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EFFECTIVENESS OF ORALLY USE TILAPIA (*Oreochromis niloticus*) ON WOUND LENGTH AND FIBROBLAST DENSITY ON INCISION WOUND OF WISTAR RAT (*Rattus norvegicus*)

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ABSTRACT

Background: *The incidence of a wound in Indonesia has increased from 7.5 percent in 2007 to 8.2 percent in 2013 according to Riskesdas. Wound care that widely used is povidone-iodine, but in case, it has a toxic effect on healthy cells around the wound. The albumin, amino acids, and fatty acids in Tilapia (Oreochromis niloticus) are the potential substances to accelerate the wound healing process. This study aims to investigate the efficacy of Tilapia (Oreochromis niloticus) on wound length and fibroblast density on the incision wound of Wistar rat (Rattus norvegicus).*
Methods: *The study was conducted in the Food and Nutrition PAU Laboratory and Pathology Anatomy Laboratory of Universitas Gadjah Mada, Yogyakarta. This post-test only control group design experimental study used 30 male white rats that randomly divided into five groups, i.e. control group K, and treatment group P1, P2, P3 and P4 that were each given Tilapia with a dose of 12.5 mg/200gBW, 25 mg/200gBW, 37.5 mg/200gBW dan 50 mg/200gBW. The length of the wound was measured by a ruler each day. The rats were terminated on the 10th day to obtain wound tissue for H&E stained histopathological sections to observe fibroblast density. One Way ANOVA, Tamhane, Kruskal-Wallis and Mann Whitney tests were used to compare the wound length and fibroblasts density.*
Results*: Incision wounds in this study showed the best wound healing on the 10th day. Based on the theory, on the 10th day, a proliferation and remodeling phase occurs. There is an effect of giving Tilapia (Oreochromis niloticus) orally at a different dose. Tilapia at dose 37.5mg/200gBW is more effective compared with the dose of 12.5mg/200gBW, 25mg/200gBW, and 50mg/200gBW to accelerate wound healing for wound length and fibroblast density in Wistar rat (Rattus norvegicus).*
Conclusion*: Consuming Tilapia (Oreochromis niloticus) could be a new alternative for wound healing treatment. The antimicrobial ability of Tilapia should be analyzed.*

Keywords*: wound healing, wound length, fibroblast density, Tilapia, Oreochromis niloticus*

INTRODUCTION

A wound is the loss or damage of a part of the tissue. This condition can be caused by the trauma of sharp or dull objects, temperature changes, chemical substances, explosion, electric shock, or animal bite. The incidence of a wound in Indonesia has increased from 7.5 per cent in 2007 to 8.2 per cent in 2013 according to *Riskesdas* [1].

The process of wound healing is divided into 3 phases, i.e. inflammation, proliferation, and remodeling [2]. The target of the biological process of the body in compensating wound is the components that act in wound healing phases. Fibroblast is one of the components of healing in fibroplasia process. Fibroplasia is a process of wound repair that involves connective tissue that has four components: formation of new blood vessels, migration and proliferation of fibroblasts, deposition of extracellular matrix (ECM), maturation and organization of fibrous tissues (remodeling) [2,3].

Wound care that widely used is povidone iodine. The use of povidone-iodine in many cases has a side effect that can be toxic to the healthy cells around the wound[4]. Alternative wound care is needed to improve wound healing. Indonesia is a biodiversity country, one of them is Tilapia [5]. Tilapia with the contents of albumin could improve healing percentage and fibroblast density on wound [5]. Albumin can regulate osmotic pressure in the blood, maintain the presence of water in the blood plasma so that it can maintain blood volume in the body, and as a means of transporting or transporting. Irwanda et al. has shown that albumin content in Toman fish (*Channa micropeltes)* could improve nutrition and cure postoperative wounds. [6] Alauddin has shown that albumin content in Snakehead fish (*Channa striata*) was effective in accelerating incision wound of Wistar rat. [7]. Thus, this study was conducted to investigate the efficacy of Tilapia (*Oreochromis niloticus*) on wound length and fibroblasts density on incision wound of Wistar rat (*Rattus norvegicus*).

MATERIALS AND METHODS

This was an experimental study with post-test only control group design using male white rat (*Rattus norvegicus*) as subject research. Ethical clearance approval No.58/EC/FK/XI/2018 was obtained from The Research Ethics Committee of Faculty of Medicine, Universitas Swadaya Gunung Jati, on 21th November 2018. The study was conducted in the Food and Nutrition PAU Laboratory and Pathology Anatomy Laboratory of Universitas Gadjah Mada, Yogyakarta, Indonesia.

