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THE EFFECT OF ORALLY ADMINISTERED CATFISH (*Clarias gariepinus*) SKIN AND MEAT ON EPITHELIALIZATION THICKNESS AND COLLAGEN DENSITY IN INCISION WOUND OF WISTAR RAT (*Rattus norvegicus*)

Muhammad Irsyad Baihaqi*, Muhammad Duddy Satrianugraha, Tiar M Pratamawati, Donny Nauphar

Faculty of Medicine, Universitas Swadaya Gunung Jati, Cirebon, Indonesia

*Corresponding author's email: baihaqimuhammadirsyad@gmail.com

ABSTRACT

Background: *Alternative medicine using animal is still rare whereas Indonesia have a lot of potential natural resources. Catfish is easily found animal in Indonesia and is common consumed as nutriment sources contains albumin, amino acid and fatty acids which plays an essential role of wound healing process. This study will determine effect of orally administered catfish (Clarias gariepinus) skin and meat on epithelialization thickness and collagen density in incision wound of white male rat (Rattus norvegicus).*
Methods: *This experimental post-test only group design used 30 white male rat (Rattus norvegicus), that randomly divided into 4 tratment goup and one control group. The treatment group was given 12,5 mg/g W, 25 mg/g W, 37,5 mg/g W and 50 mg/g W dose of catfish (Clarias gariepinus) skin and meat flour, in the other hand the contol group were given aquades. Non-parametric analyses using Kruscall-Wallis and Mann Whitney test were used to compare epithelization thickness and collagen density.*
Result: *The comparison results of epithelialization thickness between group C with TGI, C with TG2, C with TG3 and C with TG4 showed significant differences (p<0.05). TG4 was the thickest of all groups. Significant differences were also observed in collagen density result between C with TGI, C with TG2, C with TG3 and C with TG4 (p <0,05) TG4 showed the highest density of all groups.*
Conclusion: *Catfish (Clarias gariepinus) skin and meat flour with 50 mg/200g W dose is effective for wound healing and increasing epithelial thickness and collagen density in Wistar rat (Rattus norvegicus) incision wounds.*

Keywords: *Epithelial thickness, Collagen density, Wound, Catfish (Clarias gariepinus)*

INTRODUCTION

Wounds are lost or damaged in part of the body's tissues. This situation can be caused by sharp or blunt force trauma, temperature changes, chemicals, explosions, electric shock, or animal bites. The wound healing process is divided into five stages, including the stage of homeostasis, inflammation, migration, proliferation, and maturation. According to Basic Health Research (2013), the prevalence of injuries in Indonesia is 8.2 percent, with the highest prevalence found in South Sulawesi (12.8%) and the lowest in Jambi (4.5%) [1]. Comparison of the 2007 Basic Health Research results with 2013 Basic Health Research results showed a trend of an increase in the prevalence of injuries from 7.5% to 8.2% [1].

The use of topical drugs such as povidone iodine for wound healing is often used for wounds. Antiseptics such as povidone iodine, hydrogen, and acetic acid aim to make the wound sterile, the main problem that arises is that the antiseptic not only kills existing pathogenic microorganisms but also kills leukocytes and fibroblast tissue that forms new skin tissue. This can cause interference in the wound healing process [2].

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. Catfish is a fish that is widely consumed in Indonesia. One of the content in catfish (*Clarias gariepinus*) is albumin, which is a globular protein that is often applied clinically to repair nutrition and wound healing. 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 form body tissues, for example, postoperative wounds, burns and when hurt. 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. Omega-3 fatty acids and omega-6 fatty acids can help speed up the healing process of wounds in the legs of chronic diabetic mice [3,4,5,6]. This study aims to determine effect of orally administered catfish (Clarias gariepinus) skin and meat on epithelialization thickness and collagen density in incision wound of white male rat (Rattus norvegicus).

