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Research Report

The effects of golden sea cucumber extract (*Stichopus hermanii*) on the number of lymphocytes during the healing process of traumatic ulcer on wistar rat's oral mucous

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ABSTRACT

Background: *Indonesia is a country with the world's biggest potential and producer of sea cucumbers. Golden sea cucumber contains glicosaminoglycans, such as heparan sulphate and chondroitin sulphate, which could have a positive implication on wound healing process. This acceleration of wound healing process could be observed through the increasing of lymphocytes on ulcus traumaticus.* ***Purpose:*** *This study aims to analyze the effects of golden sea cucumber extract on the number of lymphocytes during the healing process of traumatic ulcer on Wistar rat's oral mucous.* ***Method:*** *Golden sea cucumber extrat was made with freeze-dried method, and then gel was prepared using PEG 400 and PEG 4000 solvent. Twenty male rats with mucosal ulcus made were divided into a control group and three treatment groups with 20%, 40% and 80% golden sea cucumber extracts. All samples were euthanized on day 4 and then a preparation for histopathological examination was made to examine the number of lymphocytes.* ***Result:*** *The biggest number of lymphocytes was found in the treatment group with 40% golden sea cucumber extract, while the lowest one was found in the control group. The results of one way Anova test then showed a significant difference between the control group and the treatment groups. And, the results of Tukey HSD showed a significant difference between the control group and the treatment group with 40% golden sea cucumber extract.* ***Conclusion:*** *It can be concluded that 40% golden sea cucumber (Stichopus hermanii) extract can increase the number of lymphocytes during the healing process of traumatic ulcer on Wistar rat's oral mucous.*

Keywords: *Wound healing; traumatic ulcer; Stichopus hermanii's extract; glicosaminoglycans, lymphocytes*

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INTRODUCTION

Ulcer is a lesion eroding epithelial tissue with clear border. Ulcer mostly found in several cases in communities is traumatic ulcer triggered by trauma and stomatitis aphtosa recurrent (SAR) that can happen spontaneously and reccurently.[1,2] Traumatic ulcer on the mucous membranes of the oral cavity is a clinical appearance of inflammation indicated by an area with exudat and surrounded by connective tissue. Inflammation is a process of destroying antigens and microorganisms moving into the body or a damaged tissue.[3] Inflammation process, moreover, involves the roles of blood vessel and inflammatory cells, such as leukocytes (netrophil, eosinophil, basophil, monocytes, and lymphocytes) and macrophage. Lymphocytes have an important role in destroying micoorganisms moving into the body (antigen) during the process of inflammation.[4]

Traumatic ulcer, furthermore, can be treated with certain medical therapies, namely topical corticosteroid, sodium bicarbonate with water, or mouthwash with antiseptics, such as 0.2% chlorhexidine gluconate or benzydamin hydrochloride. Unfortunetely, the side effect of using chlorhexidine in a long term is the change of tooth colour.[5]

Indonesia is a country with the biggest sea cucumber potential in the world. The production of sea cucumber in

Indonesia in 1994 was about 1.318.000 kg. The newest data showed that the production of sea cucumber was 42 ton during 2004.[6]

Golden sea cucumber has been used in China and Malaysia as traditional medicine and has been known as an effective tonic for wound and burn healing process.[7] Golden sea cucumber contains big bioactive materials, one of which is glycosaminoglycan. Many previous researches show that glycosaminoglycans (GAG) sulphate, such as chondroitin sulphate and heparan sulphate, have a positive effect on the wound healing process.[8]

Moreover, lymphocytes act in the process of wound healing by realeasing lymphokines affecting the number of other inflammatory cells. One of the lymphokines produced is interleukin -2 (IL-2). IL-2 can bind with heparan sulfat that can help the proliferation process of T lymphocytes.[9] T lymphocytes will secrete transforming growth factor-β (TGF-β). TGF-β functionates to stimulate fibroblast proliferation which plays a role on wound healing.[10] Many previous researches show that the water extract of golden sea cucumber could increase the number of fibroblast cells with optimal concentration of water extract from golden sea cucumber as much as 40% on the traumatic ulcer of Wistar rats.[11]

As the biggest producer of sea cucumber, thus, Indonesia should harness the potential. The acceleration of wound healing can be observed by the increasing of lymphocytes. Therefore, this study aims to analyze the effects of water extract from golden sea cucumber with the concentrations of 20%, 40%, and 80% on the number of lymphocytes during the wound healing process of Wistar rat.

