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**EFFECTIVENESS OF ORALLY USE CATFISH (*Clarias gariepinus*) SKIN AND MEAT ON WOUND LENGTH AND FIBROBLAST DENSITY IN INCISION WOUND OF WISTAR RAT (*Rattus norvegicus*)**

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**ABSTRACT**

**Background:** Treatment for wounds that are currently often given is use povidone-iodine. But, this antiseptics also kills fibroblast tissue which is useful for forming new tissue. One of the other ways to treatment for wounds is use catfish. The contents found in catfish (*Clarias gariepinus*) is albumin which is a globular protein that is useful in the formation of body tissues, such as postoperative wounds and burns. This study aims to determine the effect of skin and meat of catfish (*Clarias gariepinus*) on wound length and fibroblast density in incision wounds of Wistar rat (*Rattus norvegicus*).

**Methodology:** This study is an experimental study with Post Test Only Control Group Design using Wistar rats which are divided into five groups. The control group (aquadest) and treatment groups 1, 2, 3 and 4 are given the skin and meat of catfish (*Clarias gariepinus*) orally at a dose of 12.5 mg / 200 g BW, 25 mg / 200 g BW, 37.5 mg / 200 g BW, and 50 mg / 200 g BW. After 10 days, the rats are killed to take the wound tissue for histological preparations to observe fibroblast density. Then, it is analyzed by ANOVA test or the alternative.

**Results:** There is an effect of giving catfish skin and meat (*Clarias gariepinus*) orally at different dose. The administration of catfish skin and meat (*Clarias gariepinus*) at a dose of 37.5 mg / 200 g BW and 50 mg / 200 g BW is better than other doses assessed from wound length and fibroblast density in Wistar rat incision wounds (*Rattus norvegicus*).

**Conclusion:** The use of catfish for wound healing can be applied. This method can reduce wound length and increase fibroblast density at certain doses.

**Keywords:** Fibroblasts, incision wounds, catfish, *Clarias gariepinus*, wound healing

**INTRODUCTION**

Wounds are a form of tissue damage in the skin that can cause interference in the function and anatomical structure of the body. Wounds can be caused by sharp or blunt body trauma, temperature changes, chemical substances, explosions, electric shock or animal bites [1]. Every wound that occurs is always followed by a wound healing process consisting of several phases, for instance hemostasis, inflammation, proliferation and remodeling. The wound healing process is strongly influenced by the role of fibroblasts migration and proliferation in the area of injury. Fibroblasts are cells that are widely distributed in connective tissue, producing collagen precursor substances, elastic and reticular fibers. Fibroblasts are responsible for the preparation of producing protein structure used during the tissue reconstruction process [2,3].

Current wound care, usually hospitals use povidone iodine as an antiseptic, but the main problem that actually arises is that antiseptics as well as killing microorganisms can also kill fibroblast tissue that forms new skin tissue. This can cause interference in the wound healing process. The use of animals as an alternative material in medicine has not reached significant development, but if we take a look to the

aspect of natural resources, especially waters in Indonesia, it is very potential to be developed into raw materials in medicine [4,5].

Catfish (*Clarias gariepinus*) has been widely cultivated by people in Indonesia. One of the contents found in catfish (*Clarias gariepinus*) is albumin which is a globular protein that is useful to form body tissues, such as postoperative wounds and burns. Albumin functions to regulate osmotic pressure in the blood, maintaining the presence of water in the blood plasma so that it can maintain blood volume in the body and as a means of transportation. Albumin is also useful to increase fibroblast cell proliferation which increases collagen synthesis. Alauddin A. has shown that albumin extract in the Sneakhead (*Channa striata*) was effective in accelerating incision wound contractions in Wistar rats. Amino acid and fatty acid in catfish (*Clarias gariepinus*) also has effects on wound healing. Both of these compounds can help the process of re-forming collagen and epithelial tissue in the wound. Collagen extracted from catfish skin can also functions as chemo attractant for fibroblast cells so that it can induce chemo taxis from fibroblast cells to the wound area [6,7,8]. Thus, this study was conducted to determine the effect of skin and meat of catfish (*Clarias gariepinus*) on wound length and fibroblast density in incision wounds of Wistar rat (*Rattus norvegicus*).