MATERIALS AND METHOD

The research was conducted by using male white rats Wistar (*Rattus norvegicus*) as a test material. Therefore, this study was approved by the research ethics committee of the Medical Faculty of Swadaya Gunung Jati University in Cirebon with the number of ethical clearance (NO.62 / EC / FK / XI / 2018). The researcher has also proposed a letter of approval to conduct research from the Central Laboratory of Inter-University Food and Nutrition Studies (IUFNS) at Gajah Mada University and the Anatomical Pathology Laboratory of the Medical Faculty of Gajah Mada University.

Catfish were obtained from catfish farms in Prambanan District, Sleman Regency, Special Province of Yogyakarta. The raw material in this study is catfish aged between 6-12 months. Which has been identified taxonomically at the FMIPA Laboratory, Semarang State University. Catfish are cleaned from scales, bones and stomach contents then the skin is fleshed and dried in an oven for 12 hours at 40^{o}C after being smoothed by using a blender. The 9 kg of catfish produced catfish flour of 729 grams weight.

Male white rats that had been induced by wounds were separated *randomly* into 5 groups and each group consists of 6 rats. Rats get standard feed and drink *(ad libitum)* during treatment, then rats will be given treatment by giving skin flour and catfish meat each group with a dose of 12.5 mg / 200g W, 25 mg /200g W, 37.5 mg/200g W and 50 mg / 200g W.

Testing the effect of catfish skin and meat on wounds is done by giving orally 1 time a day. Wound observation was carried out for 10 days by observing the wound condition in the wound area. The first day of giving flour is considered to be the 0th day. The rats were terminated on the 10th day with the cervical dislocation method, after that excision of all skin tissue from the wound site, then fixed by using 10% formalin solution and stored in organ tubes, then the preparation was made into histopathological preparations by staining hematoxylin-eosin. Histopathological preparations were observed using a binocular light microscope with an objective magnification of 400x assisted by optilab aids and raster images. The epithelium is measured from the stratum corneum to the basal stratum every preparation of each preparation seen 5 fields of view. The density of collagen fibers was calculated for each preparation of the preparations seen in 3 fields of view, after which they were given a score of 0 = No collagen fibers were found, 1 = Density of collagen fibers in the low wound area (less than 10% per field of view), 2 = Density of collagen fibers in the area of moderate injury (> 10 to 50% per field of view), 3 = Density of collagen fibers in the area of tight wounds (> 50 to 90% per field of view), 4 = Density of collagen fibers in the wound area very tight (> 90 to 100% per field of view), then calculated the average epithelial thickness and collagen density score of each preparation in each treatment group [7,8].

The first data analysis after an observation was the Shapiro-Wilk normality test, because the abnormal data distribution was continued by the Kruskal-Wallis non-parametric test then continued and the Mann-Whitney post hoc test to find out which groups were different.

RESULTS

Macroscopic wound observations

Can be seen in Figure 1. Macroscopic skin differences in incision wound closure were seen. C is a control group that is given aquades. TG.1 is the treatment group 1 which is given catfish flour (*Clarias gariepinus*) at a dose of 12.5 mg/200g W, TG.2 is the treatment group 2 which is given catfish flour (*Clarias gariepinus*) at a dose of 25 mg/200g W, TG.3 is the treatment group 3 which is given catfish flour (*Clarias gariepinus*) at a dose of 37.5 mg/200g W and TG.4 is the treatment group 4 which is given catfish flour (*Clarias gariepinus)* at a dose of 50 mg/200g W.

C

TG.1

TG.2

TG.3

TG.4

Figure 1. Macroscopic description of the control group, treatment groups 1, 2, 3 and 4.

Microscopic observations

Epithelial thickness in incisional wounds can be seen in Figure 2. It can be seen that epithelialization of the control group was 45.90 µm, treatment group 1 with a dose of 12.5 mg/200g W was 56.90 µm, treatment group 2 was 25 mg/200g W , 54 µm, treatment group 3 with a dose of 37.5 mg/200g W was 107.54 µm and treatment group 4 with a dose of 50 mg/200g W was 129.26 µm. Observations were carried out with 400x magnification.

