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The effect of administration of siamese catfish (pangasius hypophthalmus) extract on fibroblast cells after tooth extraction in wistar rats

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Abstract

Objective: The study aimed to determine the effect of administration of Siamese catfish extract orally on the number of fibroblast cells in wound healing after tooth extraction of Wistar rats.
Material and Method: This research was a true experimental laboratory with posttest only control group design. 24 male Wistar rat were divided into 6 groups. After the mandibular left incisor was extracted, rats in groups 1, 2, and 3 were given Siamese catfish extract and groups 4, 5, and 6 were given aquadest orally once a day. Groups 1 and 4 were treated for 3 days, groups 2 and 5 were treated for 5 days, and groups 3 and 6 were treated for 7 days, then the rats were euthanized and made histological preparations. Fibroblast cells were calculated using Image Raster software and analyzed using One Ways ANOVA and Post Hoc LSD tests.
Results: The number of fibroblast cells increased on the 5th day and reached its peak on the 7th day. ANOVA test showed a significant difference in the mean of fibroblast number between the treatment and control groups ($p<0,05$).
Conclusion: Administration of Siamese catfish extract orally can increase the number of fibroblast cells in wound healing after tooth extraction of Wistar rats.

Keywords: Fibroblast, Siamese catfish, Wound healing
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Introduction

Tooth extraction is a common treatment in dentistry and causes tissue injury that leading to wound healing. The wound healing process is a complex and dynamic process with continuous, overlapping, and precisely programmed phases, consisted of hemostasis, inflammation, proliferation, and remodelling.[1,2]

Hemostasis phase occur immediately after injury, with vasoconstriction and formation the fibrin clot. This phase aim to keep the vascular system intact and also to provide matrix for invading cells are needed for next phase of healing. Inflammatory phase begins in late phase of hemostasis and immediately after it, this phase aims to minimize microbial contamination and prevent wound infection, which is characterized by infiltration of neutrophils and macrophages. Neutrophils and macrophages play role in microbial phagocytosis and celullar debris in the wound area, furthermore macrophages also play role in promote the transition of inflammatory phase to the proliferation phase of healing.

The proliferation phase starts 3 days after injury and lasts for about 2 weeks thereafter, which is aims to covering the wound surface, restoring the blood vessel, and forming the granulation tissue at the site of injury. Granulation tissue consist of fibroblasts, granulocytes, macrophages, and blood vessels. This tissue plays role in replacing provisional wound matrix composed of fibrin and fibronectin that formed during hemostasis phase. In proliferation phase, one of the most prominent cell type is fibroblast cells. Fibroblast cells first appear in the wound site on the 3rd day after injury and their play role in produce proteinase to degrade the provisional matrix and also produce collagen and extracellular matrix components, such as proteoglycans, hyaluronic acid, glycosaminoglycans, and fibronectin. The final phase of wound healing is remodelling phase that starts at the end developmental of the granulation tissue until 1 or 2 years. The end result of this phase is the formation of mature scar tissue as avascular and acellular environtment with high tensile strength.[23]

The wound healing process is influenced by multiple factors, which are generally categorized into local and systemic. Nutrition is one of the systemic factors that influence wound healing. One of the most important nutrient factors influencing wound healing is protein, deficiency of this nutrient causes distruption of blood vessel formation, fibroblast proliferation, colagen and extracellular matrix synthesis, and remodelling phase in wound healing process.[4] Siamese catfish is one of the freshwater fish that is widely consumed by Indonesian people, especially in South Sumatra. Local people of South

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Sumatra process siamese catfish into traditional food known as "*Pindang*".[4] Siamese catfish contains all types of essential amino acids and saturated and unsaturated fatty acids.[5] Study by Sudirman et al. showed that the dominant polyunsaturated fatty acid in Siamese catfish is omega-6 fatty acid (linoleic acid).[4]

Results of research conducted by Sudirman et al.[4] showed that the dominant polyunsaturated fatty acids in Siamese catfish are omega-6 fatty acids (linoleic acid). Omega-6 plays role in wound healing such as induce inflammatory cells migration and promote angiogenesis.[6] The ability of Siamese catfish in wound healing process is still unknown. The aim of this study is to determine the effect of administration of Siamese catfish extract orally on the number of fibroblast cells in wound healing after tooth extraction of Wistar rats.