## MATERIALS AND METHODS

This study is an experimental study with post-test only control group design using male white rat (*Rattus norvegicus*) as subject research. Ethical clearance approval No.62/EC/FK/XI/2018 was obtained from The Research Ethics Committee of Faculty of Medicine, Universitas Swadaya Gunung Jati, on 22th November 2018. This research was conducted at the PAU Food and Nutrition Laboratory and the Anatomical Pathology Laboratory of Gajah Mada University, Yogyakarta, Indonesia. Catfishes were obtained from catfish farms (*Clarias gariepinus*) in Prambanan Subdistrict, Sleman Regency, Yogyakarta, which have been identified in the FMIPA Biology Laboratory, Semarang State University. The skin and meat of catfishes are small in size and dried in an oven for 12 hours at 40°C, then mashed using a blender [9].

Thirty male white Wistar rats (*Rattus norvegicus*) are divided into 5 groups. Those are 1 control group and 4 treatment groups. At first, all the rats were wounded by 2 cm length incision at the back of the rat which previously administered with 1 cc of ketamine anesthesia intraperitoneally. Control group was 6 rats without any treatment. Meanwhile the 4 treatment groups consist of group P1 which was administered orally by 12,5 mg/200 g BW catfish, group P2 with 25 mg/200 g BW of catfish, group P3 with 37,5 mg/200 g BW of catfish, and group P4 with 50 mg/200 g BW of catfish.

The treatment was done for 10 days (once a day at 8 a.m) and on days 3, 6 and 10 the length of incision wound is measured using a ruler and the wound healing process is observed visually. At the 10th day, all rats were terminated by cervical dislocation [10]. The wound area was excised and fixated by formalin 10%. Hematoxylin and Eosin (H&E) stained sections were prepared for all groups according to the laboratory protocols. Histopathological preparations are observed using a binocular light microscope Olympus CX23 with ocular lens 100x and 400x. Fibroblasts are calculated in 5 fields of view for each preparation in each treatment group.

## RESULTS

### *Macroscopic incision length analysis*

All incision wounds are measured for 10 days by observing the wound condition in the area of the rat's back on days 0, 3, 6, 10 and the length of the wound on day 10 of all groups compared. Table 1 shows the wound length of all groups and figure 1 shows the macroscopic wound.

Table 1. Wound length

Groups	Mean $\pm$ SD Wound length (cm)			
	Day of Observation (Days)			
	0	3	6	10
Control	2	1.94 $\pm$ 0.032	1.85 $\pm$ 0.039	1.50 $\pm$ 0.144
P1: 12.5 mg/200 g BW	2	1.04 $\pm$ 0.037	0.78 $\pm$ 0.079	0.33 $\pm$ 0.087
P2: 25 mg/200 g BW	2	0.88 $\pm$ 0.386	0.82 $\pm$ 0.056	0.15 $\pm$ 0.047
P3: 37.5 mg/200 g BW	2	1.10 $\pm$ 0.034	0.62 $\pm$ 0.085	0.03 $\pm$ 0.041
P4: 50 mg/200 g BW	2	1.04 $\pm$ 0.017	0.59 $\pm$ 0.020	0.02 $\pm$ 0.040

The results of the mean wound length seen on the 10th day in the table 1 showed that group P4 at a dose of 50 mg / 200 g BW which most affected the acceleration of wound healing with  $0.02 \pm 0.040$  cm wound length followed by group P3 at a dose of 37.5 mg/200 g BW with a length of  $0.03 \pm 0.041$  cm, then followed by group P2 at a dose 25 mg/200 g BW with a length of  $0.15 \pm 0.047$  cm, then group P1 at a dose 12.5 mg / 200 g BW with a length of  $0.33 \pm 0.087$  cm and control group with a length of  $1.5 \pm 0.144$  cm.

