

EFFECTIVENESS OF CORK FISH EXTRACT GEL ON FIBROBLAST PROLIFERATION IN SOCKET WOUND AFTER TOOTH EXTRACTION

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ABSTRACT

Objective: Socket wound healing involves soft tissue cells that play an important role, namely fibroblasts. In the wound-healing process, proteins are needed that can accelerate wound healing, one of which is albumin. Cork fish contains the highest albumin than other fish. Albumin contains natural proteins and collagen that make up the extracellular matrix, which has the role of inducing growth factors, cell migration and adhesion, and tissue regeneration. The purpose of this study was to examine the effect of 1%, 3%, 5% and 7% cork fish extract gel on fibroblast cell proliferation in socket wound healing after tooth extraction.

Methods: This study was a laboratory experimental study with 30 Wistar rats that were grouped into 5 groups. The treatment group was given cork fish extract gel, namely 1%, 3%, 5% and 7%, and the negative control group was only given base gel was then the tissue was tested using *Hematoxylin Eosin* to observe fibroblasts on the 3rd, 7th and 14th day.

Results: Based on the results of the one-way ANOVA test, it showed that cork fish extract with a concentration of 1%, 3%, 5% and 7% has an effect on fibroblast proliferation in socket wound healing after tooth extraction.

Conclusion: Based on the results of the one-way ANOVA test, it showed that 3% cork fish extract had an influence on fibroblast proliferation in healing socket wounds after tooth extraction.

Keywords: Cork fish extract, Wound healing, Tooth extraction, Fibroblasts

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INTRODUCTION

Wounds are defined as an injury to the skin's tissue brought on by bringing in contact with heat sources, the results of medical actions, or changes in physiological conditions [1]. Tooth extraction is one of the minor surgical procedures performed by dentists to remove teeth from their sockets if the teeth can no longer be treated [2]. Based on data obtained from basic health research (RISKESDAS) in 2018, the proportion of the population who received dental extractions in North Sumatra was 6.41%, which was around 1,822 people [3]. Wound healing under normal circumstances is repairing tissues or other organs after injury. The wound healing process is dynamic and complex to achieve homeostasis and tissue integrity. The hemostasis phase, the inflammatory phase, the proliferation phase, and the remodeling phase are the typical phases that make up the wound healing process [4, 5]. Every tooth extraction often causes complications. A wound that is left untreated can cause pain and discomfort, which can affect a person's quality of life. Therefore, medications are needed to treat and accelerate wound healing after tooth extraction [6]. The World Health Organization (WHO) estimates that up to 80% of people worldwide rely on traditional medicine to fulfill their medical needs [7]. The use of animals as alternative ingredients in medicine has not experienced significant development in Indonesia, even when viewed in terms of natural resources, especially Indonesian waters, are very potential to be developed into a source of raw materials in medicine [8]. The utilization of marine and river animals as medicinal materials are still in the development stage, especially using fish as raw materials for treatment. Cork fish (*Channa striata*) is a productive freshwater and brackish fish. Scientific research has demonstrated that cork fish are beneficial and effective in accelerating the healing of wounds following surgery [6]. The content of cork fish

extract that is very instrumental in the wound healing process is albumin, Zn, and unsaturated fatty acids because the albumin protein in cork fish is 6.22% higher than other types of fish [6]. Albumin plays an essential role as an anti-inflammatory, it keeps blood plasma's amount of water constant, which helps to keep up blood volume and prevents fluid from the outside of the cell from entering and inducing cell swelling [9]. Albumin works by binding Zn and acting as an antioxidant, while Zn maintains the immune system by synthesizing proteins and in cell multiplication and maintaining the integrity of connective tissue [6, 10]. Zn plays a role in increasing cell proliferation, epithelialization processes, and collagen strength [7]. The extract gel also contains anti-inflammatory unsaturated fatty acids that play an important role in the regulation and synthesis of prostaglandins, chemical mediators that appear during inflammation, activating macrophages and as vasodilators of blood vessels thereby regulating the infiltration and activation of neutrophils in thus accelerating the inflammatory process [6, 9]. Haruan fish extract, also known as cork fish, comprise compounds necessary for the process of tissue formation, include albumin, minerals such as zinc (Zn), copper (Cu), and iron (Fe), and unsaturated fatty acids such as omega-3, omega-6, and omega-9 [5]. Zn is an antioxidant that protects cells, accelerates the wound healing, regulates expression in lymphocytes and proteins. Cork fish's animal protein content is examined to have high biological values, including healing and inhibiting inflammatory processes [11]. About 60% of human plasma protein is composed of albumin which makes it the main protein in human plasma. It plays a major role in accelerating the wound healing process, acting as an anti-inflammatory and accelerating proliferation [9].

