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Echinometra mathaei cream activity against blood profile of male white rats induced by grade II burns

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Abstract
Administration of Echinometra mathaei cream has been shown to heal second-degree burns in previous studies. This study aims to determine the effect of the concentration of the ethanolic extract of Echinometra mathaei in the form of cream on changes in the blood profile of rats induced by second-degree burns, compared with sulfadiazine cream. This research is a true experimental design with post test control group design. The animals used were 30 male wistar rats which were divided into 6 groups including normal rats, negative control group, positive control given sulfadiazine cream, and 3 groups of Echinometra mathaei cream with concentrations of 1%, 3%, and 5%. Burn induction was given with a 20 mm diameter hot plate for 15 seconds, at a temperature of 1000C. Blood sampling through the heart was carried out on the 7th day after administration of burn cream. Data were analyzed using ANOVA and followed by a post hoc test with LSD. The results showed that the administration of Echinometra mathaei extract cream did not show significant differences in the count of leukocytes, erythrocytes, hematocrit, platelets, and hemoglobin ($p<0.05$). The data showed an improvement in the levels of leukocytes, erythrocytes and hemoglobin in the group of rats given Echinometra mathaei extract cream and silver sulfadiazine, compared to the negative control group. The conclusion of this study is that the administration of Echinometra mathaei extract cream for 7 days did not give a significant difference to the negative and positive controls on the blood profile data of male white rats induced by second degree burns.

Keywords: burns; echinometra mathaei; blood profile; cream; silver sulfadiazine

Introduction

Burns are a form of tissue damage or loss caused by contact with heat sources such as fire, hot water, chemicals, electricity and radiation with high morbidity and mortality that require special management from the beginning (the shock phase) to the advanced phase [1]. Burns that often occur at home and are the most common are second-degree burns.

Second-degree burns (Partial Thickness Burn) experience tissue damage involving the epidermis and dermis in the form of an inflammatory reaction accompanied by an exudation process [2]. In second-degree burns, it will be painful, pale when pressed, and marked by the presence of bullae filled with fluid exudate that comes out of the blood vessels because of the increased permeability of the walls. In general, treatment of burns can use various forms of drugs to help the healing process of burns such as bioplacenton, silver sulfadiazine, and bacitracin as antimicrobial agents [3]. As time goes by, the use of irrelevant antibiotics will cause resistance, so it is necessary to develop new drugs using marine life, namely sea urchins.

Sea urchins are a group of soft-shelled animals. Including members of the Phylum Echinoderms consisting of several classes, one of which is the class Echinoidea which is a marine animal that is round in shape and has spines on its skin that can be moved [4]. Sea urchins have a hard shell and the inside is symmetrical five sides, certain types of sea urchins have a shell. which is coated by a stable black liquid pigment [5].

Echinometra mathaei is one of the most common types of echinoderms. Sea urchin gonads can be used as an alternative food source because they contain 28 kinds of amino acids, vitamin B complex, vitamin A, minerals, omega-3 and omega-6 fatty acids, while the shell has potential as anticancer, antitumor and antimicrobial [6]. Pork contains high protein compounds, flavonoids, triterpenoids, steroids, saponins, and alkaloids [1]. Sea urchins also have a secondary metabolite, namely napthoquinone which has anti-inflammatory, antioxidant and antimicrobial activity [7]. Echinometra mathaei ethanol in the healing process of second-degree burns by looking at the blood profile data.

Methods

This study uses a research design with a control group post test only design. The research was carried out at the Pharmacology Laboratory of the Pharmacy Study Program, Faculty of Medicine, Hang Tuah University, hematology examination at the Veterinary Clinical Pathology Laboratory, Airlangga University. This research has been ethically approved by the Research Ethics Commission of Hang Tuah University, Faculty of Dentistry with the number: Sket/006/KEPK-FKGUHT/II/2021.

Research subject

The experimental animals used were male white rats (Rattus novergicus) wistar strain, aged 10-12 weeks with a body weight of 150-250 grams, which were climatized for 7 days, fed and drank according to laboratory standards. Determination of the number of test animals in each group using the formula: (n-1) (t-1) 15 (n = number of animals, t = number of groups). Experimental animals were divided into 6 groups, namely:

Normal Rats: Rats are in good health without being treated (burns) and smeared cream base.
Negatif Control: Rats that have been induced by burns and applied cream base.
Positif Control: Rats that have been induced by burns and applied Silver sulfadiazine cream.
Cream 1% Extract: The rats that had been induced by burns and were given sea urchin cream with 1% concentration.
Cream 3% Extract: The rats that had been induced by burns and were given sea urchin cream with 3% concentration
Cream 5% Extract: The rats that had been induced by burns and were given sea urchin cream with 5% concentration.