Tilapia (*Oreochromis* niloticus) was obtained from fish farm in Sleman, DIY, Indonesia, confirmed by a taxonomist of Universitas Negeri Semarang, Central Java, Indonesia. The collected fishes were washed under running water, separated from the head and stomach contents, took the skin and meat, then cut into pieces, and dried by the oven for 12 hours with 40^0C. The dried fish was mashed up with a blender.

Thirty-five male white rats (*Rattus norvegicus*) of 180-200 grams of weight randomly divided into 5 groups, i.e 1 control group and 4 treatment groups. At first, all the rat were wounded by 2 cms 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 12.5mg/200gBB Tilapia, group P2 with 25mg/200gBB of Tilapia, group P3 with 37.5mg/200gBB of Tilapia, and group P4 with 50mg/200gBB of Tilapia.

The treatment was done for 10 days (once a day at 8 a.m), and on 3rd, 6th, 10th day, the length of incision wound was measured by a ruler and the healing process was analyzed visually. At the 10th day, all rats were terminated by cervical dislocation. 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. Histological analysis was performed by using a binocular light microscope Olympus CX23 with ocular lens 100x and 400x. Fibroblasts were counted in 5 viewing fields for each section slide of each group.

RESULTS

Macroscopic incision length analysis

All incision wounds at the 0th day were 2cm, then measured by a ruler at the 3rd, 6th, and 10th. Table 1 shows the wound length of all groups.

Table 1. Mean wound length

Groups	Mean ± SD Wound length (cm)			
	0th day	3rd day	6th day	10th day
Control	2	1.94±0.03	1.85±0.04	1.50±0.14
P1: 12.5mg/200gBW	2	1.07±0.02	0.91±0.03	0.38±0.07

Groups	Mean ± SD Wound length (cm)			
	0th day	3rd day	6th day	10th day
P2: 25mg/200gBW	2	1.06±0.02	0.87±0.02	0.21±0.02
P3:37.5mg/200gBW	2	1.04±0.03	0.70±0.04	0.04±0.03
P 4: 50mg/200gBW	2	1,06±0.04	0.49±0.04	0.05±0.03

The table showed that mean wound length on the 3rd day showed the worst wound length was at the control group which is 1.94±0.03SD cm, and the best wound length was at P4 which is 1.06±0.04SD cm. The 6th day showed the worst wound length was also at the control group which is 1.85±0.04SD cm, and the best wound length was at P4 which is 0.49±0.04SD cm, and on the 10th day, the worst wound length was at the control group which is 1.50±0.14SD cm, and the best wound length was at P3 which is 0.04±0.03SD cm.