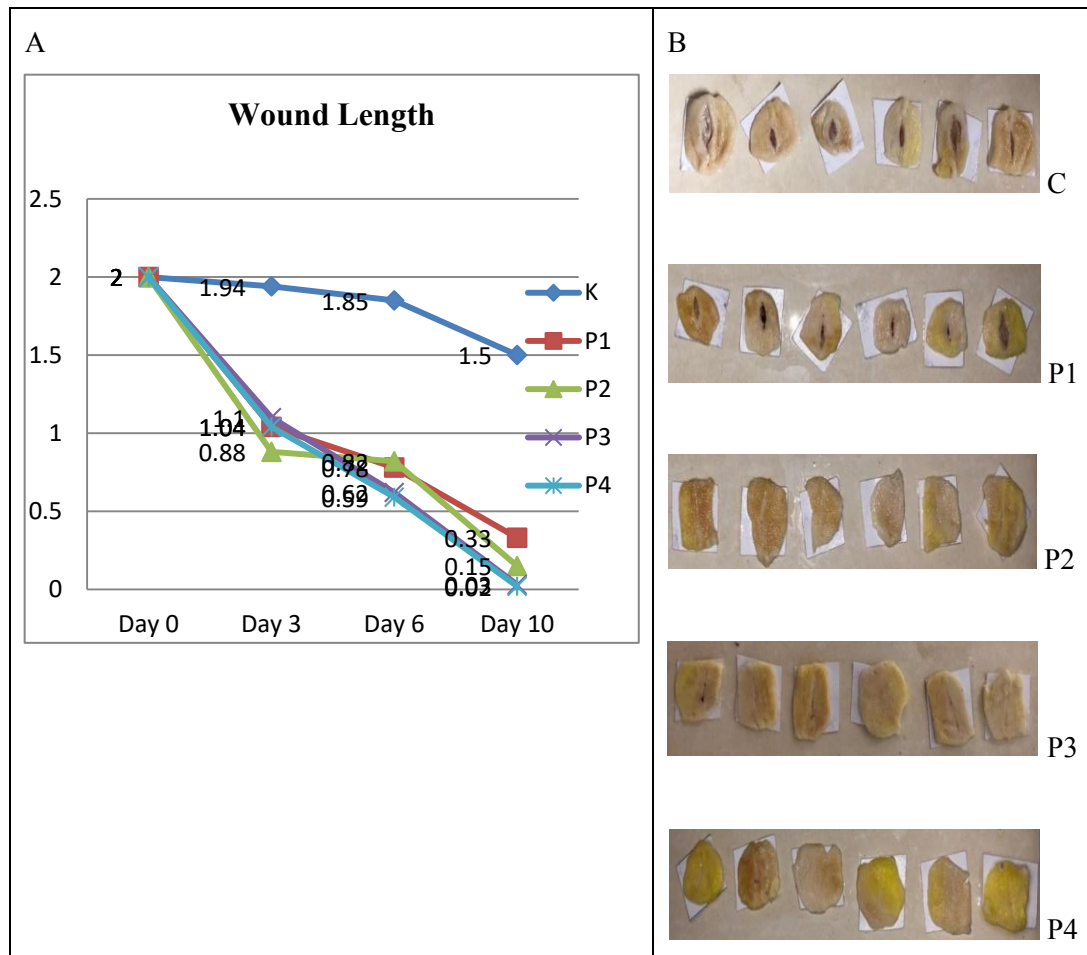


Figure 1. Macroscopic observation on incision wound. A. Graph showing length shortens of incision wound measured using a ruler. B. Incision wound of all groups taken after 10 days of treatment.

Mean length of incision wound at the 10th day of all rat groups were compared by using nonparametric Kruskal-Wallis analysis. With Confidence Interval (CI) 95%,  $p$  value = 0.001 has shown that there are

at least 2 groups which have a significant difference in the length of incision on day 10. Post-hoc Mann-Whitney was performed to see which pair groups have difference shown in Table 2.