Manufacture of Sea urchin Extract
The extraction method used in this research is the cold extraction method, namely maceration, using 70% v/v ethanol as solvent. Sea urchin (Echinometra mathaei) powder was taken as much as 500 grams then dissolved in 70% v/v ethanol in a ratio of 1:10 with 5 L covered and left for 3 days protected from light and sometimes stirred. In the maceration process, replication was carried out 3 times, after that it was filtered to take the filtrate and concentrated using an evaporator.

Making Seaweed Extract Cream
In this study, a topical formulation of sea urchin extract cream was made with a graded concentration of sea urchin extract, namely 1%, 3% and 5% with the addition of a cream base to the extract, with the type of cream o/w.

Creation of a Grade-II Burn
In the process of making burns, rats were anesthetized intramuscularly with a ketamine cocktail dose of 0.1 mL/100gBW. The ketamine used is a cocktail ketamine which is a mixture of 87.5 mg/kg Ketamine and 12.5 mg/kg Xylazine. Then the mice's fur was shaved and marked with a circle with a diameter of 20 mm.
Burns were made by inducing the skin on the rat's back with a heat-inducing device which is a metal plate with a diameter of 20mm heated for ±15 minutes on a hot plate until the temperature reaches ±100°C, then affixed to the rat's back for 15 seconds. The resulting burns are classified as IIB degree burns.

Blood Sampling
The mice that had been given treatment for 7 days in each test group were then taken blood samples. Previously, the rat was dislocated in the neck and blood was taken from the heart of the rat using a 1cc syringe. After the blood has been collected, it is immediately put into the EDTA tube and shaken around like a figure eight to prevent blood clots from forming. After all the blood has been collected, it is analyzed using a hematology analyzer.

Hematology Analysis Assays
The collected blood samples were then analyzed using a hematology analyzer to determine the values of erythrocytes, leukocytes, platelets, hemoglobin and hematocrit in male white rats.

Data analysis technique
The data obtained were tested for normality and homogeneity first. If it is normally distributed, then the One Way ANOVA statistical test is carried out and then using the LSD Post-Hoc test. The results are said to be meaningful if the p value <0.05. SPSS 22.0 statistical software tool was used for Data Analysis.

Results
The extract cream was made using a standard cream base with the heating method, and the cream made remained stable on the 30th day after manufacture. The cream made is of the o/w type, where the extract of Echinometra mathaei is oil, so it is dissolved in the oil phase.
Based on the results of the analysis that has been carried out, it can be seen in Table 1, it was obtained that the average range of total leukocytes from all groups of mice was in the normal range of leukocyte values. Leukocytes play a role in the body's defense process against exposure to foreign objects or infections. Giving cream for 7 days has improved inflammation / infection indicated by normal leukocyte levels.

Table 1: Data of Leukocyte and Erythrocyte Profile of Mice All Groups after 7 Days of Treatment

Groups	leukosit± SD (.10^3)	Normal Leukosit Value	Eritrosit ± SD (.10^6)	Normal Eritrosit Value
Normal rat	15,5 ± 0,49	7,0-15,0 x 10^3 / mm³	8,0 ± 0,70	6,0-9,0 x 10^6 / mm³
control -	10,3 ± 2,25		4,3 ± 1,04	
control +	9,3 ± 6,87		5,3 ± 1,11	
Krim 1% extract	12,8 ± 2,37		6,1 ± 1,19	
Krim 3% extract	9,3 ± 1,15		5,6 ± 1,30	
Krim 5% extract	8,1 ± 4,75		5,5 ± 3,15	

Table 2: Data on Platelets, Hematocrit, and Hemoglobin Profiles of Rats in All Groups after 7 Days of Treatment

Groups	Platelet ± SD (.10^3)	Normal Platelet Value	HCT ± SD (%)	Normal Hematocrit Value	Haemoglobin ± SD (g/dL)	Normal Hemoglobin Value
Normal rat	312,5 ± 6,36	150 -350 x 10^3/ mm³	50,5 ± 2,12	37-51 %	14,1 ± 0,21	12,4-14,6 g/dL
control -	262,0 ± 5,56		42,0 ± 10,39		8,4 ± 4,45	
control +	257,3 ± 67,27		46,0 ± 11,13		10,7 ± 0,6	
Krim 1% extract	266,6 ± 31,46		43,6 ± 3,51		13,1 ± 0,72	
Krim 3% extract	305,0 ± 4,35		40,6 ± 6,42		10,6 ± 3,49	
Krim 5% extract	307,6 ± 4,50		41,0 ± 3,00		12,3 ± 1,48	

