FORMULATING CREAM OF DUMBO CATFISH SKIN EXTRACT (Clarias gariepinus) FOR ACCELERATION OF Slice Wound Healing in WHITE MALE RATS (RATTUS NOVERGICUS) WISTAR STRAIGHT

Nurjannah Mopangga[1)], Moh Aprianto Paneo[2)], and Srimuliani Arbie[3)]
[1,3)]Bina Mandiri University Gorontalo
[2)]Gorontalo State University
Email : puputmopangga666@gmail.com

ABSTRACT

Wound is a condition in which the skin tissue is damaged and some of the skin tissue is lost. Catfish skin is one part that contains collagen.One of the ingredients that has been widely used to maintain a moist atmosphere in wounds is collagen This study aimed to determine the effect of African catfish skin extract cream to determine the effectiveness of collagen from catfish skin extract in accelerating the healing process of cuts in male white rats.

The method in this research is experimental laboratory using one sample t-test test data analysis to see the effect of the catfish skin extract cream formula. The research design used was a post test only control group. The experimental group was treated by giving the catfish skin extract cream formula, while the negative control group was not given any treatment. Each cream formula has three concentrations, namely 5%, 7.5% and 12.5% and cream base as a negative control.

The results showed that the cream formulation with a concentration of 12.5% had an effect on wound healing on the seventh day, this was seen from physical observation by looking at the changes in the length of the wound which was reduced and the wound had dried. African catfish skin cream has an effect on wound healing with a healing process within 7 days The effectiveness of African catfish skin cream at a concentration of 12.5% has a faster effect on wound healing compared to the 7.5% and 5% formulas

Keywords: cuts, African catfish skin, collagen

INTRODUCTION

A cut is a damage that occurs to the layer of skin tissue caused by sharp objects such as knives, razors, axes, and swords. When skin tissue is injured, there are several effects such as bleeding and hemostasis, loss of some organ functions, contamination of microorganisms, and cell death [8].

One of the natural remedies is the use of catfish. Catfish (Clarias gariepinus L) is one of the fisheries trade that is quite popular among the people. The nutritional composition of catfish includes 17.7% protein content, 4.8% fat, 1.2% minerals and 76% water. The advantage found in catfish when compared to other animal products is that catfish is rich in leucine and lysine. Leucine is one of the essential amino acids. Leucine is also useful in the overhaul and formation of protein in muscles. While lysine is one of the essential amino acids needed in the

growth and recovery of damaged tissue [5].

One of the ingredients that is widely used to maintain a moist atmosphere in wounds is collagen. Collagen plays a very important role in the process of wound healing in skin tissue. Collagen is one of the proteins found in animal tissues. About 30% of the total protein in the animal body is collagen which can be found in muscles and skin [7].

Collagen is a protein found in animal tissues. About 30% of the total protein in the animal body is collagen which can be found in muscles and skin [1].

Collagen from skin and bones in cattle and pigs is commonly found nowadays. The use of collagen from cows and pigs has problems, both in terms of health and religion. Collagen derived from cows can cause contamination of Bovine Spongiform Encephalopathy (BSE) and Transmissible Spongiform Encephalopathy (TSE) [13].

This condition opens the opportunity to look for collagen from other raw material sources. Potential raw materials to be used as a source of collagen are fish skin and bones [2].

Fish collagen has a smaller structure compared to collagen made from beef or pork so it is easier to be absorbed by the body. [6]

Fish skin structure is softer than fish bones. This causes the extraction process on fish skin to take place faster than fish bones. In addition, the value of collagen from fish skin is higher than the value of collagen from fish bones [5].

Extracts that contain a lot of collagen, one of which is found in catfish skin with the obtained collagen content of 25.18%. This percentage of collagen is close to the percentage of collagen from channel catfish (Ictlaurus punctaus) which is 25.8%. This shows that catfish skin can be used as a source of collagen [5].

Collagen extraction can be carried out chemically, enzymatically, or a combination of both. Chemical extraction is carried out with acidic or basic solvents. The enzymatic extraction process used pepsin as a solvent. Chemical processes with acid solvents are suitable for raw materials that have a collagen structure with less strong bonds such as pork skins and fish skins, while alkaline solvents are generally used for materials with stronger and more complex bonds such as fish bones and cow skins. 7].