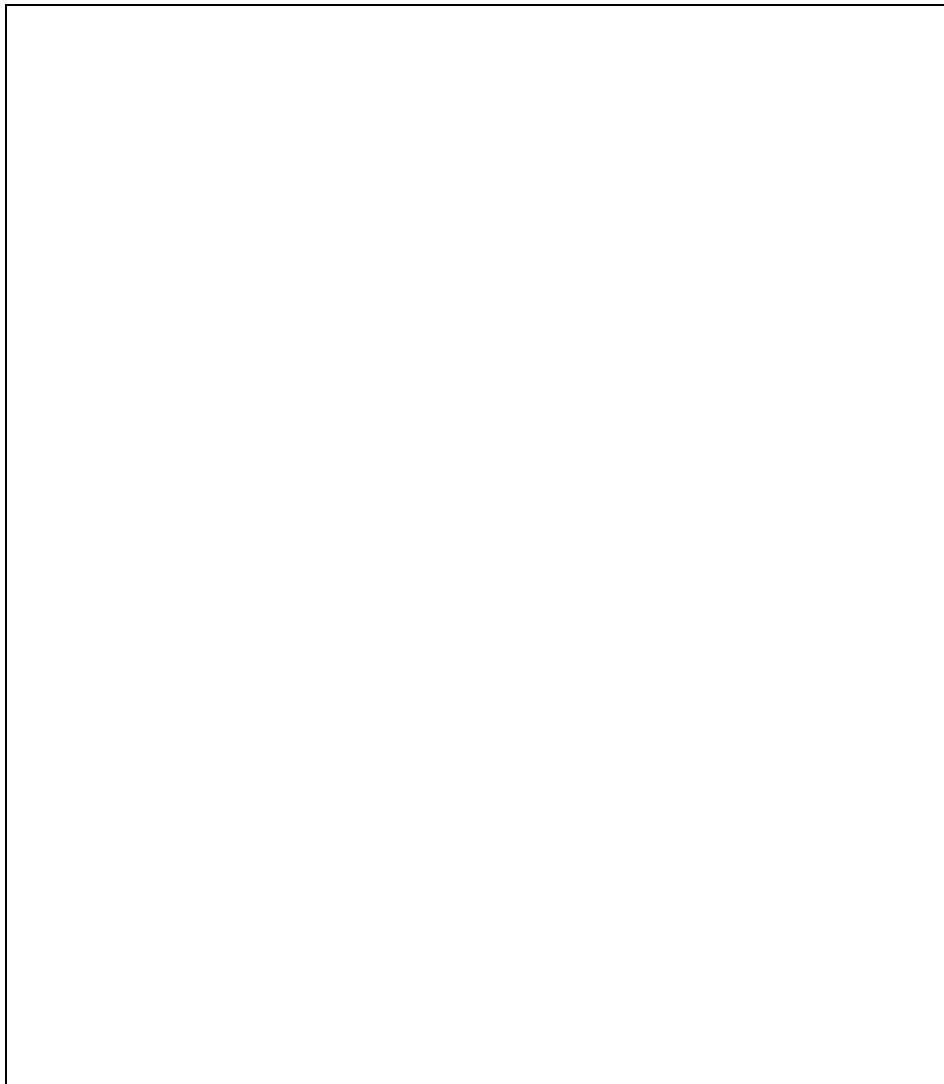
Table 2. Mann-Whitney test

	C	P1	P2	P3	P4
C	#				
P1	0.004	#			
P2	0.004	0.004	#		
P3	0.004	0.004	0.006	#	
P4	0.003	0.003	0.004	0.441	#

Based on the results of the Mann-Whitney post hoc analysis as shown in table 2, it was found that 9 pairs of treatment groups (C with P1, C with P2, C with P3, C with P4, P1 with P2, P1 with P3, P1 with P4, P2 with P3 and P2 with P4) have a value of  $P < 0.05$ .

***Microscopic analysis of fibroblasts density***

Microscopic analysis was done by counting fibroblasts density in 5 viewing field of each H&E stained sections prepared from all groups (Fig. 2.). The fibroblasts observed in all groups were counted and compared by using statistical analysis.