Therefore, the aim of this research is determine the effect of administering snakehead fish (*Channa striata*) and comparison

extract gel with a concentration of 1%, 3%, 5% and 7% on fibroblast cell proliferation on days 3, 7 and 14.

MATERIALS AND METHODS

Preparation of cork fish extract gel

Saturate the mortar and pestle for 10 min in boiling water. Once the outside of the mortar is hot, discard the water and dry the mortar with a tissue. Pour 10 ml of hot water into the first mortar. Put 0.125 g of carbopol into the hot water in the first mortar evenly and wait for about 3 min or until the carbopol expands. Pour 10 ml of hot water into the second mortar. Put 0.125 g HPMC into the hot water in the second mortar evenly and wait for about 3 min or until HPMC expands. After expanding, stir the carbopol and HPMC clockwise until it becomes homogeneous. To produce the first mixture, put 1.5 g TEA and 2 g glycerin into the first mortar and stir until homogeneous. To produce the second mixture, put 0.02 g nipagin and 0.02 g nipasol into the second mortar and mix until homogeneous. Slowly pour the second mixture into the first mixture while still stirring in a clockwise direction until both mixtures are homogeneous. Slowly add 1 g and 3 g of cork fish extract and stir until homogeneous. Flush with 5 ml of distilled water if there is any residual extract in the container. If the gel is too thick, gradually add 3 ml of distilled water until the desired viscosity is reached. In making cork fish extract gel, 1% extract is 1 g in 100 g gel x 20 g extract. For the manufacture of 3% cork fish extract gel is 3 g of extract in 100 g gel x 20 g so 0.6 extract.

Animals

The subjects used in this study were wistar strain rats (*Rattus norvegicus*). The eligible rats were male sex aged 2-3 mo, the average body weight of the rats was 200-250 grams. Experimental animals were kept in animal cages in plastic cage tubs with husk mats, with the top of the cage covered with strimin wire in such a way that the rats could not escape. The temperature of the experimental room was 18-26 °C and the room was adequately ventilated. The humidity of the maintenance room is 65-75%. Feed the rats with pellets (Charoen Pokphan 511 Starter) and drink tap water, each given ad libitum. The feed container is a glass bowl, while the drinking water container is a glass bottle with a rubber lid equipped with a drinking pipe. Sanitization 2x a week. [27] Animal experiments have been approved by the animal research ethics committee, Faculty of Mathematics and Natural Sciences, University of North Sumatra (No. 0069/KEPH-FMIPA/2023) and can be approved for implementation after considering its relevance to human health guided by ethically tested animal research principles for health research using experimental animals.

Rat tooth extraction procedure

The feed container is a glass bowl, while the drinking water container is a glass bottle with a rubber lid equipped with a drinking pipe. Sanitization 2x a week [27].

Wistar rats were anesthetized using a combination of ketamine 9.1 mg/kgBB and xylazine 9.1 mg/kgBB intraperitoneally with an anesthetic dose of 0.1 mg/100 g of Wistar rat. After that, the mandibular left incisor tooth of Wistar rats were extracted using an artery clamp with luxation movement carefully to avoid fracture of

the tooth. Irrigation of the socket with distilled water after tooth extraction to clean the socket from debris or remnants of tooth extraction [12].

