



SNAKEHEAD FISH (CHANNA STRIATA BLOCH) EXTRACT OINTMENT AND EMULSION DAILY USAGE DOSE OPTIMIZATION FOR WOUND HEALING STAGE II ON WISTAR STRAIN WHITE MALE RATS (RATTUS NORVEGICUS)

Mohamad Andrie¹, Wintari Taurina*¹ and Elvie Rusmiyanto Pancaning Wardoyo²

¹Department of Pharmacy, Faculty of Medicine, Tanjungpura University, Pontianak, Indonesia.

²Department of Biology, Faculty of MIPA, Tanjungpura University, Pontianak, Indonesia.

***Corresponding Author: Wintari Taurina**

Department of Pharmacy, Faculty of Medicine, Tanjungpura University, Pontianak, Indonesia.

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ABSTRACT

Wounds are physical injuries that cause tears and damage to skin tissue.^[1] The basic principle of optimal wound healing to minimize tissue damage is by providing proper nutrition with moist wound healing environment to restore the function of damaged tissue.^[2] Snakehead fish extract emulsion that were given orally and snakehead fish extract ointment that were given topically (20 days) can accelerate the wound healing process on Wistar strain white male rats.^[3,4] This research aims to see the effect of variations in daily doses of the use of snakehead fish extract ointment and emulsion on Wistar strain white male rats that were given acute wounds close stage II. Wistar strain white male rats divided into 6 groups such as 1, 2, 3 times a day emulsion and 1, 2, 3 times a day ointment. Analysis result with Post Hoc Test-LSD indicates that there is a significant difference between the groups that were given snakehead fish extract emulsion that indicates that the increase of the frequency of daily emulsion giving the greatest healing effect of wounds that occurred at 3 times a day emulsion. Whereas between groups of daily doses of ointment had a significant difference that indicated that the increase of the frequency of giving ointments in a day gave a significant wound healing effect, the biggest wound healing effect occurred in the administration of 3 times a day ointment. Albumin levels in the blood greatly determine the process of wound healing, by giving snakehead fish extract emulsion will increase the level of albumin in the blood.

KEYWORDS: Snakehead fish extract, emulsion, ointment, wound healing.

INTRODUCTION

Nowadays snakehead fish extract is used in medicine as a postoperative wound healing and burn injuries.^[5] Wounds are physical injuries that cause tears and damage to skin tissue.^[1] The basic principle of optimal wound healing to minimize tissue damage is by providing proper nutrition with moist wound healing environment conditions to restore the continuity of the anatomy and function of damaged tissue in a short time.^[2] So far, drugs on the market tend to rely on the use of antibiotics to speed up the process of wound healing, such as topical preparations containing antibiotics such as neomycin sulfate. This can trigger bacterial resistance, so the researchers feel it is important to conduct research of the effectiveness test of the water-oil phase combination snakehead fish extract ointments and emulsion and to optimize the concentration and comparison of water-oil phase in the preparation of ointments and emulsion.

Fish oil contains especially fatty acids such as omega-3 and omega-6, minerals and vitamins as nutrients in

wound healing that can reduce inflammation by increasing connective tissue function and collagen synthesis that accelerate revitalization, neocapillarization, and infiltrating inflammatory cells in the wounded area.^[6] Other content that is important compounds in the process of tissue synthesis is albumin and minerals such as Zn, Cu, Fe.^[7] Albumin is a globular protein that is useful in the formation of new body tissues at the age of growth and accelerates healing of body tissues, for example after surgery, burn injuries, and when sick.

MATERIALS AND METHODS

Materials

The materials used for making ointment and emulsion are snakehead fish meat (*Channa striata* Bloch), an ethanol extract of green betel leaf (*Piper betle*), clove oil (*Syzygium aromaticum*), adeps lanae, vaselin flavum, cera flavum, DMDM Hidantoin, BHT (butyl hydroxytoluene), aquadest, PGA, and Na CMC. While the materials used to test the effects of wound healing are cotton, 70% alcohol, and 10% ether.

Methods

Sample Preparation

Snakehead fish meat steamed for ± 30 minutes in temperature $65-70^{\circ}\text{C}$. Furthermore, repeated pressing using a hydraulic press tool to produce the extract. The extract was centrifuged for 60 minutes at 6000 rpm, after that extract separated into water and oil phase.