The use of extracts directly on the skin is very inconvenient and impractical, therefore this research was conducted to make a cream formulation from catfish skin extract. Cream is a semi-solid dosage form containing one or more drug ingredients dissolved or decomposed in an appropriate base [3].

The thing that needs to be considered in the manufacture of cream preparations from catfish skin extract is that it must be adjusted to a suitable base, because the base must be able to mix both physically and chemically with the active substance, not damage or inhibit the therapeutic action of the drug and can release the drug on the affected area. being treated.

Based on the explanation above, the author is interested in conducting research on catfish skin extract containing collagen. In this study, the author wanted to examine the "Formulation of Dumbo Catfish Skin Extract Cream for Accelerating Healing of Cuts in White Male Rats (Rattus novergicus) Wistar strain".

RESEARCH METHODS

The equipment used includes: stirring rod (pyrex), Separating Funnel (pyrex), Erlenmeyer (pyrex), Beaker (pyrex), Magnetic strirer, pH Meter, Scalpel, Viscometer (Brookfield).

The ingredients used are Aquadest, Stearic Acid, Adeps Lanae, Ethanol,

Dumbo Catfish Skin, Methyl Paraben, Mg, NaOH, Ninhydrin, Propyl Paraben, Liquid Paraffin, TEA.

The type of research carried out is a type of quantitative research using the Laboratory of Pharmaceutical Preparation Technology, University of Bina Mandiri Gorontalo for 2 months.

The subjects used in this study were 12 white male rats (Rattus novergicus) Wistar strain.

The research design used was a post test only control group. The experimental group was treated by giving the catfish skin extract cream formula, while the negative control group was not given any treatment

The catfish skin was macerated as much as 200g using 1000 ml of 2% HCl solvent for 3x24 hours, after extraction the sample was filtered using a 0.5mm filter cloth and part of the filtrate was taken, then the filtrate was neutralized using 1 M NaOH to close to a neutral pH of about 7 and allowed to stand for 24 hours. Furthermore, collagen fibers will be visible at the bottom of the beaker, and filtered using size filter paper±1 m to obtain wet collagen fibers.

The isolated sample was put in a test tube, then 1M NaOH was added, then the 1% Ninhydrin reagent was heated, and the changes were observed. The positive results indicated were purple, blue, and pale yellow based on the type of amino acid contained.

Formula Design

Table 1. African cat fish skin extract cream formulation

No	INGREDIENTS	F1	F2	F3	F4
1	Catfish Skin Extract	0	5%	7.5%	12.5%
2	Stearic Acid	15%	15%	15%	15%
3	Adeps Lanae	0.25%	0.25%	0.25%	0.25%
4	TEA	2%	2%	2%	2%
5	Paraffin Liquid	45%	45%	45%	45%
6	Methyl Paraben	0.1%	0.1%	0.1%	0.1%
7	Propyl Paraben	0.2%	0.2%	0.2%	0.2%
8	Aquadest	Ad	Ad	Ad	Ad

Source: Nuri, 2019

All cream ingredients are weighed according to their respective concentrations. The ingredients in the formula are separated into two groups where the water phase is also the oil phase.The oil phase is stearic acid, liquid paraffin and adeps lanae are separated in a porcelain cup, added with propyl paraben, then melted on a water bath, after that the formula for the aqueous phase is TEA and aquadest is added to a beaker added with methyl paraben. The melted oil phase was transferred on a magnetic stirrer and gradually added to the water phase until it formed a creamy mass.

In the organoleptic test, the examination includes color, odor and texture and in the pH test, pH is measured using pH Universal paper. The change in color of the pH paper is observed.

In the dispersion test, 0.5 g of cream was placed on a closed slide and allowed to stand for 1 minute to get the cm of the area formed, added loads of 50g, 100g, and 150g, and observed the diameter of the area formed.

In the adhesion test, the cream was placed on a slide, then covered with another slide. Let it sit and then lift the slide, then observe the time (seconds) for the cream to separate from the other slides

In the homogeneity test, each sample was smeared on a clean and dry slide. Covered with a glass slide. Observing the texture of the cream, it is said to be homogeneous if there are no granules and lumps in the cream

The experimental animals were acclimatized for 1-2 days and given feed from grains such as corn, rice and fruits that contain a lot of water such as papaya. Feed and drink every day. Fasted for 12 hours to 24 hours before the animals were treated. The hairs around the back of the mice were shaved with a diameter of 2 cm using a scalpel, then cleaned with alcohol.