A. *Epithelial thickness*

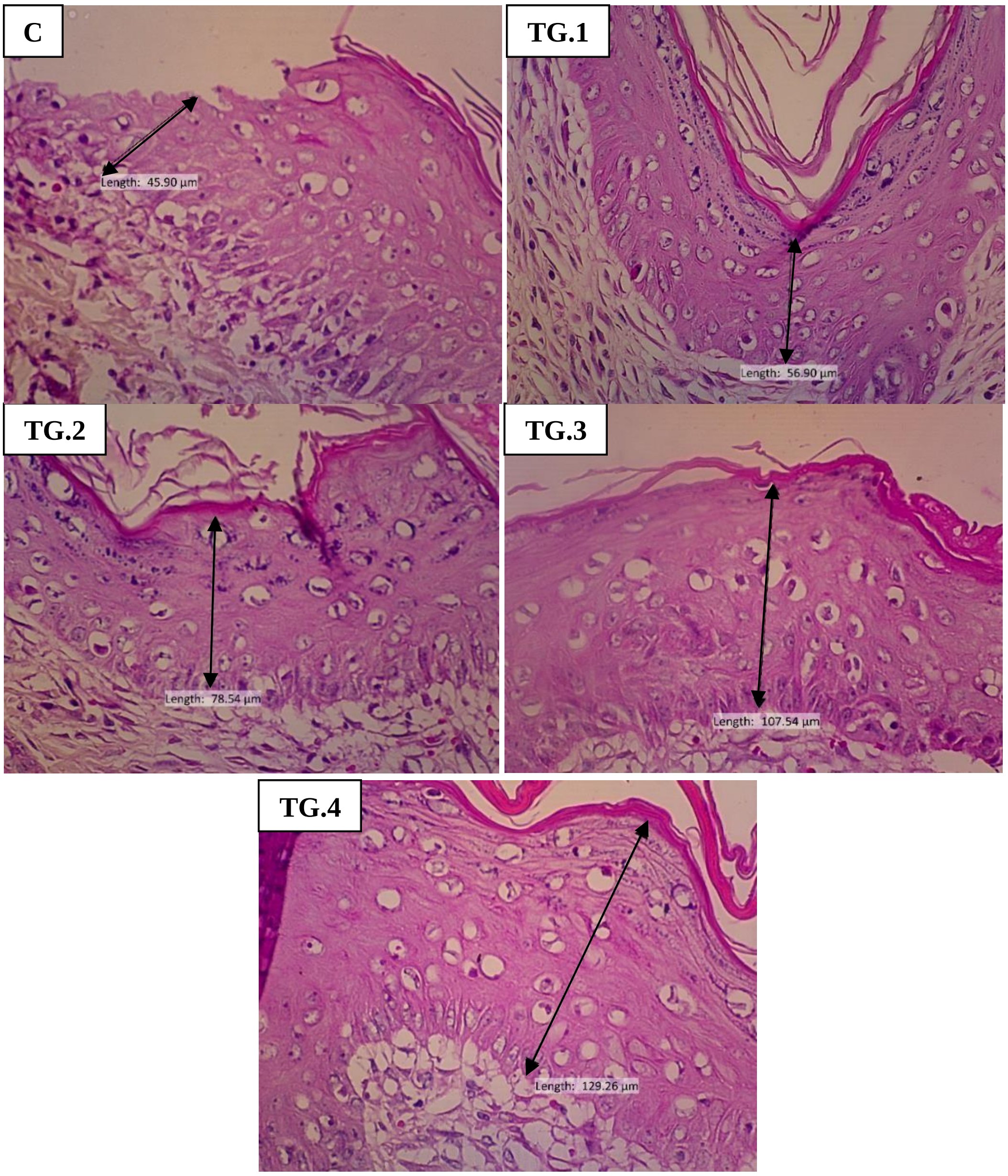


Figure 2**.** Microscopic description of epithelial thickness of the control group (C), and the treatment groups TG.1, TG.2, TG.3 and TG.4.