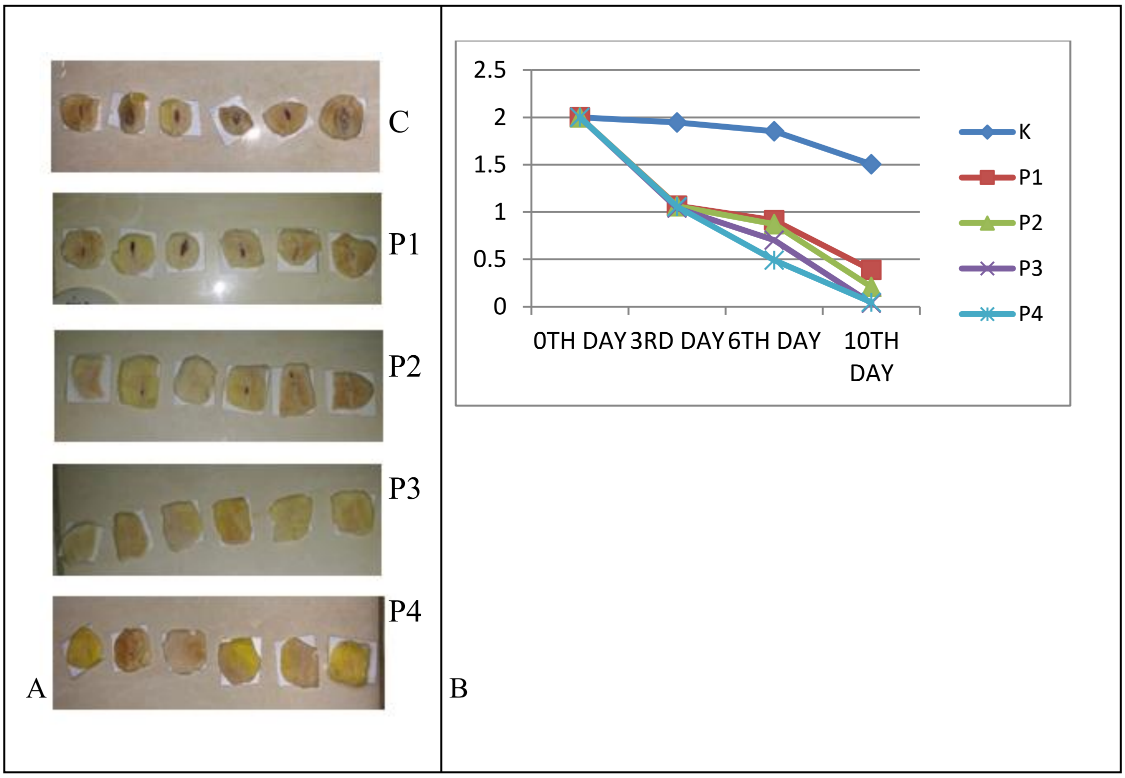

Figure 1. Macroscopic observation on incision wound. A. Incision wound of all groups taken after 10 days of treatment. B. Graph showing length shortens of incision wound.

Mean length of incision wound at the 10th day of all rat group were compared by using parametric One Way ANOVA 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 Tamhane test was performed to see which pair groups have difference shown in Table 2.

Table 2. Tamhane test

	C	P1	P2	P3	P4
C	#				
P1	0.001	#			
P2	0.001	0.009	#		

	C	P1	P2	P3	P4
P3	0.001	0.001	0.001	#	
P4	0.001	0.001	0.001	1.001	#

The results show that 9 pairs of treatment groups (C with P1, C with P2, C with P3, C with P4, P.1 with P.2, P.1 with P.3, P.1 with P.4, P.2 with P.3 and P.2 with P.4) has significant *p-value* (<0.05), while a pair of treatment group (P3 with P4) has insignificant *p-value* (>0.05).

Microscopic analysis of fibroblast 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.

