THE EFFECT OF GIVING EXTRACT AFRICAN CATFISH MEAT (*CLARIAS GARIEPINUS*) ON THE LENGTH OF TIME FOR CUTS HEALING PROCESS AMONG WHITE MALE WISTAR RATS (*RATTUS NORVEGICUS*)

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ABSTRACT

African catfish is one of the freshwater fish with high albumin which plays a role in the process of new cell formation, so that, it can help the process of cell growth and can accelerate the healing of damaged skin cells due to an incision.

This study aims to find out the effect of giving extract African Catfish meat on the length of time for the cuts healing process and the dose of African catfish extract that can provide the best effect in the cut healing process is given orally.

This research is a laboratory experimental study with a cross-sectional research design with a post-test only control group design using 20 white rats as research subjects which were divided into a 25 ml dose treatment group, 50 ml, and 75 ml/200 grBB rats and negative control. Observations of cuts healing were carried out macroscopically with the parameters of reduced redness and edema and measuring the length of the incision using a ruler.

Data analysis used the Kruskal walis test and post hoc test, the results of the analysis showed that there was a significant difference ($p<0.05$) between the negative control group and the P1, P2, and P3 groups.

This shows that African Catfish meat extract affects wound healing. The P3 group (75 ml extract) had more effect on the cuts healing process than the P1 group (50 ml extract) and the P2 group (50 ml extract).

Keywords: Albumin, *Clarias gariepinus*, Incision wound, Wistar strain.

INTRODUCTION

Wound treatment in the community generally uses synthetic chemical drugs that contain lots of chemicals to heal wounds without considering the side effects of using these drugs for health. One of the synthetic chemical drugs that is often used in wound treatment is the use of chemicals with 10% povidone iodine content. Povidone iodine content can be toxic to fibroblasts, povidone iodine will affect the process of collagen formation which will be responsible for the formation of new tissue in the wound, so that it will inhibit the wound closure process [2].

The public does not know that there is an alternative wound treatment by utilizing natural resources, especially water sources in Indonesia, as in previous studies using snakehead fish which is one type of freshwater fish in healing cuts and burns [1]. And there are also previous studies showing that the skin and flesh extract of

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African catfish (Clarias gariepinus) has activity in helping the healing process of cuts in wistar rats seen from the reduced length of the wound and the density of fibroblasts in the wound [7].

Wounds are diseases that cause the skin to lose its constituent tissue structure. Physical and chemical trauma that occurs in the body can increase the risk of injury [15]. One type of open wound is Vulnusscissum or cut. Vulnus scissum is a cut or iris wound which is characterized by the edges of the wound in the form of a straight or regular line and are usually found in daily activities. Cuts can occur as a result of intentional (surgical) or unintentional (accidental) injuries caused by contact with sharp objects [10]. The speed of wound healing in general is very dependent on many factors, one of which is nutrition.

One of the nutritional content found in African catfish is albumin which is often applied clinically for nutritional improvement and postoperative wound healing. Albumin has a role to increase the proliferation of fibroblasts thereby increasing the synthesis, accumulation and remodeling of collagen. Albumin is needed to maintain osmotic pressure inside and outside the cell. The role of albumin in the maturation phase is as a basic material for the formation of collagen. Albumin is also useful as a means of transporting nutrients and oxygen that the body needs to form new tissues at the proliferative stage [9]. African catfish also contains fatty acids and amino acids which also have a role in helping the process of new tissue growth in wound healing [6].

Based on this, researchers are interested in conducting further research to see the effect of giving African catfish (Clarias gariepinus) meat extract on the length of time the wound healing process in male white rats (Rattus norvegicus) wistar strain.

RESEARCH METHODS

This type of research is a quantitative study carried out in an experimental laboratory to see the effect of giving African catfish meat extract (Clarias gariepinus) on the length of the wound healing process in male white rats (Rattus novergicus) with a post-test only control group design.

The test animals used in this study as research subjects were male white rats (Rattus novergicus) Wistar strain which were given 20 slashes with 5 each in each treatment group.