Material and Methods

This research was a true laboratory experimental with posttest only control group design. Extraction was conducted at the Biochemistry Laboratory, Faculty of Medicine, Sriwijaya University, treatment on animals were performed at the Animal House Laboratory, Faculty of Medicine, Sriwijaya University, and manufacture of histological preparations and observation was done at the Dyatnitalis Anatomical Pathology Laboratory Palembang. This research was approved by Health Research Review Committee of Mohammad Hoesin Hospital and Faculty of Medicine, Sriwijaya University No: 082/kepkrsmhfkunsri/2020.

Twenty four male Wistar-strain rats, aged 2-3 months with weight of 200-250 g. Inclusion criteria comprised of healthy rat (active movement and good appetite) while exclusion criteria comprised of death rat and unhealthy rat (limp and weight loss more than 10%). Samples were randomly divided into 6 groups consisting of two treatments, namely Siamese catfish extract and aquadest.

Fresh Siamese catfish weighing 500-1000 g were obtained from the Siamese catfish cultivation area in Sungai Rengit Village, Talang Kelapa District, Banyuasin Regency, South Sumatra. Fishes were cleaned and the abdominal contents were cut out. Fish meats were sliced into small pieces using transversal cut with a thickness of 0,5-1 cm without taken out the bones. Aquadest was put first into steaming pot in the ratio between fish meats and aquadest 1:1. Siamese catfish meats was placed in a stainless steel container and placed on the sieve then steamed with a temperature 70°C for 50 minutes. Siamese catfish extract was yellowish liquid that was collected in container. Extract was filtered and then placed in a closed container.

The rats were adapted in laboratory for a week at room temperature (20°C-25°C). Before the tooth was extracted, the rats were anesthetized intramuscularly using Ketamine 40 mg/kgBW. The left mandibular incisor was extracted using lecron and needle holder, then the tooth socket was irrigated with aquadest to remove debris or remains of tooth extraction. Thereafter, sterile cotton pellet were applied to the tooth socket with light pressure to stop bleeding. Rats in groups 1, 2, and 3 were given Siamese catfish extract, whereas groups 4, 5, and 6 were given aquadest orally once a day using gastric sonde. Siamese catfish extract dosage was 2,5 ml/250gBW. Rats in groups 1 and 4 were treated for 3 days, groups 2 and 5 were treated for 5 days, and groups 3 and 6 were treated for 7 days.

Rats were euthanized on the 3rd, 5th, and 7th days after tooth extraction using chloroform. Each tooth socket on the left mandibular of the rat was cut with a No. 11 scalpel blade and fixated in 10% neutral-buffered formalin for 24 hours to maintain cell structure and component. The observation of fibroblast cells on the histological preparation that was stained using Hematoxylin and Eosin staining was performed using light microscope (*Olympus CX-33*®, Japan) at 400 times magnification on 3 fields of view. The number of fibroblast was calculated using Image Raster software. Data were tested with Saphiro-Wilk normality test and Levene homogeneity test, then analyzed using One Way ANOVA test and continued with LSD post-hoc test with a value of $p<0,05$ was considered statistically significant.

Results

Observation of histological preparations for counting the number of fibroblast cells were performed at 400 times magnification on 3 fields of view. Based on the results of observation, the increase in the mean number of fibroblast cells occured in all groups at each day of observation. The mean number of fibroblast cells in the treatment groups receiving Siamese catfish extract were higher than the control groups receiving aquadest. The lowest mean number of fibroblast cells was found in the 3rd day control group, whereas the highest mean number of fibroblast was found in the 7th day treatment group.

Figure 1 shows that the number of fibroblast cells on the 7th day appear more than 3rd and 5th days in both of the treatment and control groups. Histopatological view of fibroblast cells on the 3rd