Albumin Identification Test

5 mL of water phase snakehead fish extract was heated in a water bath for 30 minutes. Water phase contains albumin if there is a white clump floating on the top of the water phase.^[8]

Formulation Of Snakehead Fish Extract Ointment And Emulsion

Ointment Formulation

Oil phase	20%
Freeze dry powder water phase	5%
Aquadest	50%
Adeps lanae	2,5%
Betel leaf/ clove oil ethanol extract	10%
DMDM Hidantoin	1%
BHT	0,1%
Cera flavum	5 %
Vaselin flavum	ad 100%

Emulsion Formulation

Oil phase	20 %
Freeze dry powder water phase	10 %
PGA	50 parts of the oil phase
BHT	0,1%
DMDM Hidantoin	1%
Na CMC	1%
Aquadest ad	100%

Preparation Of Snakehead Fish Extract Ointment And Emulsion

The method of making ointment follows the rules of making ointment number 2 and 3. Ointment is made as much as 30 grams.^[7,8] While the emulsion is made as much as 50 g, stirred until homogeneous.^[10]

Dose Determination of Snakehead Fish Extract Ointment And Emulsion

Dose Determination of Ointment (One Time Use)

Dose determination of one time use ointment based on orientation results that is 0.16 g/cm^2 area of the wound.

Dose Determination of Ointment (One Day Use)

The dose of one day uses ointments, use 3 dose variation such as 1, 2, and 3 times a day, topically about 0.16 g/cm^2 ointment per area of the wound and observed for 20 days (time needed for wound healing).

Dose Determination of Emulsion (One Time Use)

Dose determination of emulsion is closely related to the volume of administration. In rats, the volume of administration for 1 time is 0.5 mL, meaning that if given to humanity the volume of administration for 1

time use is to 0.5 mL: $0.018 = 27.78\text{ mL}$ (about 2 tablespoons). This is considered to be reasonable if it is given to humans. So the dose of emulsion for 1 time use is 0.5 mL/ 200g BB of rats. While the oil phase concentration is equal to x% of the volume of administration. x% will be obtained from the estimation results of the oil phase concentration in the current study.

Dose Determination Of Emulsion (One Day Use)

The dose of one day uses emulsion use 3 dose variation such as 1, 2, and 3 times a day orally about 0.5 mL/ 200g BB of rats and observed for 20 days (time needed for wound healing).

Effectiveness Test Wound Healing Stage II Of Ointment And Emulsion

Grouping of Experimental Animals

Group 1: 1 time a day ointment
Group 2: 2 times a day ointment
Group 3: 3 times a day ointment
Group 4: 1 time a day emulsion
Group 5: 2 times a day emulsion
Group 6: 3 times a day emulsion

Making Wounds in Rats Skin

Rats are anesthetized using 10% ether with an inhalation pathway. Then the hair around the back is shaved with 4 cm diameter and cleaned with 70% alcohol. Wound stage II is made by making a round wound 2 cm in diameter (3.14 cm^2) using a round mold, cut in a round shape to remove the printed skin.^[11] This injury was carried out in the back area of the rat and was carried out equally in each group of test animals.

Measurement of Wound Area Using The Macbiophotonic Image J Program

Round wounds with a diameter of 2 cm in rats were photographed perpendicular with a high resolution camera. Each photo was quantified using the parameters of the wound area (cm^2). Quantification assisted by the Macbiophotonics Image J program until the result of wound area (cm^2) was obtained.

Data Analysis

The data obtained from this study are qualitative and quantitative data. Qualitative data include observing the wound healing process of rats. While quantitative data in the form of the percentage of wound healing power that is calculated based on changes in the area of the wound from day to day. Furthermore, percentage data of wound healing power are presented descriptively in the form of tables and line graphs, then statistically tested using One Way ANOVA assisted by SPSS 22 for Windows computer program. If the data obtained is included in the parametric category, the data are normally distributed, has a homogeneous variant and the sample is taken randomly, then the analysis will use One Way ANOVA with Post Hoc LSD. If the data obtained is included in the nonparametric category, which does not meet the

three requirements that have been determined, then the analysis will use Kruskal-Wallis Post Hoc Wilcoxon.^[12]

RESULTS AND DISCUSSION

Sample Preparation

± 2.8 kg snakehead fish are cleaned from the head, stomach contents, and scales. Extraction of snakehead fish is done by steaming and pressing. Steaming is carried out at a temperature of 65-70° C for 30 minutes. Steaming below 65° C resulted in the cells from the flesh of the snakehead fish being difficult to break so that the oil will released slightly. Steaming above 70° C will cause denaturation protein in snakehead fish. The steamed snakehad fish meat, then wrapped in a cloth napkin and put into a hydraulic press. The working principle of the hydraulic press tool is to put pressure on the material placed on the underside of the tool, then the upper and lower sides are closed after being pressurized by the hydraulics that are fused to the underside of the tool.

The snakehead fish extract then centrifuged at a speed of 6000 rpm for 60 minutes. The result of the centrifugation will be seen in 3 layers in the test tube. The lowest layer is impure, the middle layer is the water phase, and the upper layer is oil phase. Oil will form a layer that is separated from water due to differences in density where the density of water is greater than the density of the oil. The oil phase and water phase are separated and stored in containers covered with aluminum foil. Then the water phase was freeze dried at a temperature below 0° C with a pressure of 27-133 pA for 24 hours. The yields of extract, oil phase, water phase, and freeze dry powder obtained can be seen in Table 1.