The equipment used is a blender, jar, separating funnel, pipette, volume pipette, stirring rod, 50 ml glass beaker (Pyrex), 500 ml glass beaker (Pyrex), 1000 ml glass beaker (Pyrex), porcelain cup, measuring cup (Pyrex).), filter cloth, water bath, analytical balance, ohaus scale, shaver, thermometer, ruler, scalpell blade sterile No.11, sonde, tweezers, 3ml injection syringe.

The materials used were catfish meat, extract (water phase) of catfish meat, aquadest, 70% alcohol, 10% ether, 1% HCL, hexane and aluminum foil.

The sample used was African catfish (Clarias gariepinus) obtained from catfish ponds located in West Limboto District, Gorontalo Regency, Gorontalo Province. Fish The part used is the meat as much as 4.5 kg.

Fresh African catfish (Clarias gariepinus) is processed in the laboratory for the separation process of catfish meat. African catfish cleaned (separated and removed scales, gills, entrails, head, tail, bone and meat) after that washed until there is no more blood and mucus in the fish meat. The meat of the fish obtained is cut into small pieces and the bones are removed. The weight of the sample to be used was re-weighed as much as 4.5 kg of

African catfish meat. Then the sample was cut into small pieces and puree using a blender by adding a solvent in a ratio of 1: 1 v/w (100 mL of water solvent: 100 g of fish) for the crude extract.

Albumin extraction is using 1% HCl solvent with a ratio between solvent and fish porridge 1:1 w/v using heating at a temperature of 50-60^{O}C for 30 minutes. Then filtered to separate the liquid and residue, then the liquid was added with 200 mL of hexane, and shaken for 30 minutes. So that two phases are formed, the oil phase and the aqueous phase (liquid extract). The oil phase is separated and collected in a holding container. The obtained extract liquid (aqueous phase) is ready for analysis.

The aqueous phase of the catfish extract obtained was taken as much as 5 ml, heated on a water bath for 30 minutes. Look at the changes that occur in the extract. The extract was positive for albumin if there were white lumps floating on the top of the extract [8].

Grouping of Test Animals A total of 20 male white rats of wistar strain were divided into 4 groups. each group of 5 rats.

The dose group was 25 ml/ 200 g BW. African catfish extract was given orally 3 times a day. Dosage group 50 ml/ 200 g BW was given African catfish extract orally 3 times a day. Dosage group 75 ml/ 200 g BW was given African catfish extract orally 3 times a day. Group (negative control) was given aquadest 3 times a day orally.

Rats were anesthetized using ether by inhalation. Furthermore, the back of the test animal was shaved with a diameter of 3 cm and cleaned with alcohol before being injured. The test animals used were 20 animals which were divided into 4 groups. Injuries were performed on the back of the rat by making a 2 cm long incision with a depth of 0.4 mm using a sterile scalpel number 11. This treatment was carried out the same for all test animals.

African catfish meat extract was given 3 times a day as much as 3 ml each in each treatment group orally until all doses in each group were met. Observations were made by measuring the length of the wound by measuring the sides of the wound using a ruler since the injury was first performed until the wound closed completely after being given treatment. In addition, macroscopic observations were also made of the presence/absence of redness around the wound and the presence/absence of swelling (edema) around the wound.

Data analysis was carried out statistically using the Kruskal Wallis test in the SPSS 16.0 for windows program. Testing continued with the Post Hoc Test Man Whitney.

RESEARCH RESULT

Extraction of African catfish meat using hydrochloric acid (HCL) as a solvent by heating at a temperature of 50-600C. After obtaining the aqueous phase of the African catfish meat extract, the percentage of the yield was calculated, the extract yielded 90.47% of the 950 ml extract (water phase). The calculation of the yield of African catfish meat extract using 1% HCl is shown in Table 1

Table 1. African catfish meat extract marinade

Sample weight (kg)	Extract Weight	Yield Yield (%)
4.5 kg	950 ml	90.47%

Source: Processed Data (2021)