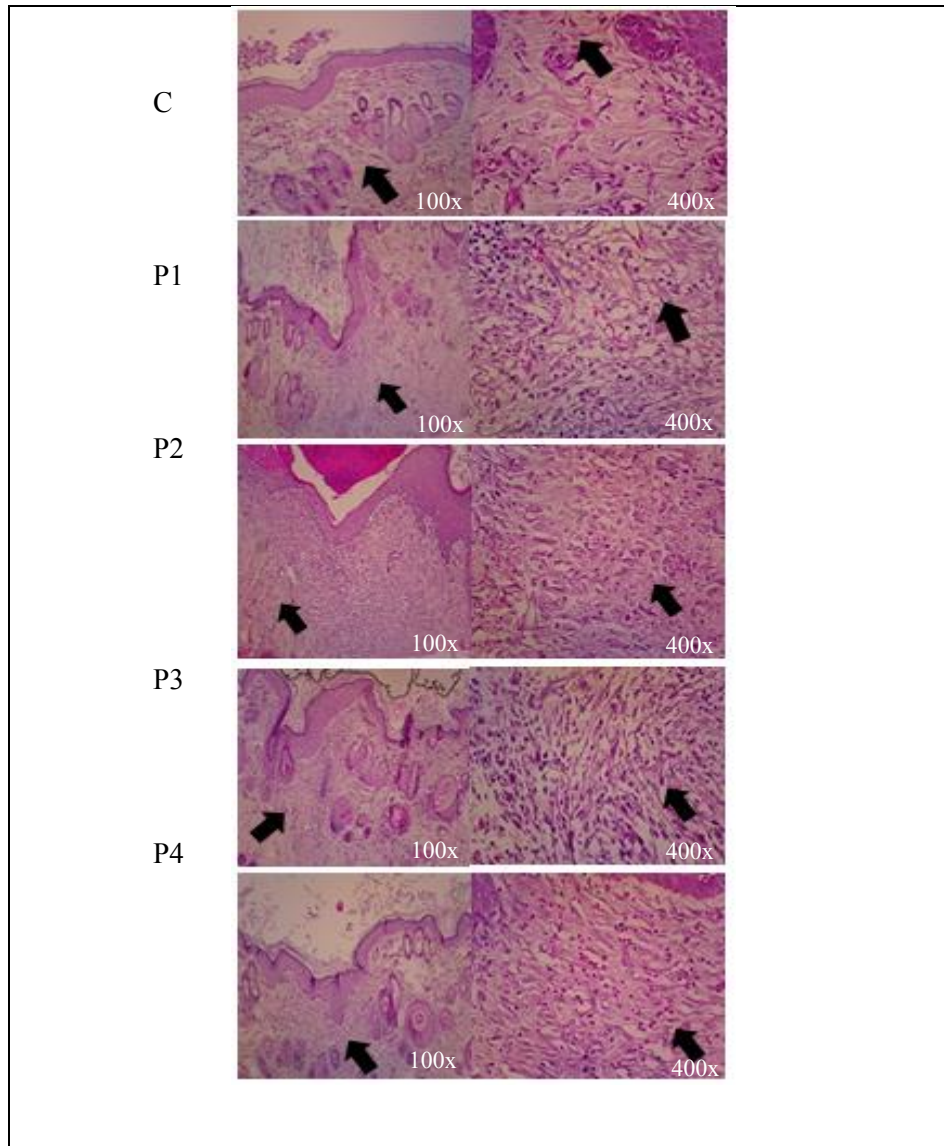


Figure 2. Microscopic observation on incision wound of all groups.