Table 1: Extract yield, oil phase, water phase, and freeze dry powder.

Active Ingredients	Acquisition	Yield (%)
Snakehead fish extract	540 mL	25,71
Oil phase	10 mL	1,85
Water phase	530 mL	98,14
Freeze dry powder	34,75 g	6,55

Identification Results of Water Phase Albumin of Snakehead Fish Extract

Water phase of snakehead fish extract contains positive albumin, it is characterized by white clumps or foam.

Formulation and Manufacture Snakehead Fish Extract Ointment and Emulsion

Ointment is formulated to extend the contact of the drug with the skin, so that it is expected that more drugs are released from the base to the skin. Ointment itself is expected to provide a moisturizing effect because it is formulated with a base which is one of the moisturizing agents, so that thick and hard crusts are not formed which can interfere with the penetration of the drug into the skin and interfere with the granulation and epithelialization processes so that wound healing can last longer. Snakehead fish extract consists of water phase

and oil phase that cannot mixed. Therefore, it is made in the form of an emulsion that makes it possible to form a stable preparation between the water phase and the oil phase by adding emulsifier.^[9]

Injury Process and Treatment on Wistar Strain White Male Rats

Rats that used in the study were acclimatized for 7 days. This is intended for accustomed rats to their new environment to minimize stress that may arise. 24 Wistar strains white male rats were divided into 6 groups. Rats are anesthetized using 10% ether with an inhalation pathway to minimize animal movements when wounding. Then the hair around the back is shaved with 4 cm diameter and cleaned with 70% alcohol. Wound stage II is made by making a round wound 2 cm in diameter using a round mold, cut in a round shape to remove the printed skin. The injury is in the form of an acute wounds open stage II. Based on the time of healing the wound is an acute wound or a wound that immediately gets treatment and the healing period does not require a long time. Based on the depth of the wound at the stage II is the loss of the skin layer in the epidermal layer and the upper part of the dermis.

Effectiveness Test Result of Acute Wounds Close Stage II of Snakehead Fish Extract Ointment and Emulsion

The area of the wound is measured using the Macbiophotonic Image J program. The working principle of the Macbiophotonic Image J program is to determine and quantify the wound area of the test animal in mm² units. Photo of the wound area was taken with the help of a rectangular frame with a scale. The frame position must be parallel to the wound surface of the rats so that the measurement scale can represent the actual size of the wound of the rats. Taking photos of the wound starts from day 2. This is because on day 0 and day 1 the wound has not dried so the skin around the wound area is still moving so that the area of the wound can still change. The results of wound area obtained from quantification using the MacbiophotonicImage J program were converted into a percentage wound healing power with the following formula:

$$\% \text{ wound healing power} = \frac{\text{initial wound area} - \text{final wound area}}{\text{initial wound area}} \times 100\%$$

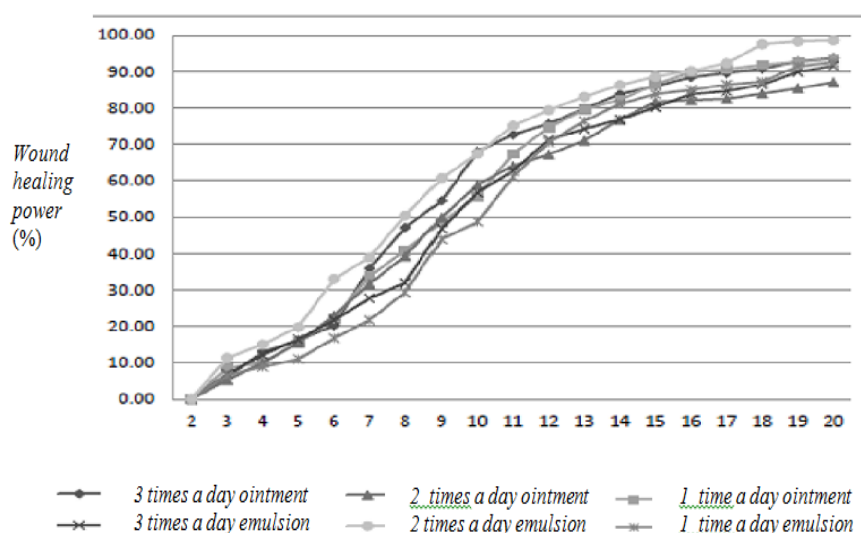


Figure 1: Graph of Average Acute Wounds Close Stage II Wound Healing Percentage.