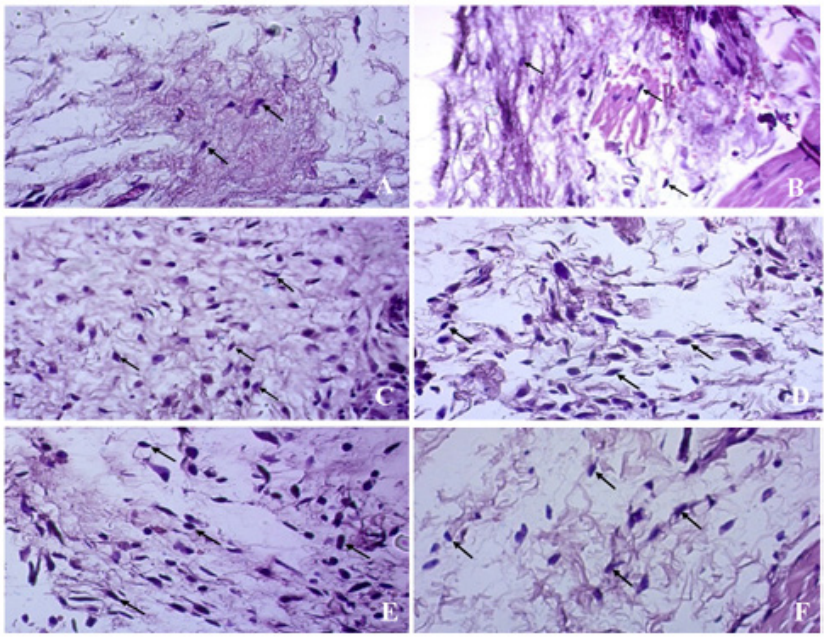

Figure 1. Histopathological features of fibroblast cells on the 3rd day of the treatment group A. Control group, B. Histopathological features of fibroblast cells on the 5th day of the treatment group, C. Control group, D. Histopathological features of fibroblast cells on the 7th day of the treatment group, E. Control group, F. Fibroblast cells are pointed by black arrows. HE staining, magnification 400x.

day of control group show the lowest number of fibroblast cells, therefore the fibroblast cells look like far apart from each other, whereas on the 7th day of treatment group show the highest of fibroblast cells number so the fibroblast cells look like dense and close to each other.

Data were analyzed with Saphiro-Wilk normality test and Levene homogeneity test. Based on normality and homogeneity tests, data were normally distributed and had a homogeneous variance which was indicated by a value of p<0,05. Based on the results of normality and homogeneity tests, analysis data was continued with One Way ANOVA test and the results can be seen in Table 1. The next test was LSD post-hoc test to determine the significance of the difference in the mean value of number of fibroblast cells between each observation group.

The results of the data analysis by One Way ANOVA test in Table 1 showed that the mean value

Table 1. Analysis of One Way ANOVA test of fibroblast cells number post tooth extraction in each observation group

Groups	Mean of fibroblast cells number (Mean ± SD)			N	P value
	3rd day	5th day	7th day		
Treatment (Siamese catfish extract)	52.918 ± 21.403	84.165 ± 27.969	88.665 ± 23.645	4	0.014*
Control (Aquadest)	44.250 ± 7.696	45.835 ± 7.631	51.665 ± 22.248	4	

*There was significant difference

Table 2. Analysis of least significant difference test of fibroblast cells number after tooth extraction inter-groups

Day	Groups		Mean difference	95% Confidence interval		P value
	Treatment	Control		Minimum	Maximum	
3rd	Siamese catfish extract	Aquadest	8.668	-21.112	38.452	0.549
5th	Siamese catfish extract	Aquadest	38.330	8.545	68.115	0.015*
7th	Siamese catfish extract	Aquadest	37.000	7.215	66.785	0.018*

*There were significant differences

of fibroblast cells between siamese catfish extract group and aquadest group was significantly difference, which was indicated by a value of p<0,05. Based on the One Way ANOVA test results, it can be concluded that there was an effect of administration of siamese catfish extract on the number of fibroblast cells after Wistar rat tooth extraction.

The results of LSD post-hoc test in Table 2 showed that on the 3rd day, there was no significant difference between treatment group and control group with p=0,549 (p>0.05). The results on the 5th day and 7th day, there were significant difference between treatment group and control group with significance value of 0.015 and 0.018 (p<0.05).

Discussion

Showed that on the 3rd day after tooth extraction, both the treatment and control group had seen the

presence of fibroblast cells in wound area. Starting from the observation on the 3rd day, the mean number of fibroblast cells continued to increase until it reached its peak on the 7th day. During first 3 days after the tissue injury, fibroblast cells in the surrounding tissue are stimulated to proliferate and then they migrate into the site of injury. On the 3rd day after injury, fibroblast cells first appear in the wound site. In the wound site, fibroblast cells proliferate continuosly and also produce collagen and extracellular matrix components.[2]

That in the treatment group and control group, the mean number of fibroblast cells increased on 5th day and reached the highest number on 7th day. These indicate that the lowest number of fibroblast cells was found in the 3rd day after injury and the highest number of fibroblast cell was found in 7th day after injury. An increase in the number of fibroblast cells is an indicator of the wound healing process because the increasing number of fibroblast cells indicates an increase of cell density which play role in the formation of new tissue so that wound healing can be achieved.[7] On the 3rd day after injury, the number of fibroblast cells was the lowest, this is happened because fibroblast cells first appear in the wound site on the 3rd day after injury.[2]