Fibroblast density in incisional wounds can be seen in Figure 2. C is a control group that is given aquades. P1 is the treatment group 1 which is given catfish flour (*Clarias gariepinus*) at a dose of 12.5 mg/200g BW, P2 is the treatment group 2 which is given catfish flour (*Clarias gariepinus*) at a dose of 25 mg/200g BW, P3 is the treatment group 3 which is given catfish flour (*Clarias gariepinus*) at a dose of 37.5 mg/200g BW and P4 is the treatment group 4 which is given catfish flour (*Clarias gariepinus*) at a dose of 50 mg/200g BW. It can be seen that the higher the dose, the more influential in increasing the density of fibroblasts in incisional wounds of Wistar rat (*Rattus norvegicus*).

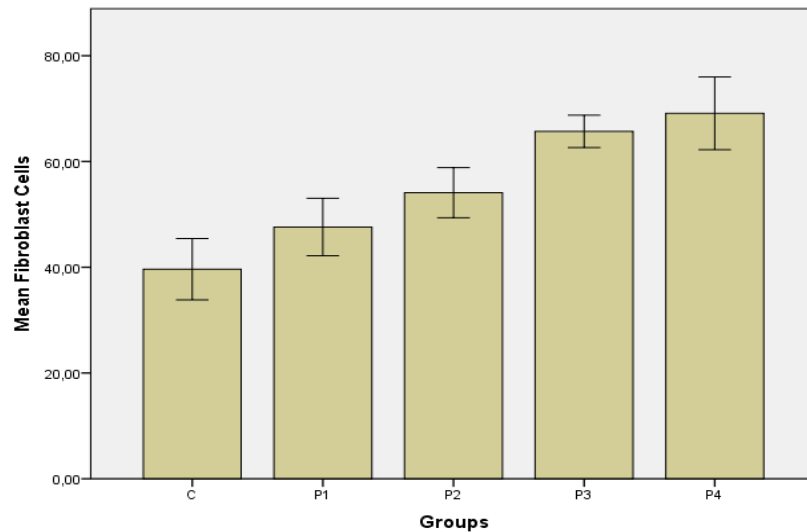


Figure 3. Mean fibroblast density after 10 days of treatment on each group.

Figure 3 shows the results of the mean fibroblast density in the control group at  $39.6 \pm 5.53$  cell, group P1 with a dose of 12.5 mg/200 g BW obtained mean fibroblast density of  $46.7 \pm 5.17$  cell, group P2 with a dose of 25.5 mg/200 g BW obtained mean fibroblast density of  $54.1 \pm 4.51$  cell, group P3 with a dose of 37.5 mg/ 200g BW obtained mean fibroblast density of  $65.7 \pm 2.91$  cell and group P4 with a dose of 50 mg/ 200 g BW obtained mean fibroblast density of  $69.1 \pm 6.56$  cell.

Mean fibroblast density of incision wound after ten days treatment (fig.3) were compared by using parametric One Way Anova analysis with Confidence Interval (CI) 95%,  $p$  value = 0.000 has shown that there are at least 2 groups which have a significant difference in the length of incision on day 10. Post-hoc Bonferroni test was performed to see which pair groups have difference shown in Table 3.

Table 3. Bonferroni test

	C	P1	P2	P3	P4
C	#				
P1	0.176	#			
P2	0.000	0.134	#		
P3	0.000	0.000	0.003	#	
P4	0.000	0.000	0.000	1.000	#

Based on the results of the Bonferroni post hoc analysis as shown in table 3, it was found that 7 pairs of treatment groups (C with P2, C with P3, C with P4, P1 with P3, P1 with P4, P2 with P3 and P2 with P4) have a value of  $P < 0.05$ .

## DISCUSSION

Every wound that occurs is always followed by a wound healing process consisting of several phases, such as the inflammatory phase, proliferation, and remodeling. The inflammatory phase lasts from the time of injury until the fifth day. At first, the broken blood vessels in the wound will cause bleeding, and the body tries to stop it with vasoconstriction, retraction, and hemostasis reactions. The proliferation phase lasts from the end of the inflammatory phase to approximately the end of the third week. The proliferation phase is also called the fibroplasia phase because what stands out is the process of proliferation of fibroblasts. Fibroblasts originate from undifferentiated mesenchymal cells, producing mucopolysaccharides, glycine amino acids, and proline which are the basic content of fiber collagen

that will link the edges of the wound. The next phase is remodeling. It is a maturation process consisting of excessive tissue reabsorption, shrinkage and finally re-modeling of new tissue. This phase can last for months and all signs of inflammation will disappear after this phase ends. [11].