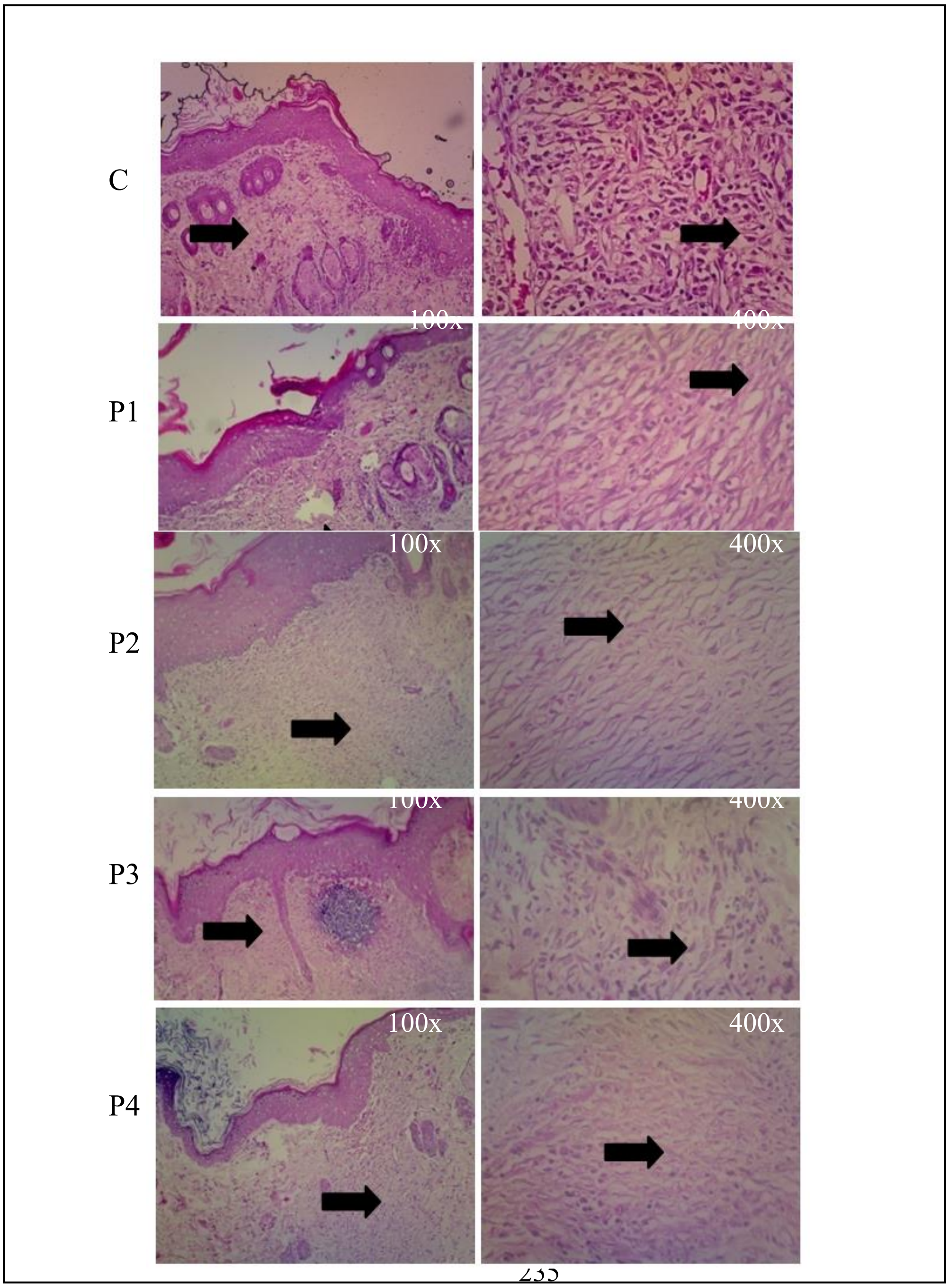

Figure 2. Microscopic observation incision wound

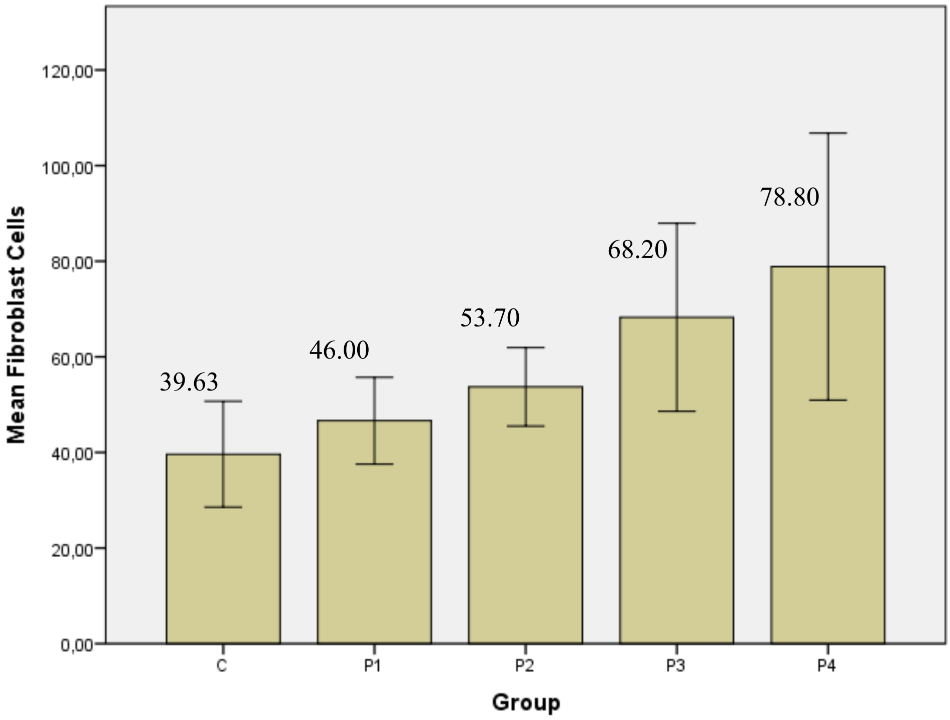

Figure 3 showed mean fibroblast density of control group is 39.63 cells, then P1 is 46.00 cells, P2 is 53.70 cells, P3 is 68.20 cells and P4 is 78.80 cells. Mean fibroblast density of incision wound after ten days treatment (fig.3.) was compared by using parametric Kruskal-Wallis analysis, with Confidence 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 different shown in Table 3.