MATERIALS AND METHOD

This study is a laboratory experimental research with Randomized Post test only control group design. This study was conducted with both variables after treatment using random sampling, and a negative control group as a comparison. This study used 20 male rats aged around 2-4 months old and weighed 200-300 grams as samples (*Rattus norvegicus)* with certain criteria, such as normal lower lip mucous and no abnormalities in physical condition.[12]

Ulcer was made by inhalation anesthesia. Wistar rat's lower lip mucous was wounded by no 4 burnisher with 2 mm diameter that had been heated for 1 minute, and it was then touched to Wistar rat's lip mucous for 1 second.[11] The samples were divided into four treatment groups, namely the 1st treatment group with 80% golden sea cucumber extract, the 2nd treatment group with 40% golden sea cucumber extract, the 3rd treatment group with 20% golden sea cucumber extract, and the 4th group with aquadest as the control group.

Furthermore, the golden sea cucumber extract gel with 20%, 40%, and 80% concentrations was then applied one time to ulcer as much as 0.1 mg for each treatment group. Afterward, those rats were sacrificed using ether in lethal doses on the 4th day. The lower lip mucous of those rats was cut until the corner of mouth including the ulcer part and the normal part, and then put in fixation solution, while the dead rats were burried.[11]

Moreover, the number of lymphocytes was measured on histometric paraffin block preparation under compound light microscope with 400x magnification. It means that cell counting was conducted using graticulae dividing visual from light microscope to a certain size for easy reading and preventing cell duplication. Data obtained were derived from the mean calculation in five visual areas of the graticulae.[13] Normality test with one-sample Kolmogorov-Smirnov Test was then conducted on each sample. The results showed that the samples had a normal distribution ($p>\alpha=0.05$). Homogenitas test was also conducted. The results showed that the samples were homogen ($p>\alpha=0.05$). Next, Anova test was conducted to examine the significance of the difference between groups. Finally, Tukey HSD test was conducted to examine the significance of the differences between one group with the others ($p<\alpha=0.05$).

RESULTS

Figure 1 shows lymphocytes on Wistar rat's traumatic ulcer during the histopathological preparation of the treatment groups. The red arrows shows lymphocytes. The mean and standard deviation of lymphocytes on each group can be seen in Figure 2 and Table 2.

The biggest number of lymphocytes was found in the treatment group with 40% golden sea cucumber extract, while the lowest one was in the control group. Moreover, the results of Tukey HSD test showed that there was a

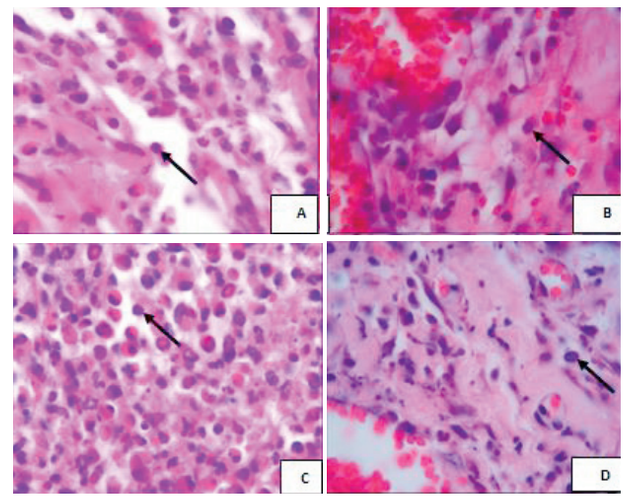

Figure 1. Histological illustration of traumatic ulcer lymphocytes on Wistar rat's oral mucous. Male Wistar using HE with 400x maginification. A) control group; B) 20% gold sea cucumber extract. C) 40% gold sea cucumber extract. Illustration shows a significant rise of lymphocytes. D) 80% Golden sea cucumber extract.

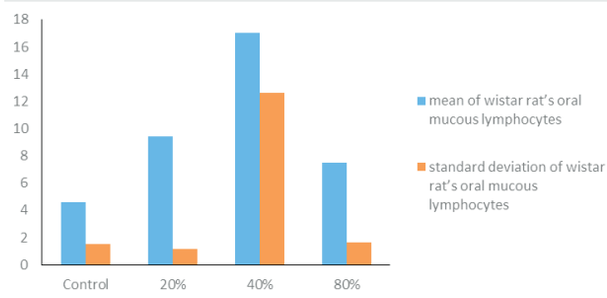

Figure 2. The mean and standard deviation of wistar rat's oral mucous lymphocytes.

Table 1. The mean and standard deviation of lymphocytes on day 4

Group	$\overline{X}$ ± SD
Control	4.6 ±1.51
20%	9.4 ± 1.14
40%	17.0±12.63
80%	7.5 ± 1.64

Table 2. The results of tukey HSD analysis

	Control	20%	40%	80%
Control	-	0.628	*0.028	0.869
20%	0.628	-	0.257	0.958
40%	*0.028	0.257	-	0.095
80%	0.869	0.958	0.095	-

(*)= a significant difference/ significance ($p<\alpha=0.05$)

significant difference between the treatment group with 40% golden sea cucumber extract and the control group. Meawhile, there was no significant difference between the treatment groups with 20% and 80% golden sea cucumber extracts and the control group. The analysis of Tukey HSD results can be seen in Table 2.