An incision is made 1 cm long with a depth of 0.1 mm

The information analysis technique used the one sample t-test method by seeing the occurrence of a significant difference <0.05% <95%. Then proceed with the post hoc LSD method to see the effect of the concentration of one formula with another.

RESEARCH RESULT

Table 2. The results of the calculation of the yield of African catfish skin

Sample weight (g)	Extract Weight	Yield (%)	Yield
268.28 g	1.01 g	0.38%	

Source: Data in Processed, 2021

Table 3. Results of collagen phytochemical screening on African catfish skin

Phytochemical Screening	**Test**	**Test results**	**Conclusion**
Collagen	Ninhydrin	Pale yellow	(+)

Source: Data in Processed, 2021

Cut Length Measurement Results

After conducting the research, the results obtained from the measurement of the length of the incision which were seen physically from day 1 to day 11, the length of the incision in Male White Rats (Rattus novergicus) can be seen in the following table:

Table 4. Measuring the length of the cut

Cream Sample	H0	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11
F1(5%)	1.0	0.98	0.88	0.82	0.70	0.60	0.44	0.32	0.22	0.0		
F2 (7.5%)	1.0	0.96	0.86	0.79	0.64	0.54	0.32	0.20	0.00			
F3 (12.5%)	1.0	0.93	0.81	0.75	0.54	0.46	0.20	0.0				
Negative Control	1.0	0.99	0.90	0.88	0.85	0.74	0.60	0.44	0.36	0.20	0.12	0.0

Source: Data in 2021

Evaluation Results of Dumbo Catfish Skin Extract Cream Preparations

Table 5. Organoleptic test of cream preparation

Test	**F1 (5%)**	**F2 (7.5%)**	**F3 (12,5)**
Smell	Rancid	Rancid	Rancid
Color	Broken white	Broken white	Broken white
Texture	Bit rough	Gentle	Gentle

Source: Data in 2021

Table 6. Cream stickiness test results

Cream Formula Preparations	Adhesion (Second)
F1 (5%)	33.43 seconds
F2 (7.5%)	36.44 seconds
F3 (12,5)	36.87 seconds

Source: Data in 2021

Table 7. Spreadability test results

Assigned Burden	F1 (5%)	F2 (7.5%)	F3 (12.5%)
only glass	3.06 cm	3.72 cm	3.03 cm
50g Beban Load	3.53 cm	3.46 cm	3.46 cm
Load 100g	3.80 cm	3.63 cm	3.80 cm
150g Beban Load	3.96 cm	3.86 cm	4 cm
Average	3.58 cm	3.66 cm	3.57 cm

Source: Data in 2021

Table 8. Homogeneity test results

Cream Preparation Formula	Observation result
F1 (5%)	(-) Inhomogeneous
F2 (7.5%)	(+) Homogeneous
F3 (12.5%)	(+) Homogeneous

Source: Data, 2021

Table 9. Preparation ph test results

Cream Preparation Formula	Preparation pH
F1 (5%)	5
F2 (7.5%)	6
F3 (12.5%)	6

Source: Data in 2021

DISCUSSION

The purpose of this study was to see whether there was an effect on the

concentration of the cream formula of African catfish skin extract (Clarias gariepinus) on wound healing in male white rats (Rattus novergicus) Wistar strain. This experimental laboratory research was chosen because the samples used were not too many studies carried out, and the process in the research was more measurable and the effect of treatment was more reliable.

The acid soluble method is the extraction method used in this study. The reason for choosing an acid solvent to be used in maceration is because acid solvents are more suitable for raw materials that do not have a collagen structure with not too strong a bond. There was a fluctuation in the sample weight at the time of immersion this was due to a swelling effect that would facilitate the extraction process. The degree of swelling experienced by collagen tissue depends on the pH of the solution used [9].

After the extraction process is complete, the sample is filtered to separate the filtrate and residue, then the filtrate is purified by purification using a salting-out technique with 1 M NaOH for 24 hours to produce wet collagen fiber deposits.