Figure 3 shows the results of the average epithelial thickness in the control group at 57.02 µm, treatment group 1 with a dose of 12.5 mg/g obtained an average epithelial thickness of 76.27 µm, treatment group 2 with a dose of 25.5 mg/g was obtained the average epithelial thickness was 83.31 µm, treatment group 3 with a dose of 37.5 mg/g obtained an average epithelial thickness of 96.74 µm and treatment group 4 with a dose of 50 mg/g obtained an average epithelial thickness of 108.61 µm.

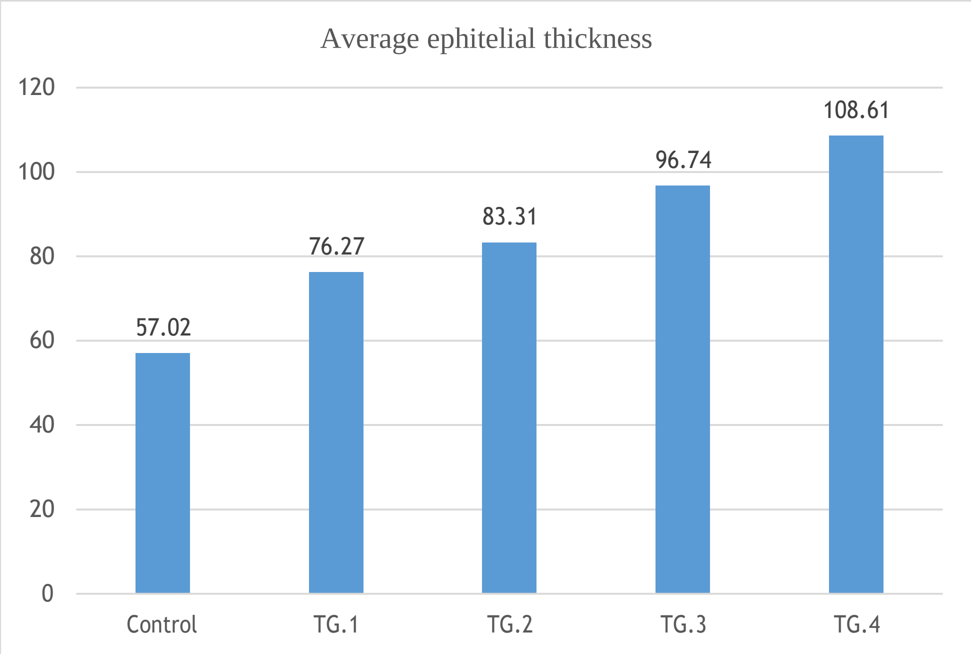


Figure 3. Average ephitelial thickness in control and treatment group.