The meat extract of African catfish (Clarias gariepinus) was positive for albumin, which was indicated by the

presence of white lumps at the top of the extract. This can be seen in Table 2

Table 2. Qualitative Analysis Test

Qualitative Analysis Test	Test	Test results	Conclusion
Albumin	Heating At 90oC	White Blob	(+)

Source: Processed Data (2021)

The results of the observations obtained data on the length of time for wound healing in male white rats of the Wistar strain based on the parameters of reduced redness (Erythema), reduced swelling (edema) around the cut area and reduced length of the incision. For more details, the results of observations of wound healing in male white rats of the Wistar strain are explained as follows:

Table 3. Mean Time (days) parameter of redness reduction (erythema)

Mouse	Time (Day)			
	Class I	Class II	Class III	Class IV
1	16	11	9	5
2	16	12	8	5
3	16	11	8	6
4	15	12	9	5
5	15	12	9	6
Average	15.6	11.6	8.6	5.4

Source: Processed Data (2021)

Based on the data in Table 3 shows that. The higher the dose of African catfish meat extract, the faster the healing time (days) for the wound or the reduced redness each day in the area of the incision wound for each group of white rats.

Table 4. Average Time (days) of Wound Healing Swelling parameter

Mouse	Time (Day)			
	Ex I	Class II	Class III	Class IV
1	12	6	5	4
2	11	7	6	4
3	12	6	5	5
4	12	7	6	4
5	11	7	5	4
Average	11.6	6.6	5.4	4.2

Source: Processed Data (2021)

Based on the data in Table 4, it shows that the higher the dose of African catfish meat extract, the faster the healing time (days) for the wound or the reduced swelling at the edges of the cut for each group of white rats.

Table 5. Mean Time (days) of the cut length reduction parameter

Mouse	Time (Day)			
	Class I	Class II	Class III	Class IV
1	16	12	10	6
2	16	13	10	7
3	16	12	9	7
4	15	13	10	7
5	15	13	10	7
Average	15.6	12.6	9.8	6.8

Source: Processed Data (2021)

Based on the data in table 5 shows that the higher the dose of African catfish meat extract, the faster the time (days) for wound healing or the smaller/reduced length of the cut in each group of white rats until the wound closed completely.

Table 6. Kruskal walis test results

Group	U	Erythema	Edema	Cut Length
K	5	0.000	0.000	0.000
P1	5			
P2	5			
P3	5			

Source: Processed Data (2021)

Based on the data in table 6 shows that the results of the Kruskal Wallis test with the parameters of erythema, edema and reduction in the length of the incision in white rats with wistar strain showed $p < 0.05$, then Ho was rejected so that there was a significant effect or difference between treatments (negative control, extract 25 ml, Extract of 50 ml and Extract of 75 ml of African catfish meat) on the length of time for wound healing in male white rats of wistar strain in reducing the length of the incision. Furthermore, to see which groups were significantly different, further tests were carried out, namely the post hoc test and the post hoc test results showed ($p < 0.05$) meaning that there was a significant difference in the length of time (days) of wound healing in the three parameters.

DISCUSSION

In wound healing, it requires the fulfillment of high nutritional intake to accelerate the process of wound healing. The nutritional content needed in accelerating the wound healing process is met by utilizing the extract of African catfish meat (Clarias gariepinus) in several dose ratios, namely doses of 25 ml, 50 ml, and 75 ml. Clarias gariepinus) on accelerating the process of wound healing in male white rats (Rattus norvegicus) and a more effective dose of accelerating the process of wound healing in male white rats (Rattus norvegicus) wistar strain.

The extraction process in this study used hydrochloric acid (HCL) as solvent, because the use of HCL solvent in albumin extraction was better than other solvents. This is in accordance with previous studies using HCL solvent in extracting Channa striata resulting in albumin levels of 20.8% [5]. To obtain the extract (aqueous phase) of African catfish meat, the extraction process was carried out first with the heating method at a temperature of 50-600C with the temperature maintained not exceeding 900C in order to prevent protein denaturation. The resulting extract is a liquid extract (aqueous phase) of African catfish meat. Taking liquid extract (aqueous phase) because albumin itself is a globalular protein dissolved in water. African catfish meat extract produced a yield of 90, 47% of 950 ml extract (aqueous phase). The marinade obtained was more than the marinade in previous studies using extracts from snakehead fish meat, namely 12.08% using the same sample weight of 4.5 kg of fish meat [1].