A total of 100 g of African catfish skin (Clarias gariepinus) was extracted using 800 ml of 2% HCl solvent to produce an extract of 0.81 g, and for the second extraction it produced 0.05 g of extract with 200 ml of solvent blackish brown in color. .

In table 2 the yield on African catfish skin is obtained as much as 0.38%.In this case, the yield on African catfish skin research has a lower yield than other types of fish.

The obtained wet collagen fibers were then tested for phytochemical screening usingninhydrin test. This can be seen in table 3 where the ninhydrin test shows a color change from clear to yellow, which theoretically positive results for ninhydrin are blue, purple and yellow, depending on the type of amino acid constituent. This test aims to determine whether in a sample solution there are free amino acid groups.

Proline is a type of amino acid that only has a secondary amino group. The main benefit of proline is to form collagen in the body. Collagen is a smooth and flexible tissue that provides bonds to bones like glue. Collagen is the main structural protein found throughout the human body. In conjunction with another amino acid, namely lysine, proline is a precursor to hydroxyproline and hydroxylysine. Hydroxyproline is used by the body to make collagen, tendons, ligaments, and also heart muscle. Collagen in our body consists of 15% proline [8].

Wound healing is a process that has interrelated and interdependent parts. Hemostasis or cessation of bleeding is the first process in the wound healing process. Platelets and clotting factors are the main intravascular hemostatic factors. Collagen is a very efficient hemostatic agent, because platelets adhere to collagen, swell and release substances that will later start the hemostatic process [10].

Platelets not only initiate the hemostatic process, but also release a number of biologically active substances including extracellular matrix molecules, including fibronectin and several growth factors such as platelet derived growth factor (PDGF) [13].

Fibroblasts are the most abundant elements in granulation tissue. Collagen synthesis and deposition is an important part of the proliferative phase and of wound healing in general. Collagen is secreted into the extracellular space in the form of procollagen. This form then divides at the terminal segment and is called tropocollagen. Tropocollagen can combine with other tropocalagen molecules to form collagen filaments. These filaments then combine to form fibrils [13].

These collagen fibrils then combine to form collagen fibers. The form of filaments, fibrils, and fibers occurs in a matrix of glycosaminoglycans, hyaluronidase acid, chondroitin sulfate, dermatan sulfate and heparin sulfate produced by fibroblasts [13].

The body has a protective barrier against environmental changes, namely the skin. If the external factor is not able to be restrained by the protector then an injury occurs. In response to these wounds, the body has a physiological function of wound healing. The wound healing process consists of inflammatory, proliferative and maturation phases [2].

The body will try to normalize all scar tissue due to the healing process, edema and inflammatory cells will be absorbed, young cells will mature, new capillaries will close and be reabsorbed, excess collagen will also be absorbed and the rest will shrink according to the existing strain. , and at the end of this phase the skin condition is able to withstand stretching up to 80% of normal skin's ability

Physiologically the body can repair damaged skin tissue (wounds) by itself. Collagen plays an important role at every stage of the wound healing process [2].

Tissue strengthening in scars is done by remodeling collagen and elastin, causing upward pressure on the surface of the injured skin, followed by itching and the appearance of epithelial protrusions (keloids) [12].

Collagen has abilities such as hemostasis, interaction with platelets, interaction with fibronectin, increasing fluid exudation, increasing cellular components, increasing growth factors and promoting the fibroplasia process. The benefit of collagen in the medical field is to accelerate the growth of new tissue [10].

In this study, based on the type of wound, namely the type of clean wounds, where the cuts in these rats do not have inflammation or infection that is large enough. These wounds have the potential for infection of about 1-5%.

