3^-\)) are very strong oxidizing agents.

B. Soaking Time with 7% NaCl Concentration (Table Salt) for Incision Recovery

This study tested the difference in the duration of soaking the wound with 7 and 15 minutes. The results found that for the 7-minute soaking group with 7% NaCl concentration, wound closure was faster than 15 minutes.
In this study, the wound was soaked using NaCl (7% table salt), which had been tested for chemical analysis at the Hasanuddin University Science Research and Development Laboratory. This is different from a study on the difference in macroscopic wound healing using povidone-iodine in the Sprague dawley strain of white male rats (Rattus norvegicus), a significant difference in wound healing time between WJMSCs extract and povidone-iodine was found, and there was no local infection or allergic reaction. This is in line with the theory that says that normal saline is recommended for wound cleaning, a physiological fluid that will not harm the wound fluid.

Cuts are wounds caused by sharp-edged objects or tools that occur with light pressure and scratches on the body surface. The general picture of a cut wound is characterized by flat, line-shaped edges and walls, no tissue bridges and a line or point-shaped wound base. In this study, the wound was made after the adaptation process was carried out, the incision was made for all groups, the wound area was 1.5 cm. One hour after the incision, the wound was soaked. Specifically for wound immersion, they were divided into four groups, namely the intervention group (6 rats) for 7 minutes of soaking, the intervention group (6 rats) for 15 minutes of soaking, the control group (6 rats) for 7 minutes of soaking and the control group. (6 rats) for 15 minutes of immersion. This immersion was carried out on days 1-9 to determine the effect of immersion using 7% NaCl solution.

In the 7 minute wound immersion, the wound closure process began to occur on the third day. The literature noted that the wound healing process occurs through the migration of fibroblasts along the wound's edges by forming fibrin threads, then synthesizing collagen, which reaches its peak on day 5 to 7. Fibroblasts produce fibres that are the basis for forming ordinary connective tissue that produces internal substances. These cells have a cytoplasmic pathway that is mostly basophilic by actively synthesizing proteins that form a connective tissue that contains many cytoplasmic vacuoles containing short collagen fibres and proteolytic enzymes. This proves that fibroblasts participate in the formation of fibre bodies through resorption in the formation of collagen. Furthermore, sodium chloride (NaCl) concentration of 7% in water will ionize to form hydrogen ions which have important properties in all enzyme system activities in the body and protein systems as an effect of acid-base balance. Thus it can be said that 7 minutes of immersion gave a better closing effect than 15 minutes of immersion in experimental animals.

Wound soaking for 7 and 15 minutes did not experience infection, while the control group had an infection because the control group experienced longer wound closure. Wound closure lasts a long time due to infection and hypoxia. Eventually, fibrosis and scarring occur in the inflammatory phase, in Matrix Metalloproteinase (MMPS) in acute and chronic wounds. MMPS plays a role in inhibitors regulating the degradation and deposition of extracellular matrix, which is very important for wound healing. Excessive protease activity can cause the wound not to recover; therefore, with 7% NaCl immersion, there will be an acid-base balance so that the skin's pH is at a normal threshold of around 7.4 so that acidosis and alkalosis do not occur in body tissues. In addition, it also functions as an antimicrobial, and by soaking for 7 to 15 minutes, the wound becomes moist so that healing is better (35–40). Based on this, it can be said that for wound healing, wound moisture is needed to prevent germs from surviving. In addition, wound immersion using a NaCl concentration of 7% is very good in wound care because of the bacteriostatic properties of sodium chloride.

Research on wound care with cell therapy, curcumin, compressing the wound with moist medicinal Gauze, biological Gauze based on biomaterials serves as a substitute for tissue engineering. This study proved its ability to increase granulation formation, collagen deposition, tissue remodelling and wound contraction. Furthermore, soaking and cleaning the wound using a NaCl concentration of 7% could reduce infection and germs in the wound; this could be seen in perfect wound closure both inside and outside the wound.

V. CONCLUSIONS

The incision area and fibroblast of the intervention group were recovered quickly than the control group. In addition, there was a significant difference between the mean reduction in wound area of mice in the treatment or intervention group compared to the control group. Finally, NaCl solution with a concentration of 7% of table salt was significantly different in healing time seen from the length of soaking.

SUGGESTIONS

Further research is needed to conduct pharmacodynamic tests on a 7% concentration of people's salt. Also, further research is needed on postpartum mothers with first and second-degree perineal wounds with the treatment of washing the wound or lapping using NaCl with a concentration of 7% table salt.
REFERENCES


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