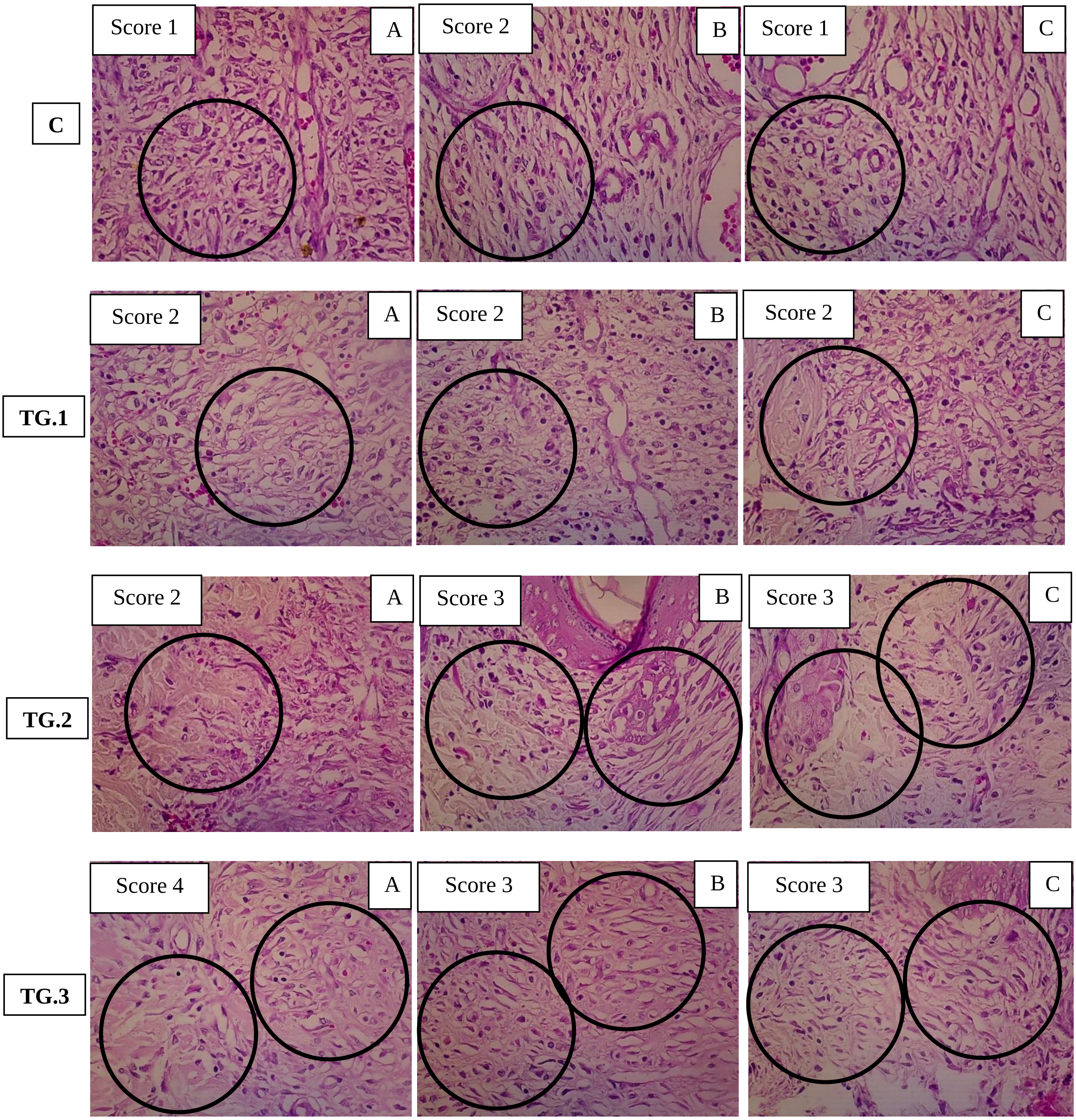
Data analysis

Table 1. *Post hoc Mann-Whitney* epithelial thickness test

	C	TG.1	TG.2	TG.3	TG.4
C	#				
TG.1	0.004	#			
TG.2	0.004	0.004	#		
TG.3	0.004	0.004	0.006	#	
TG.4	0.004	0.004	0.004	0.262	#

The results are then analyzed by *Mann-Whitney post hoc* can be seen in table 1, the results show that 9 pairs of treatment groups (C with TG.1, C with TG.2, C with TG.3, C with TG.4, TG.1 with TG.2, TG.1 with TG.3, TG.1 with TG.4, TG.2 with TG.3 and TG.2 with TG.4) has a value of P <0.05.