The extract (aqueous phase) has been tested to give positive results containing albumin which is indicated by the presence of white lumps on the top of the extract (aqueous phase) of African catfish meat. This is because albumin will coagulate and form white clumps with heating above 900C [11]. This is in accordance with previous research using snakehead fish meat extract which stated that there were white lumps at the top of the extract which indicated the presence of albumin content in the snakehead fish meat extract [1].

Formation of a cut on the back of a white rat Wistar strain 2 cm long with a depth of 0.4 mm, the wound is given based on the wound at stage 2, namely the wound causes the epidermis to separate from the dermis and/or affects part of the dermis [11]. For the parameters of wound healing observed in this study macroscopically by observing the reduction in redness and

swelling around the cut wound area and observing the reduction in the length of the incision in male white rats of Wistar strain after giving treatment to the test animals.

The presence of redness and swelling around the cut area is due to the fact that when the wound is formed there will be an inflammatory reaction. This is in accordance with the previous theory that after an injury, an inflammatory reaction will occur which is marked by a reddish color (rubor) because the capillaries will widen, then swelling (tumor) will occur until various inflammatory mediators are released, causing itching around the area of the incision wound [14].].

The wound healing process consists of 3 phases, namely the inflammatory phase, the proliferative phase, and the maturation or remodeling phase. In the inflammatory phase, observations were made macroscopically including the reduction in redness and swelling around the cut area. The research data for the parameters of reduced redness and swelling around the wound showed that in the negative control group with oral aquades given there were 3 samples that did not experience redness on the 16th day, and 2 samples that did not experience redness on the 15th day. For the parameter of reduced swelling around the edges of the wound, it showed that in the negative control group there were 3 samples that had no swelling on the 12th day and 2 samples on the 11th day. Although the negative control group (aquadest) was not given treatment, however, the wound healing process in the negative control group was still ongoing, marked by reduced redness and swelling around the cut area in rats. This is because a healthy body has a natural ability to recover itself so that the wound healing process occurs even without special treatment for wounds [3]. However, the negative control group needed a longer time (days) of wound healing compared to the treatment group or the extract group. This is because a healthy body has a natural ability to recover itself so that the wound healing process occurs even without special treatment for wounds [3]. However, the negative control group needed a longer time (days) of wound healing compared to the treatment group or the extract group. This is because a healthy body has a natural ability to recover itself so that the wound healing process occurs even without special treatment for wounds [3]. However, the negative control group needed a longer time (days) of wound healing compared to the treatment group or the extract group.

In the P1 group (extract 25 ml) which was given orally for the parameter of reduced redness around the cut area, it showed that there were 3 samples that had no redness on the 12th day and 2 samples on the 11th day. For the parameter of reduced swelling around the edges of the wound, it showed that in group P1 there were 2 samples that were no longer swollen on the 6th day and 3 samples had no swelling on the 7th day. In the P2 group (50 ml extract) which was given orally for the parameter of reduced redness around the wound area, it showed that there were 2 samples that had no redness on the 8th day and 3 samples had no redness on the 9th day. For the parameter of reduced swelling around the edges of the incision, it showed that there were 3 samples that had no swelling on the 5th day and 2 samples that had no swelling on the 6th day. In the P3 group (75 ml extract) which was given orally with the parameter of reduced redness around the cut area, it showed that there were 3 samples that had no redness on the 5th day and 2 samples on the 6th day, and for the parameter of swelling around the edge of the cut area showed that there were

4 samples that had no swelling on the 4th day and 1 sample on the 5th day.