Treatment procedure

In group I, cork fish extract gel with 1% concentration was applied, in group II cork fish extract gel with 3% concentration was applied, in group III 5% cork fish extract gel was applied, in group IV 7% cork fish extract gel was applied and in group V base gel was applied. By placing directly on the socket wound using a 1 ml abocath syringe 12 since the occurrence of the wound which is counted as day 1. The application of cork fish extract gel was carried out twice a day, namely in the morning at 08.00-10.00 AM, and in the afternoon at 04.00-06.00 PM for 14 d.

Hematoxylin Eosin (HE) tissue staining procedure

After each day has been reached, rats are euthanized by dislocating the neck and the lower jaw of the rat will be excised and the alveolar bone in the socket is taken. Next, the excised tissue will be decalcified using 10% EDTA for 10 d. All groups were preceded by tissue preparation and *Hematoxylin Eosin* (HE). In order to avoid counting male Wistar rats repeatedly, tissue preparations of fibroblast cells were analyzed using a 400 X magnification binocular microscope and ocular graticule positioned inside a lens divided into three fields [6].

Fibroblast proliferation measurement procedure

The excised tissue was fixed in a 10% Buffered Neutral Formalin (BNF) solution with the ratio of tissue to 10% BNF solution is 1:10. Labels were given to the container and then fixation was carried out for 12-48 h, after which the tissue was decalcified; namely the tissue was immersed in 10% EDTA solution for 10 d. The excised tissue was then cut using a scalpel and microtome with a thickness of 4 mm; the tissue was placed in tissue cassettes and put into a basket. The basket was put into an automatic processor and continued to the dehydration machine to dehydrate the tissue: 70% ethanol for 1 h; 95% ethanol for 1 h (twice); and 100% ethanol for 1 h (twice). Afterwards, the basket was rinsed with xylol for 1 h (twice) and infiltrated with paraffin for 1 h (twice). The next stage is embedding. The paraffin-infiltrated tissue is removed from the cassette and placed in the mould. Fill the mould with liquid paraffin until the entire tissue is submerged in paraffin. Leave the paraffin for at least 15 min to freeze into a paraffin block on a cooling machine. Remove the paraffin block from the mould and store at room temperature. Next, cut the paraffin block with a thickness of 4-5 µm using a microtome machine and place the cut in a water bath at 45 °C. Take the tissue pieces with a slide glass. Label the glass slides and arrange them in a special rack. Slides were placed in a 37° incubator to dry overnight. Slides are stored in a dry box at room temperature until the preparations are ready to be stained with haematoxylin and eosin. The stages of staining the preparations were as follows: Xylol I, Xylol I, Absolute ethanol I, Absolute ethanol II, 95% ethanol I, 95% ethanol II, Rinsed with running water, Hematoxylin solution, Rinsed with running water, Decolourised in acidic alcohol (1 ml 37% HCl in 100 ml 70% ethanol), Rinsed with running water. Eosin, Rinsed with running water. 95% ethanol III, 95% ethanol IV, 100% ethanol III, 100% ethanol IV, Xylol III [13].

Table 1: Mean fibroblast proliferation in socket wound healing after tooth extraction on days 3, 7 and 14 after tooth extraction

Observation	Treatment	N	mean±SD (σ)	P-value
1%	Day 3	3	27.600±5.048	0.343
3%	Day 7	3	22.333±18.966	0.032*
	Day 14	3	13.267±0.833	
5%	Day 3	3	12.533±2.730	0.913
	Day 7	3	27.200±9.849	
7%	Day 14	3	11.533±1.677	0.135
	Day 3	3	23.733±5.644	
Base gel	Day 7	3	19.800±2.615	0.289
	Day 14	3	20.533±19.667	
	Day 3	3	20.800±6.315	
	Day 7	3	24.467±4.500	
	Day 14	3	15.600±1.637	
	Day 3	3	22.667±12.401	
	Day 7	3	19.667±8.582	
	Day 14	3	10.667±0.987	

*One-way ANOVA test; p<0.05; significant.