DISCUSSION

Like the previous researches, golden sea cucumber extract with the concentrations of 20%, 40%, and 80% was given to Wistar rat's lower lip mucous in this research. The previous researches show that the 40% golden sea cucumber extract is optimal to increase the number of fibroblasts in the healing process of traumatic ulcer.[11] Euthanasia was conducted on the 4th day to examine the number of lymphocytes after the administration of golden sea cucumber extract on the treatment groups compared to the control group expected to achieve the highest number from day 5 to day 7 during the wound healing process.[14] The administration of golden sea cucumber extract, consequently, is expected to accelerate the wound healing process.

Research data showed that golden sea cucumber extract could increase the number of lymphocytes on the treatment groups compared to the control group. On the 4th day, the number of lymphocytes significantly increased. This indicates that the administration of golden sea cucumber extract could accelerate the wound healing process. Moreover, the results of histological examination results showed that the biggest number of lymphocytes was found in the treatment group with 40% golden sea cucumber extract. 20% golden sea cucumber extract could not affect the number of lymphocytes, while 80% golden sea cucumber extract could decrease the number of lymphocytes. Therefore, it indicates that the concentrations of golden sea cucumber extract can differently affect, so the dose should be given correctly.[15] Every drug has their own concentration rules. Putranti's research proves that 40% golden sea cucumber extract is the most optimal concentration to increase angiogenesis on the Wistar rat's traumatic ulcer healing process.[16]

The increasing of lymphocytes compared to control group because of glycosaminoglycans contained in golden sea cucumber extract. Glycosaminoglycans, usually known as mucopolysaccharides, is a complex carbohydrate molecule which interacts with protein and involved in various physiological and pathological process. GAG consists of two types, namely GAG sulphate and GAG non sulphate.[17] Examples of GAG sulphate are chondroitin sulphate, dermatan sulphate, keratan sulphate, heparan sulphate, and heparin. Chondroitin sulphate and heparan sulphate have positive effects on wound healing process.[18]

Heparan sulphate located in organs and tissue is part of the extracellular matrix, such as colagen, fibronectin and laminin. Various types of protein can bind with heparan sulphate, such as extracellular matrix, growth factor, cytokine and chemokine. Heparan sulphate is involved in various physiological processes, such as proliferation, migration, differentiation, and interaction between cells. Heparan sulphate is known for its substansial role in a variety of cell interactions, and its bond with various types of protein can put those proteins on the cell surface.[19] The binding of cytokine with heparan sulphate can modulate the bioactivity of cytokine itself. Heparan sulphate can bind with various types of cytokines such as IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, Interferon-γ (IFN- γ), and fibroblast growth factor.[20]

Lymphocytes is the centre of cell immunity system that has an important role in the inflammation process by destroying microorganisms entering the body (antigen) and forming immunity (antibody) in the form of immunoglobulin.[4] The role of lymphocytes is to release lymphokines triggering the other inflammation of cell population. Some of the lymphokines released, consequently, can stimulate the agregation of macrophage in wound healing process. T lymphocytes then secrete various

types of cytokine, one of which is IL-2. IL-2 function to activate macrophage by stimulating the synthesis of IFN-γ.[21] T lymphocytes also produce TGF-β that function on the proliferation of fibroblast.[10]

Some of the cytokines that can bind with heparan sulphate are IL-2 and IFN-γ. A research shows that the binding of heparan sulphate with IL-2 can trigger activation and proliferation of T lymphocytes. IL-2 is an autocrine growth factor for T lymphocytes. IL-2 is produced by lymphocytes. IL-2 has a role for the growth factor of lymphocytes itself.

On the other hand, a research shows that binding of heparan sulphate with IFN-γ can affect bioactivity of IFN-γ by protecting from proteolytic degradation. It can also trigger IFN-γ to bind with it's receptor. IFN-γ is derived from the stimulation of IL-2. IFN-γ can activate macrophage. Consequently, it is also known as macrophage activating factor.[21] In addition, IFN-γ is considered as a major cytokine stimulant to activate monocytes and macrophage, as a result, the activated macrophage will activate lymphocytes and other cells that will work together in the inflammation process until the inflammation is gone.[22]

Furthermore, heparan sulphate has a great effect on the immune response through the modulation of antigen presenting cell (APC). On of the APC, there are macrophages. When many macrophages are activated, they will release more cytokines, one of which is IL-1. IL-1 can contribute to the activation of lymphocytes.[21] Thus, the number of lymphocytes will increase. Finally, it can be concluded that golden sea cucumber extract could increase lymphocytes, and 40% golden sea cucumber extract is the most optimal concentration in increasing lymphocytes during the process of Wistar rat's oral mucous traumatic ulcer healing.

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