Catfish contain substances that are important for the process of tissue synthesis and wound healing, such as albumin, amino acids and fatty acids. Catfish albumin with an amount of 3.77 g / dl has a role to increase the proliferation of fibroblasts thereby increasing the synthesis, accumulation and remodeling of collagen. Albumin contains a large number of collagen amino acids, namely glycine and proline. This greatly affects fibroblasts to synthesize collagen so that it accelerates the process of forming new tissues on proliferation and maturation. Albumin is also useful as a means of transporting or transporting nutrients and oxygen needed by the body to form new tissue at the proliferation stage [12,13]

Catfish has Lauric acid content of 37.24%, and linoleic acid is 13.52%. Lauric acid and linoleic acid are unsaturated fatty acids. Unsaturated fatty acids can modulate cells or tissues and respond to infections, injuries and inflammation. Fatty acids in catfish play a role in the addition of energy in the process of re-forming myelin sheaths and the formation of cell membranes in tissue growth in wound healing [14,15]

Catfish also have 67.66 g / 100 g amino acid content. Amino acids have been known to play a role in wound healing, particularly arginine and glutamine. Arginine is a proline precursor which plays a role in collagen production during the proliferation phase. Meanwhile, glutamine plays a role during the inflammatory phase in the process of leukocyte apoptosis, superoxide production, and phagocytosis [16,17].

Incision wounds in this study showed that group P4 with a dose of 50 mg / 200 g BW was the best group to give the wound healing effect on the 10th day compared to the dose of 12.5 mg/200 g BW, 25 mg/200 g BW, 37.5 mg/200 g BW and control groups. This study is in concordance with previous study by Alauddin.A which revealed that the best wound healing effect occurred on the 10th day [6]. Based on the theory, on the 10th day, the wound undergoes a cell proliferation process whereas on 3rd day, the wound is still experiencing an inflammatory process. Then on 6th day, the wound enters the beginning of the proliferation phase, so that on the 3rd and 6th day the wound still hasn't healed completely [11].

The observation of the 10th day was found that giving catfish (*Clarias gariepinus*) at a dose 37.5 mg/200 g BW and 50 mg/200 g BW were effective in accelerating wound healing but based on the result of statistical analysis, both of them showed insignificant p value. Then in the use of clinical case the lowest dose will be have the same effect. Therefore, a dose of 37.5 mg / 200 gr BW was said to be the most effective dose in healing Wistar rat incision wounds.

Wound healing in male white rats given catfish skin and meat is faster than the control group without treatment, this can occur because of nutritional content in catfish (*Clarias gariepinus*) so that the nutrients needed during the wound healing are fulfilled. Albumin, amino acids, and fatty acids are nutrients that play an important role in the inflammatory phase, proliferation and remodeling in the wound healing process. Consuming catfish for wound healing is good, this study result showing treatment group has better wound length and fibroblast density than control group. Based on this statement, it can be concluded that catfish which are widely cultivated by people in Indonesia can be used as an alternative treatment for wound healing. Wound healing process also affected by immunity, this study did not analyzed antimicrobial ability of orally administered catfish (*Clarias gariepinus*). It is recommended to analyze antimicrobial ability of orally administered catfish (*Clarias gariepinus*).

## CONCLUSION

In conclusion, the oral administration of catfish could be a new alternative for wound healing treatment. The giving of catfish skin and meat (*Clarias gariepinus*) at a dose of 37.5 mg / 200g BB and 50 mg / 200 g BB is better than other doses assessed from wound length and fibroblast density in Wistar rat

incision wound (*Rattus norvegicus*). The dose of 37.5 mg / 200 gr BB is the most effective dose in reducing wound length and increasing fibroblast density. Antimicrobial ability of orally administered catfish should be analyzed.

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