The wound healing process for the parameters of reduced redness and reduced edema around the wound area occurred with the fastest average time (days) for wound healing at P3 namely the administration of 75 ml extract. The average time (days) of reduced redness (erythema) around the cut wound area on P3 takes 5 days for wound healing or reduced redness of the cut wound so that the wound can be said to be healed and for the parameter of reduced swelling (edema) the average time (days) required So the wound is said to be healed which is 4 days. The reduction in redness and swelling around the cut area is due to the African catfish meat extract containing albumin which plays a role in the inflammatory phase by regulating osmotic pressure inside and outside the cell. When a skin injury occurs, the skin will show signs of inflammation or inflammation. Foreign objects from outside the body can enter through open wounds such as cuts, the entry of these foreign objects triggers hydrostatic pressure disturbances because intracellular fluid will enter the cells due to differences or imbalances in concentrations inside and outside the cells through osmotic pathways, causing the cells to experience edema or swelling. This condition requires the role of albumin which can maintain osmotic pressure inside and outside the cell, so that the edema or swelling that occurs around the wound area does not get worse. Foreign bodies from outside the body can enter through open wounds such as cuts, the entry of these foreign objects triggers hydrostatic pressure disturbances because intracellular fluid will enter the cells due to differences or imbalances in concentrations inside and outside the cells through osmotic pathways, causing the cells to experience edema or swelling. This condition requires the role of albumin which can maintain osmotic pressure inside and outside the cell, so that the edema or swelling that occurs around the wound area does not get worse. Foreign bodies from outside the body can enter through open wounds such as cuts, the entry of these foreign objects triggers hydrostatic pressure disturbances because intracellular fluid will enter the cells due to differences or imbalances in concentrations inside and outside the cells through osmotic pathways, causing the cells to experience edema or swelling. This condition requires the role of albumin which can maintain osmotic pressure inside and outside the cell, so that the edema or swelling that occurs around the wound area does not get worse. The entry of foreign bodies triggers hydrostatic pressure disturbances because intracellular fluid will enter the cells due to differences or imbalances in concentrations inside and outside the cell through osmotic pathways, causing the cells to experience edema or swelling. This condition requires the role of albumin which can maintain osmotic pressure inside and outside the cell, so that the edema or swelling that occurs around the wound area does not get worse. The entry of foreign bodies triggers hydrostatic pressure disturbances because intracellular fluid will enter the cells due to differences or imbalances in concentrations inside and outside the cell through osmotic pathways, causing the cells to experience edema or swelling. This condition requires the role of albumin which can maintain osmotic pressure inside and outside the cell, so that the edema or swelling that occurs around the wound area does not get worse.

The proliferative phase usually lasts from day 3 or day 4 after the injury is given for up to 2 or 3 weeks [3]. At this stage the researchers measured the length of the incision on the rat using a ruler and the measurement of the length of the cut was

carried out every day at 17.00 WITA until the wound healed (closed completely). The process of closing the wound is said to be complete if the wound undergoes a process of removing the scab. This indicates that the growth of new cells has occurred with the edges of the wound closer together. The process of removing the scab is indicated by the underlying tissue is dry and the edges of the wound begin to be pulled to the center [4].

In this study, for the 4 treatments, the incision wound closed or shrunk the fastest in the treatment with a dose of 75 ml orally for the 5 samples, on average, they had experienced complete wound closure on the 7th day with an average length reduction. incision of 0.2 cm/day. Then followed by the treatment group with a dose of 50 ml orally, the average for the 5 samples had healed on the 10th day with an average reduction of the length of the cut by 0.1 cm/day. While the cut wound healed the longest in the 25 ml dose of extract treatment group and the negative control was given orally with distilled water. For the administration of extract 25 ml of the wound closed completely on the 13th day on average with an average reduction in the length of the cut wound by 0.

The wound healing in the proliferative phase with the parameter of reducing the length of the cut wound the fastest occurred at P3 (75 ml extract) with the average for rats in this treatment the wound closed on the 7th day. This is in accordance with previous studies using African catfish skin and flesh extract orally at a dose of 37.5 mg/200 g BW and 50 mg/200 g BW gave a better healing effect than other doses and showed that the healing of cuts in 10 days after the wound was formed as assessed by the length of the wound and the density of fibroblasts in wistar rats [7].