Based on figure 1, there was an increase in the percentage of wound healing power from day 2 to day 20. The smaller the area of the wound, the greater the percentage of wound healing power. From the percentage of wound healing day to day can be

calculated the value of AUC (Area Under Curve). The AUC values describe the overall wound healing in the animal test so that it can be known which group gives the best healing effect (figure 2).

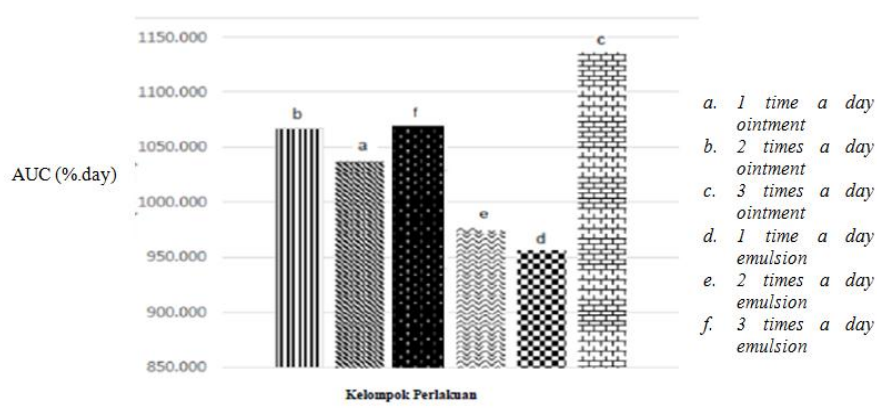


Figure 2: Total AUC Value of Each Group.

A total AUC value for each animal test was tested statistically using SPSS version 22 program. The research data was tested normality using the Shapiro-Wilk test. The test results show that the data normally distributed with a significance value more than 0.05 ($p > 0.05$). Furthermore, the data were analyzed using Homogeneity of Variance Test (Levene Test) to see if the data obtained were homogeneous. The results obtained show a significant value of more than 0.05 ($p > 0.05$) so it can be said homogeneous data. Data that is normally distributed and homogeneous is a category of parametric data. Thus, One Way ANOVA test was conducted to see the significant difference in the data obtained between treatment groups. One Way ANOVA test showed significance that data had significant differences between groups ($p < 0.05$). To know which groups show the significance value, then proceed with Post Hoc test.

Post Hoc test results with the LSD method show that that between groups of daily doses of the emulsion had a significant difference, this indicated that an increase in the frequency of daily emulsion administration gave a wound healing effect, the biggest wound healing effect occurred at 3 times a day dose emulsion. Post Hoc test results with the LSD method showed that between groups of daily doses of ointment had a significant difference, this indicated that an increase in the frequency of daily ointment giving a significant wound healing effect, the biggest wound healing effect occurred in the administration of 3 times a day ointment.

Albumin levels in the blood to determine the process of wound healing, by giving snakehead fish extract emulsion will increase the level of albumin in the blood. High amino acid content in the snakehead fish extract is also very helpful in the process of skin tissue formation.

Antibacterials in the ointment are absolutely necessary in the process of wound healing, the lower level of wound infection, the faster of wound healing. Similarly, the content of the snakehead fish extract in the ointment, rich in albumin nutrients and amino acids is very necessary in the process of formation of damaged tissue.

The method used is to make a covered (dressing) wound stage II. Covered wound will make wound condition is always moist. Moist conditions are even more needed in the process of wound healing. With moisture in the wound automatically the body will accelerate the process of fibrinolysis by neutrophil cells and endothelial cells will quickly remove fibrin threads. Furthermore, it will accelerate the process of angiogenesis or the formation of new blood vessels in the wound. In the moist atmosphere the body will accelerate the formation of active cells and will affect the presence of neutrophil invasion followed by macrophages, monocytes, and lymphocytes directly to the wound. And the last is the formation of growth factors that play a role in the wound healing process to form the stratum corneum. The process of granulation and epithelialization will also occur faster if the wound condition is moist. A balanced wound moisture condition strongly supports wound healing. Too dry wounds cause the formation of hardened fibrin to form scabs that can inhibit wound healing, while wounds that are too wet cause maceration, which is softening of the skin tissue which can cause wounds to widen and damage the skin tissue around the wound.

CONCLUSIONS

- a) Post Hoc test results with the LSD method showed that between groups of daily doses of the emulsion had a significant difference, this indicated that an increase in the frequency of daily emulsion administration gave a wound healing effect, the biggest wound healing effect occurred at 3 times a day dose emulsion.
- b) Post Hoc test results with the LSD method showed that between groups of daily doses of ointment had a significant difference, this indicates that an increase in the frequency of daily ointment giving a meaningful wound healing effect, the greatest healing effect of wounds occurred in the administration of 3 times a day ointment.

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