The research for the process of wound healing is made of cream formulations in three concentrations, namely 5%, 7.5%, 12.5% and one cream base as a negative control. The reason for using a negative control is to know how the effect is produced from the cream containing collagen extract. Based on the results of physical observations for 11 days, it was seen that on the first day at the beginning of the incision the length of the wound in each treatment was still the same, namely 1 cm. On the first day until the third day for the treatment group an inflammatory process occurred which was marked by swelling in the area around the incision wound to stop bleeding, prevent infection caused by foreign objects or bacteria. As for the negative control, it occurred until day 5, this was because the collagen contained in the African catfish skin extract could accelerate the inflammatory phase. The proliferation process for the treatment group occurred on day 4 to day 6, this proliferative phase was marked by the appearance of new blood vessels as a result of reconstruction. The negative control lasted from day 6 to day 9. Meanwhile for the maturation phase in the treatment group occurred on day 7, day 8 and day 9, and for negative control it occurred on day 11. In this maturation phase the wound was observed. physically undergo a color change from blackish red to white.

In table 5 it can be seen that the concentration of 12.5% in the cream preparation has an effect or effect on faster wound healing compared to concentrations of 5% and 7.5%. This is characterized by a reduction in wound area from day 1 to day 7 until the wound dries, and this also occurs because catfish skin cream at a concentration of 12.5%

contains more collagen than other concentrations.

The final evaluation to determine the stability of the preparation was carried out after the manufacture of the cream was completed. Evaluations that can be observed are organoleptic test, pH test, dispersibility test, dispersibility test, homogeneity test. In the organoleptic test, it can be seen in table 6 where each formula has a rancid smell, this is because in the cream preparation there is an extract of catfish skin which is known that fish skin has a rancid or fishy odor, while the color has a distinctive cream color, namely ivory white, and for the texture of the formula with a concentration of 5% it still has grains and is a little lumpy.

Physically, this cream preparation is also tested for physical homogeneity to see if there are clumps of particles in the cream preparation. The results obtained can be seen in Table 7 where the 5% cream formula is not homogeneous because there are still lumps and particles, while the 7.5% and 12.5% preparations are homogeneous because all the particles in the cream are evenly distributed.

The pH test results can be seen in table 8 for the 5% formula which has a pH of 5 which does not match the pH standard for the skin, while the 7.5% and 12.5% formulas have a pH of 6 with a pH value for both of them according to the skin's pH of 6 0.0 – 7.0, so it is safe to be applied to the skin.

The results of the adhesion test for each formula can be seen in table 9 where the 12.5% formula has greater adhesion than the 5% and 7.5% formula with the release time in the 12.5% formula, which is 36, 87 seconds, while the 5% formula is 33.43 seconds and 7.5% 36.44 seconds. The longer it takes for the two glass objects to come off, the better the stickiness of the cream preparation. The longer the cream is attached to the skin, the greater the effect.

Cream is a semi-solid dosage form containing one or more drug ingredients dissolved or distributed in a compatible basis. This term has traditionally been used for semi-solid preparations that already have a relatively liquid consistency formulated as W/O or W/W emulsions (Ditjen POM, 2014).

There are types of cream that are A/M and there are M/A. As an emulsifier can be anionic-cationic and non-ionic surfactants. Oil-in-water emulsion (vanishing cream) is a base that can be washed off with water. The water-washable base will form a thin, semi-permeable film, after the water has evaporated at the place where it is usedThe formulation of cream from catfish skin extract uses an A/M base, the reason for using an A/M base is because it is seen from the active substance in the fish skin extract where the active substance is collagen. Collagen is a compound that is difficult to dissolve in water, so it is easier to use an A/M basis to make it into cream preparations.

When a drug is used topically, the drug will come out of the carrier and diffuse to the surface of the skin tissue. The type of base that has a high viscosity will cause the diffusion coefficient of a drug in the base to be low, so that the release of the drug from the base will be small.

Good quality is stable, soft, easy to use and evenly distributed. A cream is said to be stable if it is free from incompatibility, stable at room temperature and humidity in the room. Soft means that all substances are in a smooth state and all products become soft and homogeneous because the cream will be used on skin that is easily irritated. The stability of the cream is damaged if the mixing system is disturbed, mainly due to changes in temperature and changes in composition due to the addition of one phase in excess or the mixing of two types

of cream if the emulsifying agents are not mixed with each other. As a cream stabilizer, antioxidants and preservatives can be added that can be used, namely nipagin 0.12% - 0.18% and nipasol 0.02% - 0.05%.

In this study, a cream base with a water-in-oil cream group was used. The advantage of this group base is that it is inert and only absorbs a little water and formulations or skin and can form a waterproof film that can prevent water evaporation so that the skin is not easily dry and cracked. The weakness of this base is the low water absorption capacity, easy to become rancid (rancid) and the ability to penetrate the skin is small.