Processing and data analysis

The data obtained were Residual Socket Volume (RSV) observed clinically to assess the acceleration of wound closure and mean cell count of fibroblasts observed under a microscope to assess fibroblast cell proliferation. The first statistical analysis performed was the Shapiro-Wilk test to determine whether the data distribution was normal or not. Levene's test was performed to see the homogeneity of the socket wound volume on day 1 in each group. One-way ANOVA and LSD tests were used to determine whether there was a significant difference in RSV values between days 3, 7 and 14 in the treatment and control groups. To see if there was a significant difference in the mean cell count of fibroblasts between days 3, 7, and 14 in the treatment and control groups, one-way ANOVA and LSD post-hoc tests were used.

RESULTS

In the treatment group, the mean and standard deviation of the number of fibroblast cells after administration of 1% cork fish extract gel on days 3, 7 and 14 after tooth extraction of Wistar rats were 27.600 ± 5.048 ; 22.333 ± 18.966 ; 13.267 ± 0.833 . Based on the results of the one-way ANOVA test, there was no significant difference in fibroblast proliferation in socket wound healing after administration of 1% cork fish extract gel on days 3, 7 and 14 ($p > 0.05$).

In the treatment group, the administration of 3% cork fish extract gel on days 3, 7 and 14 after tooth extraction of Wistar rats was 12.533 ± 2.730 ; 27.200 ± 9.894 ; 11.533 ± 1.677 . Based on the results of the one-way ANOVA test, there is a significant difference in fibroblast proliferation in socket wound healing after the administration of 3% cork fish extract gel on the 3rd, 7th, and 14th days after tooth extraction of male Wistar rats ($p < 0.05$).

In the treatment group, the mean and standard deviation of the number of fibroblast cells after the administration of cork fish extract gel with a concentration of 5% on the 3rd, 7th and 14th days after tooth extraction of Wistar rats were 23.733 ± 5.644 ; 19.800 ± 2.615 ; 20.533 ± 19.667 . Based on the results of one-way ANOVA, there was no significant difference in fibroblast proliferation in socket wound healing after the administration of cork fish extract gel with a concentration of 5% on days 3, 7, and 14 after tooth extraction of male Wistar rats ($p > 0.05$).

In the treatment group, the mean and standard deviation of the number of fibroblast cells after application of cork fish extract gel with a concentration of 7% on the 3rd, 7th and 14th days after tooth extraction of Wistar rats were 20.800 ± 6.315 ; 24.467 ± 4.500 ; 15.600 ± 1.637 . Based on the results of the one-way ANOVA test, there was no significant difference in fibroblast proliferation in socket wound healing after the administration of cork fish extract gel with a concentration of 7% on days 3, 7, and 14 after tooth extraction of male Wistar rats ($p > 0.05$).

In the control group, the mean and standard deviation of the number of fibroblast cells after the administration of base gel on days 3, 7 and 14 after tooth extraction of Wistar rats were 22.667 ± 12.401 ; 19.667 ± 8.582 ; 10.667 ± 0.987 . Based on the results of the one-way ANOVA test, there was no significant difference in fibroblast proliferation in socket wound healing after the administration of base gel on days 3, 7, and 14 after tooth extraction of male Wistar rats.

DISCUSSION

Based on table 1, the group that showed a significant increase in fibroblast proliferation occurred in the cork fish extract gel group with a concentration of 3% ($p < 0.05$). The concentration of cork fish extract has an effect on wound healing. This is because the increase in concentration causes more active substances contained in higher concentrations so that more fibroblasts are produced [14]. This is in line with the research of Atmajaya, *et al.* 2019, 100% cork fish extract has an effect on increasing the number of fibroblasts in socket wounds after tooth extraction. The study explained that the administration of 100% cork fish extract gel to post-tooth extraction socket wounds in wistar strain white rats (*Channa striata*) showed a

higher number of fibroblasts compared to wounds treated with other extract concentrations such as 25% and 50% [9].