Discussion

This study used a comparison of 1% silver sulfadiazine cream, which is the topical antibiotic of choice for burns or often referred to as the gold standard therapy. Silver sulfadiazine is a metal and antibacterial mixture that can fight fungi, dermatophytes, and other microorganisms. Sulfadiazine has a bacteriostatic effect, works by inhibiting the formation of bacterial cell membranes and silver metal works by closing and reducing pain [8]. The use of antibiotics as a burn medicine can cause drug resistance [9]. So that other alternatives are needed to prevent resistance, one of which is to use the active substances contained in sea urchins (Sea urchin).

Treatment of sea urchin extract cream Echinometra mathaei aims to accelerate the healing process of burns. Echinometra mathaei has an active compound, namely flavonoids which have anti-inflammatory activity. This anti-inflammatory activity can occur because the bisopiron ring in the flavonoid structure can bind to cyclooxygenase and lipoxygenase, in addition, if flavonoids have hydroxyl groups at C5 and C7, this group can bind to the lipoxygenase enzyme [10]. In the end, it will inhibit the formation of leukotrienes and hydroxy fatty acids, so that the production of LTB4 mediators that act as chemotactic polymorphonuclear leukocytes, eosinophils and monocytes will be reduced [11]. So that on the 7th day the leukocyte value was in the normal range.

Erythrocytes are red blood cells that transport hemoglobin in circulating body cells. In mice Ex. positive control, Ex. negative control, Ex. Normal was in the range of normal erythrocyte values as well as erythrocyte values in male white rats given Echinometra mathaei extract cream at concentrations of 1%, 3% and 5% were in the normal range. Normal erythrocyte values indicate that Echinometra matahei sea urchin extract cream is not toxic to white rats, so it does not cause rats to undergo hemolysis. Hemolysis is the rupture of erythrocytes accompanied by the release of substances contained therein, so that the serum or plasma looks reddish and can cause analysis errors [12]. Things that can cause hemolysis are erythrocyte defects, infections, drugs, chemicals, transfusions, antibodies, excessive spleen work, toxic and mechanical [13].

The average range of hemoglobin levels of male white rats in each group was in the normal range. The value of hemoglobin is directly proportional to erythrocytes, it can be seen that in this study the number of erythrocytes and hemoglobin levels in each group were in the normal range of values. The hemoglobin and erythrocyte data also showed that in the negative control group, the erythrocyte and hemoglobin levels were lower than the other groups, this indicates that the administration of SSD cream and extract cream provided an improvement in erythrocyte and hemoglobin levels. Hemoglobin has a very important function in the blood, namely transporting oxygen from the lungs throughout the body and bringing back carbon dioxide from all cells to the lungs to be removed from the body. Oxygen is one of the important elements in the wound healing process, the more hemoglobin binds oxygen, the faster the wound healing process. Low hemoglobin levels and lack of oxygen intake to the tissues can cause tissue death [14]. So that the wound healing process becomes longer.

Hematocrit percentage is the ratio of erythrocytes to total blood volume. The amount of the hematocrit percentage depends on the total number of erythrocytes and the amount of oxygen needed for body metabolism [15]. It can be seen in table 5 the hematocrit value in the group of rats Ex. positive control, Ex. negative control, and Ex. Normal was in the normal range of hematocrit values as well as the hematocrit values in male white rats treated with Echinometra mathaei extract cream at concentrations of 1%, 3% and 5% were in the normal range. Sea urchin extract cream had no significant effect on increasing or decreasing hematocrit.

Platelets or platelets are blood cells that function in the hemostasis process. From the results of the research conducted, it can be seen in table 2. That the platelet value in the group of rats Ex. positive control, Ex. negative control, Ex. Normal was in the normal range of platelet values as well as platelet values in male white rats given Echinometra mathaei sea urchin extract cream at concentrations of 1%, 3% and 5% were in the normal range.

Observation of the blood profile on the 7th day after therapy has a weakness because it does not describe the inflammatory condition experienced by rats after induced burns. It is better to observe the blood profile during the inflammatory phase (H-7) for example on the 3rd day after induction, so that the course of inflammation due to burns can be observed. On the 7th day of taking blood, it has entered the proliferative phase, so that changes in the blood profile are not very visible because it looks normal.

Conclusions and suggestions

The administration of Echinometra mathaei extract cream for 7 days did not give a significant difference to the negative and positive controls on the blood profile data of male white rats induced by second degree burns.

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