The wound healing process consists of four overlapping phases: hemostasis, inflammation, proliferation, and remodelling. All the phases in wound healing process must occur in proper sequence and appropriate time with specific duration.[1] Proliferation phase begins on 3rd day after injury and this phase aims to covering the wound surface, restoring the blood vessel, and forming the granulation tissue at the site of injury. The dominating cell in proliferation phase is the fibroblast cell, which have various different functions, such as degrade the provisional matrix by producing matrix metalloproteinases (MMPs) and produce collagen and Extracellular Matrix (ECM) substances, such as glycosaminoglycans, hyaluronic acid, proteoglycans, glycoproteins, laminin, trombospondin, and heparan sulphate. ECM formation aims to fill up the wound gap, replace provisional matrix, and provide a scaffold for support and regulates the migration and activity the growth, movement, and differentiation of cells during wound healing process.[8,9]

LSD post-hoc test on the 3rd day showed that there was no significant difference between treatment group and control group, whereas the result on the 5th day and 7th day showed significant difference between treatment group and control group. The not significant difference on the 3rd day allegedly happened because the active substances contained in the Siamese catfish extract have not worked optimally due to the short duration of administration and also on the 3rd day after injury, fibroblast cells first appear in the wound site.[2,7]

This study resulted that the mean number of fibroblast cells in treatment group was higher than control group. The highest mean number of fibroblast cells was found in the 7th day treatment group and the lowest mean number of fibroblast cells was found in 3rd day control group. The increase in the number of fibroblast cells in the control group occurred as a normal physiological wound healing response, although there was no active compound in aquadest that can accelerate fibroblast cells proliferation. In the treatment group, the increase in the number of fibroblast cells showed the presence of arginine and omega-6 fatty acids in Siamese catfish extract that can increase the number of fibroblast cells.

Arginine is one type of amino acid that plays role in wound healing. Arginine can stimulate the production and secretion of growth hormone, activate T cells, and act as a precursor for proline and nitric oxide, which are important in inflammatory phase and synthesis collagen. Arginine also promote the deposition of collagen, angiogenesis, and contraction of wound.[1,10] A results study by Fujiwara et al. showed that arginine supplementation stimulates fibroblast cell proliferation and also inhibits apoptosis in fibroblast cell. This proves that arginine play an important role in proliferation of fibroblast cell and has an anti-apoptotic effect on fibroblast cells.[11]

Omega-6 fatty acid (linoleic acid) has ability to accelerate the inflammatory phase by inducing migration of inflammatory cells as well as promoting angiogenesis in wound area.[6,12] On the 2nd until 3rd day after injury, monocyte cells are recruited to the site of injury and differentiate into macrophages that continue the phagocytosis activity and promote the transition of inflammatory phase to the proliferation phase.[2,3] Macrophages synthesis numerous potent growth factor, such as TGF-β (transforming growth factor β), FGF (fibroblast growth factor), and PDGF (platelet derived growth factor) which functions to stimulate fibroblast cells to migrate from the surrounding tissue to the wound site, proliferate, and synthesis of extracellular matrix substances.[8,13] In proliferation phase, omega-6 fatty acid promote angiogenesis in the site of injury, which is closely related to the increasing the concentration of VEGF (vascular endothelial growth factor) and angiopoietin-2, which are essential in the angiogenesis process.[12]

Angiogenesis is an important process in wound healing because the new blood vessels are needed to supply oxygen and nutrients during the wound healing process.[3] A results study by Rodrigues et al. [14] showed that oral administration of omega-6 fatty acid (linoleic acid) can hasten the inflammatory phase in wound healing process and also promote faster wound closure.[14]

This study is a preliminary study and still has several limitations. This research still cannot be able to determine the spesific active substances and the percentage of each active substance in the Siamese catfish extract in various concentrations that can increase proliferation of fibroblast cells. This research also cannot determine the standardization of Siamese catfish quality and its extract quality affecting the number of fibroblast cells, as well as the histological examination in this research to observe fibroblast cells still have not used immunohistochemistry staining.

Conclusion

Oral administration of Siamese catfish (Pangasius hypophthalmus) extract can increase the number of fibroblast cells in wound healing after tooth extraction in male Wistar rats (Rattus norvegicus).

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Conflict of Interest

The authors report no conflict of interest.

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