Based on table 1, there was a significant difference in fibroblast proliferation on days 3 and 7 as well as days 7 and 14 using cork fish extract gel between concentrations of 3%. This follows research conducted by Hanafiah *et al.* in 2022, showing that fibroblasts appeared for the first time on day 3, peaking from day 7 to day 14. This is also because on day 7 is the phase where fibroblasts produce a lot of collagen to produce an extracellular matrix that will fill the wound cavities and provide a foundation for keratinocyte migration [15]. However, in the 1% cork fish extract gel group, fibroblasts peaked on day 3. This is in accordance with research conducted by Sutiyo which says that fibroblasts will appear and increase on day 3 and gradually decrease on day 7 and day 14, on day 3, fibroblasts have been replaced with collagen so that on day 7 the inflammatory process begins again because the wound has not been completely closed due to external factors such as food irritation in the socket, then the wound healing process begins again, this is also what causes the wound on day 14 to re-open [16]. Albumin also plays a role in this phase, where albumin helps stimulate the growth of more fibroblasts and fibroblasts produce collagen, which will form the extracellular matrix so that wound healing occurs more quickly.

Based on table 1, there was no significant difference between the treatment group and the control group on days 3, 7, and 14 after tooth extraction of male Wistar rats ($p > 0.05$) but in the 7% cork fish extract treatment group, fibroblasts were seen more than the 5% cork fish extract treatment group and the base gel control group. This contradicts the results of Gusdi's research in 2012, which proved that the topical preparation of cork fish extract gel has the effectiveness of closing cuts at a concentration of 5% [16].

Fibroblasts were used as an indicator in this study because *fibroblasts* are one of the criteria for wound healing, with fibroblasts forming in the socket. Fibroblasts are very important in the wound healing, both in forming collagen fibres, connective tissue, and the subsequent differentiation process in forming osteoblasts for bone tissue formation. The use of Wistar rats as experimental animals in this study is because Wistar rats are easy to handle and also have healing reactions that are in principle similar to reactions that occur in humans. The choice of day 3 as the beginning of the study is based on the fact that fibroblasts begin to form on day 3 and can continue until day 14. Fibroblasts are the main element in the proliferation phase, which starts to increase on day 3 and reaches its peak on day 7. From day 7 to day 14, there will be a decrease in the number of fibroblasts as new alveolar bone begins to fill the socket [17, 18].

On day 3, it appears that the average number of fibroblasts in the cork fish extract treatment group with a concentration of 7% is more when compared to the average number of fibroblasts in the base gel control group (table 1). On day 7 fibroblasts still increased and reached its peak and on day 14 fibroblasts began to decline. This study is in line with Atmajaya's research in 2019, which conducted research on fibroblast observations tended to increase on day 3 and reached its peak on day 7 [6]. This is also in accordance with research conducted by Hanafiah, *et al.*, in 2021, showing that fibroblasts appeared for the first time on day 3, peaking on day 7 to day 14 [19]. The number of fibroblasts that are higher in the cork fish extract treatment group with a concentration of 7% than in the control group given the base gel indicates that these results suggest that cork fish extract gel with a concentration of 7% could accelerate the wound healing process. The higher number of fibroblasts in the 7% cork fish extract treatment group can be related to the mechanism of action of cork fish extract in wound healing.

In the treatment group with a concentration of 5% seen in the histological picture, there are many necrosis tissues. In this study, the immunity of the rat body is included in the uncontrolled variable, in cork fish extract; there are unsaturated fatty acids, namely omega 3 and omega 6. Omega 3 functions as an anti-inflammatory for all diseases. Omega 6, which functions as a pro-inflammatory is found more in the body, making it prone to inflammation. For the body not to be prone to inflammation, there must be a balance between the amount of omega 3 and omega 6 in the body. This could be since most of the mice experienced delayed

wound healing. The inflammatory response in each rat and external factors such as environmental interventions may not be the same. Differences in wound healing after tooth extraction can be influenced by many factors that play a role in the inflammatory process, both cellularly similar to the release of inflammatory mediators. This could also be due to the influence of oral hygiene factors of the rats as hygiene is one of the local factors that can affect wound healing. Systemic factors include nutrition, metabolic status, and immune status. This could be because most rats experienced delayed wound healing [20].