The basic ingredient of the cream is stearic acid. Stearic acid is a hard solid with a crystal structure, white or pale yellow, similar to waxy fat. Stearic acid is practically insoluble in water, soluble in 20 parts of 95% ethanol, in 2 parts of chloroform and in 3 parts of ether. . Stored in a closed container. Paraffin liquidum is a viscous, transparent, non-fluorescent, colorless, almost odorless, almost tasteless liquid. Paraffin liquidum is practically insoluble in water and in 95% ethanol, soluble in chloroform and in ether. Stored in well-closed containers and protected from light. Efficacy is as a laksativum. Adeps lanae is the basis for making creams. The mass is fat like, sticky, light yellow or pale yellow in color, slightly translucent and has a characteristic odor. Insoluble in water, miscible with water approximately 2 times its weight, slightly soluble in 95% ethanol, easily soluble in ether and chloroform. Stored in a well closed container and protected from light in a cool place.

Nipagin is in the form of colorless crystals or white crystalline powder, odorless or almost odorless and has a slightly hot taste. Nipagin is soluble in 5 parts of propylene glycol, 3 parts of 95% ethanol, 60 parts of glycerin and 400 parts of water. Usefulness is as a preservative.

Nipasol is a white crystal powder, odorless and tasteless. Very sparingly soluble in water, soluble in 3.5 parts of 95% ethanol, in 3 parts of acetone and in 140 parts of glycerol. Stored in a tightly closed container. Khaits properties are as preservatives and Aquadest is a clear, colorless, odorless and tasteless liquid. Aquadest is soluble in all types of solutions. Stored in a well-closed container

Stability is defined as the ability of a drug or cosmetic product to withstand the specified limits applied throughout the period of storage and use to ensure product identity, strength, quality and purity. The definition of a stable cosmetic preparation is a preparation that is still within acceptable limits during the period of storage and use, where the properties and characteristics are the same as those of the product when it was made. color, odor, phase change or separation, emulsion rupture, suspension or caking deposition, consistency change, crystal growth, gas formation and other physical changes.

Symptoms that are indicators of emulsion damage include creaming, which is a process in which the emulsion with less dense particles tends to the surface so that there is a separation into two emulsions. Flocculation is the fusion of globules which are joined in the repulsion of electrolysis (zeta potential). Coalescence or agglomeration is the process by which the internal two-phase droplets approach and combine to form larger particles. Inverse is an event where the external phase becomes the internal phase and vice versa

The incision in this study only occurs in the epidermis layer of skin. The epidermis is the first layer of the skin which consists of epithelial tissue (stratified squamous epithelium). The

epidermis has a very thin layer, 0.04 mm thick, has no blood vessels, regenerates cells every 4-6 weeks, and gets nutrients from capillary diffusion.

Based on the results of the data that the greater the collagen concentration in the African catfish skin extract cream, the faster the healing process and the reduction in the length of the wound, this is because collagen is a very efficient hemostatic agent, because platelets are attached to collagen which causes the collagen to swell and subsequently hemostasis or blood clotting occurs. Apart from being a hemostatic agent, collagen also has the ability to increase fluid exudation, increase growth factors and stimulate the process of fibroplasia and epidermal proliferation [10].

In a previous study for topical administration of catfish skin collagen extract to the number of fibroplasts in second-degree burns, healing on day 10 by means of decrease TNF- and increase the number of fibroblasts [9].

Based on the results of the normality test, it is known that the significance value is 0.459 > 0.05, so it can be concluded that the residual value is normally distributed, and for the homogeneity test the data results obtained a significance value of 0.884 > 0.05. So it is continued by using the one sample t-test parametric test which is known to have a significant value of 0.00 < 0.05, so according to the basis of decision making it can be concluded that the average value of wound repair has a significant difference.

CONCLUSION

Based on the results of research data analysis, several conclusions can be drawn, including the following: African catfish skin cream has an effect on wound healing with a healing process within 7 days. The effectiveness of African catfish skin cream at a concentration of 12.5% has a faster effect on wound healing compared to the 7.5% and 5% formulas.

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