B. Collagen density



TG.4

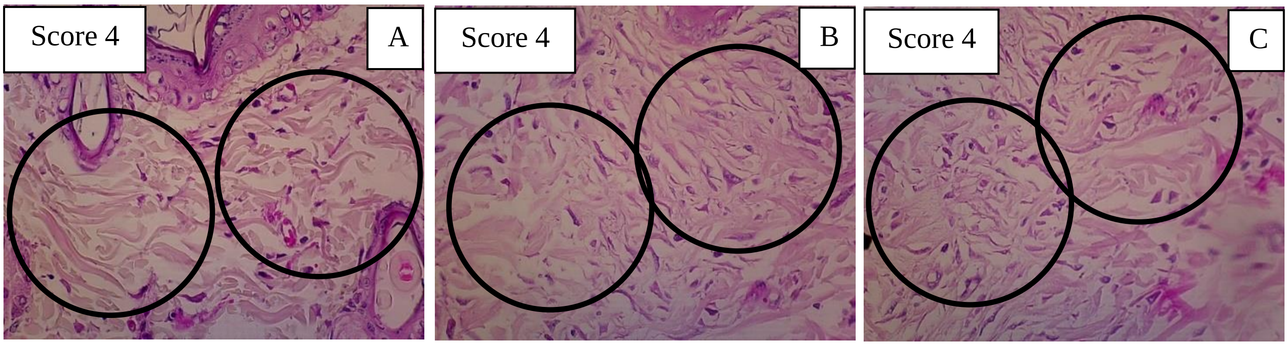


Figure 4. Microscopic description of collagen density in the control group, treatment groups 1, 2, 3 and 4.

The density of collagen appears in Figure 4 shows the density of collagen in the treatment group 1 with a dose of 12.5 mg/200g W has average score 1.3, means density of collagen fibers in the area is still low (less than 10% per field of view), treatment group 2 with a dose of 25 mg/200g W has average score 2, means density of collagen fibers in the area is moderate (> 10 to 50% per field of view), treatment group 3 with a dose of 37.5 mg/200g W has average score 3.3, means density of collagen fibers in the area is tight (> 50 to 90% per field of view) and treatment group 4 with a dose of 50 mg/200g W has the best score of all group it shows average score 4, means density of collagen fibers in the wound area very tight (> 90 to 100% per field of view) [7]. Observations were conducted with 400x magnification.

Data analysis

Table 2. Test *post hoc Mann-Whitney* collagen density

	C	TG.1	TG.2	TG.3	TG.4
C	#				
TG.1	0.004	#			
TG.2	0.003	0.002	#		
TG.3	0.003	0.008	0.128	#	
TG.4	0.003	0.003	0.003	0.119	#

Based on the results of the *Mann-Whitney post hoc* analysis as shown in table 2, it was found that 8 pairs of treatment groups (C with TG.1, C with TG.2, C with TG.3, C with TG.4, TG.1 with TG. 2, TG.1 with TG.3, TG.1 with TG.4 and TG.2 with TG.4) have a value of $P < 0.05$.

DISCUSSION

Table 3. Catfish nutritions in meat and skin [5].

Number	Nutritions	Meat and skin
1	Water (%)	78,5
2	Calori (Kal)	89,38
3	Protein (gr)	19,2
4	Fatt (gr)	1,34
5	Calsium (mgr)	1,61
6	Phospor (mgr)	1,29
7	Iron (mgr)	13,58
8	Sodium (mgr)	0,45
9	Thiamine (mgr)	0,25
10	Riboflavin (mgr)	0,09
11	Niacin (mgr)	0,05

Catfish (*Clarias gariepinus*) contains a lot of nutritions, table 3 show amount of each nutritions. Amino acids and albumin are included into proteins, catfish contain 47.65 g/100g protein essential amino acids and 21.65 g/100g protein non essentials amino acids [5,9]. Amino acid Arginine is a precursor for nitric oxide produced during the inflammatory phase [10]. Cellular activity that occurs during the inflammatory phase of the movement of leukocytes penetrates the blood vessel wall (diapedesis) to the wound due to the power of chemotaxis. Leukocytes secrete hydrolytic enzymes that help digest bacteria and wound debris. Monocytes and lymphocytes which then appear, contribute to destroy and eat wound feces and bacteria (phagocytosis). This phase is also called the slow phase because the reaction of new collagen formation is less, and the wound is only linked by very weak fibrin. Monocytes that turn into macrophages as phagocytes cells can kill bacteria with non-oxidative processes, in this process macrophages will produce a reaction that can kill bacteria with nitric oxide activity [11]. Besides nitric oxide arginine is also a precursor for proline which plays a role in collagen production during the proliferation phase. Glutamine plays a role during the inflammatory phase in the process of leukocyte apoptosis, superoxide production, and phagocytosis [10].