In wound healing, albumin is an anti-inflammatory by maintaining fluid balance in the blood vessels. The healing activity is due to the activation of T cells, which causes a decrease in macrophage cells. Albumin acts as an antioxidant through a mechanism as a radical binder and capture of reactive oxygen species (ROS), so the body will need antioxidants to guard against free radical attacks by eliminating the negative effects of these compounds. The principle mechanism of action is inhibiting radical formation by stabilising and preventing free radical reactivity. When the antioxidants produced by the human body are not enough to fight free radicals, the body requires antioxidant intake from outside. Consumption of foods that contain antioxidant agents can help the body to reduce the content of free radicals in the body [21, 22].

Albumin contains natural collagen, a constituent of the extracellular matrix, which plays a role in inducing growth factors, cell migration and adhesion, and tissue regeneration. Albumin in cork fish extract contains amino acids that makeup collagen fibres in large quantities, namely glycine and proline. The role of albumin is also as a basic ingredient for collagen formation. Collagen is one type of protein that reaches 30% of all proteins that make up the human body [1, 22, 23].

Zinc, as well as some amino and fatty acids, help albumin in its function of promoting wound healing. Zinc plays a role in wound healing starting from the inflammatory, proliferation, and remodelling phases. Zinc plays an important role in human health, essential for growth and development, metabolism, and wound healing. Various proteins whose function depends on Zn cofactors (Zn-dependent proteins) have important roles in the cell, including extracellular matrix (ECM) regulation and antioxidant defence. Antioxidants are important in wound healing because free radicals can damage the protein structure of cells, preventing cell proliferation. Antioxidants can neutralise free radicals so that cell proliferation is not disturbed. Zinc takes a part in cell proliferation and collagen synthesis during wound healing. Zinc is necessary for all cell types that proliferate, including fibroblasts, epithelial cells, and inflammatory cells. The zinc in cork fish leads to an increase in fibroblast cell count by producing fibroblast growth factor (FGF). Zn also plays a role in the inflammatory phase as Zn is required for the proper functioning of lymphocytes and plays an important role in several steps of the blood clotting process [6, 24].

Cork fish's high concentration of mandatory amino acids, as glycine, impacts its effectiveness as a material for healing wounds [6]. Glycine takes an important part in stimulating the release of growth hormone, aiding muscle development and growth and wound healing. Glycine has a function in stimulating the process of angiogenesis so that the formation of new blood vessels that will nourish the tissue [17]. Cork fish contains unsaturated fatty acids, unsaturated fatty acids such as omega-3, omega-6 and omega-9. Due to study that, omega-3 is considered to accelerate the process of collagen remodelling and re-epithelialization of the wound, it is thought to aid in wound healing. Omega-3 can mobilise macrophage cells to feed on netrophil cells and clear the debris of the phagocytosis process. Omega-3 fatty acids, especially EPA, have been shown to assist fibroblasts in synthesising collagen. Arachidonic acid (AA) is a derivative of Omega-6. AA acts as a proinflammatory and anti-inflammatory agent. With the help of the cyclooxygenase enzyme, arachidonic acid is converted to prostaglandin-type eicosanoids and their derivatives (prostacyclin and thromboxane). Prostacyclin (PGI2) functions to inhibit blood clotting and facilitate blood flow, while thromboxane (TXA2) formed in platelets causes blood pieces to coalesce and clot [25, 26].

CONCLUSION

Cork fish extract gel (*Channa striata*) with a concentration of 3% has an effect and there is a significant difference in fibroblast proliferation on days 3, 7, and 14 after tooth extraction of Wistar rats ($p < 0.05$).

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AUTHORS CONTRIBUTIONS

All the authors of the manuscript have contributed in the manuscript and the study. Design of study, collection the data and its analysis, manuscript writing, editing, and proofreading was carried out by the authors.

CONFLICTS OF INTERESTS

Declared none

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