The process of accelerating the time of wound closure in the treatment group is due to the role of albumin in the proliferative stage, at this stage the epithelium requires energy to stimulate the formation of new cell tissue and albumin which plays a role in the energy formation process so that the albumin content in African catfish has an effect on accelerating the wound healing process. I t. From this it can be seen that the extract (aqueous phase) of African catfish meat can help speed up the healing process of cuts compared to wound healing without extracts because African catfish meat contains albumin protein which can help the wound healing process.

The data obtained from the wound healing results were carried out by one way ANOVA statistical test. However, before the one-way ANOVA test was carried out, prerequisite tests were carried out, namely the Shapiro Wilk test and Levene's test to see if the data were normally and homogeneously distributed. The resulting data is $0.000 < 0.05$ ($p < 0.05$) meaning that the data is not normally distributed, so a non-parametric kruskal walis test was carried out to see the effect of giving African catfish (Clarias gariepinus) meat extract on the length of time the wound healing process in rats white male (Rattus norvegicus). The statistical test results obtained for the parameter of reduced redness have a significant value of $0.000 < 0.05$, the parameter of reduced swelling has a significant value of $0.000 < 0$, 05 and the parameter of reducing the length of the incision has a significant value of $0.000 < 0.05$, which means ($p < 0.05$). In this case, H0 is rejected and H1 is accepted, meaning that there is a significant difference or there is an effect of giving African catfish meat extract on the length of time the wound healing process in male white rats (Rattus norvegicus) is given after each treatment.

The results of the kurskal walis statistical test stated that there was an effect of giving African catfish meat extract to the acceleration of the wound healing process, so to find out which treatment was more effective in accelerating the healing of cuts in male white rats, a post hoct test was carried out. The results of this test indicate that there is a significant difference between the wound healing time in the four treatment groups. This is indicated by the p value < 0.05 in the comparison of each group on each wound healing parameter. In the parameter of reduced redness around the cut, group I (negative control) was significantly different from group II (extract 25 ml), namely (p=0.007). Group 1 (negative control) was compared with group III (50 ml extract) ie (p=0.007). Group I (negative control) was compared with group IV (75 ml extract) (p=0.007). Group II (extract 25 ml) was compared with group III (extract 50 ml) i.e. (p=0.007), group II (extract 25 ml) was compared to group IV (extract 75 ml) i.e. (p=0.007) and group III (extract 50 ml) compared to group 4 (75 ml extract) ie (p=0.007).

In the parameter of reduced swelling around the cut for group I (negative control) compared to group II (extract 25 ml) namely (p = 0.007), group I (negative control) was compared to Group III (extract 50 ml) namely (p = 0.007) , Group I (negative control) was compared with Group IV (75 ml extract) that is (p=0.007). Group II (extract 25 ml) was compared with group III (extract 50 ml) (p=0.020), group II (extract 25 ml) was compared to group IV (extract 75 ml) (p=0.007). Group III (50 ml extract) was compared with group IV (75 ml extract) (p=0.031).

In the parameter of reducing the length of the incision for group I (negative control) compared to group II (extract 25 ml), namely (p = 0.007), group I (negative control) was compared to Group III (extract 50 ml) namely (p = 0.006) , Group I (negative control) was compared with Group IV (75 ml extract) that is (p=0.006). Group II (extract 25 ml) was compared with group III (extract 50 ml) (p=0.006), group II (extract 25 ml) was compared to group IV (extract 75 ml) (p=0.006). Group III (extract 50 ml) was compared with group IV (extract 75 ml), namely (p=0.005).

Thus, based on the results and discussion of the research that has been done, the extract dose of 75 ml is proven orally to be better in treating wounds, especially cuts, compared to extract at a dose of 25 ml and extract at a dose of 50 ml. The three doses were also proven to be better in helping the healing process of cuts compared to wounds.

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that there is an effect of giving African catfish meat extract (clarias gariepinus) on the length of time the wound healing process in male white rats (Rattus norvegicus) and African catfish meat extract (clarias gariepinus) dose of 75 ml separately. Oral was proven to be better in accelerating the healing process of cut wounds in male white rats (Rattus norvegicus) compared to the 25 ml and 50 ml doses of extract.

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