Table 3. Mann-Whitney test

	C	P1	P2	P3	P4
C	#				
P1	0.055	#			
P2	0.004	0.030	#		
P3	0.004	0.004	0.008	#	
P4	0.004	0.004	0.004	0.109	#

The results show that 9 pairs of treatment groups (C with P1, C with P2, C with P3, C with P4, P.1 with P.2, P.1 with P.3, P.1 with P.4, P.2 with P.3 and P.2 with P.4) has significant *p-value* (<0.05), while a pair of treatment group (P3 with P4) has insignificant *p-value* (>0.05).

DISCUSSION

The process of wound healing is divided into 3 phases, i.e. inflammation, proliferation, and remodeling. The inflamation phase starts while the tissues injured until the 5th day. The broken blood vessels in the wound will cause bleeding, and the body tries to stop it with vasoconstriction, retraction, and hemostasis reactions [8,9]. Hemostasis occurs because platelets that come out of blood vessels are attached to one another. The attached platelets will degranulate, release the chemoattractant that attracts inflammatory cells, activates local fibroblasts and endothelial cells and vasoconstrictors [8,9].

The proliferation phase comes out from the end of the inflammatory phase to approximately the end of the 3rd week. Fibroblasts originate from undifferentiated mesenchymal cells, producing mucopolysaccharides, glycine amino acids, and proline which are the basic ingredients of collagen fiber which will link the edges of the wound. In this fibroplasia phase, the wound is filled with inflammatory cells, fibroblasts, and collagen, and new blood vessel formation (angiogenesis). The next phase is remodeling, which is a ripening process consisting of excessive tissue reabsorption, shrinkage which is in accordance with the gravitational force, and finally remodeling new tissue [8,9].

Tilapia (*Oreochromis niloticus*) contains a variety of important substrates that can accelerate the process of wound healing, including the content of albumin, collagen and fatty acids [10].

The albumin with an amount of 6.25 mg / dL has the potential to accelerate wound healing [10]. Albumin can regulate the osmotic pressure in the blood, maintain the presence of water in the blood plasma so that can maintain blood volume in the body, and transport cells nutrition. Albumin is useful for the formation of new body tissues and accelerating healing of body tissues [11].

Type I collagen was found in the skin of Tilapia (*Oreochromis niloticus*) with an amount of 70% from total protein in the skin of Tilapia. Type I collagen can improve wound healing. It forms the extracellular matrix and collagen fibers outside the cell [12].

Fat content in Tilapia is 4.17% with fatty acid components [13]. Fat can accelerate the wound healing process. Omega 3 and omega 6 are also known to increase the immune system of wounded patients so that they can avoid infection. In addition, omega 3 and omega 9 fatty acids increase pro-inflammatory cytokines. These cytokines can enhance the inflammatory phase in the process of wound healing. Overall fatty acids can also increase collagen synthesis, so the wound healing process is going faster [14].

Incision wounds in this study showed the best wound healing on the 10th day. Based on the theory, on the 10th day a proliferation and remodeling phase occurs. This was in line with Alauddin's study which stated that wounds in Wistar rat with treatment using Gabus Fish (*Channa striata*) experienced the best wound healing on the 10th day, while on 3rd and 6th day, the wound still in inflammatory processes so that the wound has not healed completely [7].

The observation of the 10th day was found that giving a dose of Tilapia (*Oreochromis* niloticus) 37.5mg/200gBW and 50mg/200gBW were effective in accelerating wound healing. However, based on the results 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. Thus, a dose of 37.5 was said to be the most effective dose in healing Wistar rat incision wounds in this study.

Consuming Tilapia (*Oreochromis niloticus*) is very useful for the wound healing process. Albumin, amino acids, collagen and fatty acids are nutrients that play an important role in the inflammatory phase, proliferation and remodeling in the wound healing process. This study result showing treatment group has better wound length and fibroblast density than control group. Wound healing process affected by amount of immunity, this study did not analyzed antimicrobial ability of Tilapia (*Oreochromis niloticus*). It is recommended to analyze antimicrobial ability of Tilapia (*Oreochromis niloticus*).

CONCLUSION

Oreochromis niloticus 37.5mg/200gBW is more effective compared with dose of 12.5mg/200gBW, 25mg/200gBW and 50mg/200gBW to accelerate wound healing for wound length and fibroblast density in Wistar rat (*Rattus norvegicus*). Thus, consuming Tilapia (*Oreochromis niloticus*) could be a new alternative for wound healing treatment. Antimicrobial ability of Tilapia should be analyzed.

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