Albumin is a protein in human plasma that dissolves in water and settles in heating and the highest concentration of protein in blood plasma [12]. Catfish contain 3.77 mg/dL albumin, the mechanism of albumin in wound healing is where at the stage of the inflammatory process albumin plays a role in regulating osmotic pressure in the blood and is almost 50% of plasma protein. When a skin tissue is injured, the skin will show signs of inflammation, it is when foreign matter get in through an open wound such as a cut, the entry of a foreign object triggers a hydrostatic pressure disorder where the intracellular fluid will enter the cell due to a difference or imbalance in the concentration inside and outside the cell via the osmotic pathway causing the cells to experience edema or swelling. This condition requires albumin nutrition which can maintain osmotic pressure inside and outside the cell, so that, the edema which occurs does not get worse. In the maturation phase, albumin acts as a basic ingredient through the body's catabolic reform to form collagen. Collagen is rapidly developing as the main factor forming the matrix. Collagen fibers at the beginning are randomly distributed to form crosses and aggregate into fibrin yarn thread which slowly causes tissue healing and increases stiffness and tensile strength. The return of stress strength runs slowly because of continuous deposition of collagen tissue, remodeling of collagen fibers forms greater collagen fibers and changes from inter-molecular cross-linking. Collagen remodeling during scar tissue formation depends on the process of continuous collagen synthesis and catabolism [9,13].

Catfish also contains fatty acids, fatty acids in catfish has play a role in the addition of energy in the process of re-forming axon myelinization and the formation of cell membranes in tissue growth in wound healing. Omega-3 fatty acids can reduce the inflammatory response resulting in vasodilation through the release of cytokines. Meanwhile, omega-6 is a precursor for the production of prostaglandin, thromboxane, and leukotriene in the inflammatory response. Disconnected blood vessels in the wound will cause bleeding, and the body tries to stop it with vasoconstriction, retraction, and hemostasis reactions. Hemostasis occurs because platelets that come out of the blood vessels are attached to one another, and together with the mesh of fibrin that forms, freezes the blood that comes out of the blood vessels. Adapted platelets will degranulate, release chemoattractants that attract inflammatory cells, activate local fibroblasts and endothelial cells and vasoconstrictors [6,14]. Omega-6 is therefore very important in platelet aggregation and vasoconstriction during the inflammatory phase in the wound healing process [10].

Nutritional content in catfish (*Clarias gariepinus*) is very useful for the wound healing process. 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 [4,5,6]. Consuming catfish for wound healing is good, this study result showing treatment group has better ephitelial thickness and collagen density than control group, Based on this statement, it can be concluded that catfish which are widely consumed 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)*. Toxicity level of orally administered catfish was also not analyzed. 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. Skin and meat of catfish (*Clarias gariepinus*) with a dose of 12.5 mg/200g W, 25 mg/200g W, 37.5 mg/200g W, and 50 mg/200g W given orally to male white Wistar strain rats (*Rattus norvegicus*) showed better epithelial thickness and collagen density than the control group in incision wounds and there were significant differences ($p < 0.05$). The skin and meat dose of catfish (*Clarias gariepinus*) which is effective in increasing epithelial thickness and collagen density in incision wounds is a dose of 50 mg/200g W. Antimicrobial ability and toxicity level of orally administered catfish should be analyze.

CONFLICT OF INTEREST DECLARATION

The authors declare that there is no conflict of interest regarding the publication of this paper.

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