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The Influence of *Channa Striata* Extract Emulgel on Incision Wound Healing in White Rats

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ABSTRACT

Snakehead fish (*Channa striata*) have high albumin content, a protein needed for cell development and the formation of new tissue. The aim of this study was to determine the influence of snakehead fish extract emulgel given topically on incision wounds in white rats. The parameters of wound healing consist of wound length, a number of neutrophils, macrophages, fibroblasts and density of collagen. The white rats divide into three groups of (n = 6), one group was given the emulgel base as the negative control, one group of povidone-iodine as the positive control, and one group of snakehead fish extract 10% emulgel. White rats were sacrificed on the third and seventh days for microscopic observations. The results showed that snakehead fish extract emulgel can accelerate incision wound healing: decrease wound length, increase the number of neutrophil and macrophages cells, increase the average number of fibroblasts and increase collagen density on white rats.
Keywords: *Channa striata*; emulgel; healing; incision wounds

INTRODUCTION

Open wounds are wounds that visible to where the blood comes out of the body. Types of open wounds include incisions, lacerations, abrasions or superficial wounds, penetrating wounds, gunshot wounds, and stab wounds. Incision wounds can occur intentionally (surgical wounds) or accidentally (accidental injuries) due to sharp objects. The surface of the incision wound is flat and bleeding. A deeper incision includes the lining of the muscularis, blood vessels, nerves, and tendons (Nagori dan Solanki, 2011).

The wound healing process consists of four phases: hemostasis, inflammation, proliferation, and remodeling. In the hemostasis phase, the wound response occurs so blood vessels themselves constrict and platelets secrete to form a stable clot that sealing the damaged blood vessels. The inflammatory response is the second phase that occurs since the blood vessels leak and expel plasma and neutrophils into the tissues. Inflammation lasts up to four days after the wound and characterized by erythema, swelling, and heat accompanied by fever. In the proliferation phase which lasts for 4 to 21 days, angiogenesis, collagen tissue deposition, granulation tissue formation, and wound contraction and epithelization occur. In the remodeling phase, the healing process involves remodeling and realignment of the collagen tissue to produce greater tensile strength. The main cells that play a significant role in this process are fibroblasts (Orstedv *et al.*, 2011).

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A fibroblast is a component of wound healing that is widespread in connective tissue, producing precursor substances of collagen, elastic fibers, and reticular fibers. Wound healing involves fibroblasts in the process of fibroplasia. Fibroplasia is a process of repairing wounds involving connective tissue consisting of four components namely new blood vessel formation, migration, and proliferation of fibroblasts, ECM (extracellular matrix) deposition, and maturation and organization of fibrous tissue (remodeling) (Masir *et al*., 2012).

Protein is needed for all stages of the wound healing process, including fibroblast proliferation, collagen synthesis, angiogenesis, and immune function (Crowe *et al.*, 2009). Snakehead fish containing 70% protein as the main constituent and 21% from total protein is albumin so snakehead fish is a good source for regenerate damaged cells (Sediaoetama, 2004).

The contents of snakehead fish extract were 17 amino acids including glutamic acid 1.87–43.13 mg/g, glycine 21.80–80.85 mg/g, leucine 7.85–40.19 mg/g, aspartic acid 13.85–44.07 mg/g, proline 9.49–45.46 mg/g, alanine 11.38–35.25 mg/g, and arginine 5.99–21.79 mg/g. The fatty acid content includes palmitic acid 3.54–26.84%, stearic acid 3.25–15.90%, oleic acid 1.40–27.68%, linoleic acid 0.51–7.82% of total protein. The research was carried out by extracting snakehead fish using a cooking pot at a temperature of 100˚C for 60 minutes which was compared with cured fish extract with water solvent and snakehead fish extract with a mixture of chloroform-methanol. This study concluded that snakehead fish contains

Table I. Formula of Snakehead Fish Extract Emulgel

No.	Ingredients	Negative control (g)	Formula (g)
1	Snakehead fish extract	-	10
2	HPMC	3	3
3	Liquid paraffin	5	5
4	Tween 80	1,08	1,08
5	Span 60	0,42	0,42
6	Propylenglycol	10	10
7	Methylparaben	0,03	0,03
8	Propylparaben	0,01	0,01
9	Aqua pro injeksi ad	100	100

bioactive components, especially amino acids and fatty acids which are the main components of antioxidants that can be used on wound healing (Daud *et al*., 2010).

The research by Sinambela *et al.* (2012), optimized the concentration of snakehead fish in the form of ointments containing 6, 8, and 10% and the best effectiveness for incision wound healing achieved at 10%. However, in their research, the organoleptic test still gave poor results, which is the distinctive odor of snakehead fish.

Topical medicine is one form of medicine that is often used in dermatological therapy. In this study, the formula chosen was in the form of emulgel for it has a good penetration function based on its ability to penetrate the hypodermic layer, so that the absorption of the skin is better and will immediately melt in contact with the skin and forms a thin layer, therefore it is good to be used in lesions on haired skin (Yanhendri and Yenny, 2012). The main advantage in a topical preparation is not a subject of the first-pass metabolism in the liver. Systemic absorption in topical therapy can be ignored so the side effects and drug interactions are rare (Brunton *et al.*, 2011).

The aim of this study was to know the influence of *Channa striata* extract emulgel on white rat incision wounds healing with the parameters, the number of neutrophils, macrophages, fibroblasts, and collagen density.

METHODOLOGY

Materials

Materials used were snakehead fish extract (Striata Group Malang), 10% povidone-iodine solution (Betadine, PT. Mahakam Farma), HPMC, paraffin liquid, Tween 80, Span 60, propylene glycol, methylparaben, propylparaben, ether, alcohol 70%, 10% formaldehyde (all chemicals were pharmaceutical grade obtained from PT. Brataco), aqua pro injection (PT. Otsuka), and hematoksillin-eosin dye. Healthy Wistar strains rats aged 3-4 months with the bodyweight of 250-300 g. Rats were kept in cages under controlled room temperature of 25oC with 75% relative humidity.

Research Methods

Characterization of Snakehead Fish Extract

Characterization of snakehead fish aqueous extract comprises of albumin content by spectrophotometry, total protein content by Kjeldahl method and trace element compound (Zn, Cu and Mn) by atomic absorption spectrophotometry

Emulgel Preparation

The formula of snakehead fish extract emulgel used is shown in Table I.

The water phase for the emulsion was made by mixing Tween 80 in the aqua pro injection. Methylparaben and propylparaben dissolved in propylene glycol and then added into the water phase. The oil phase was made by mixing Span 80 with liquid paraffin. Each phase was heated to 70-75˚C separately. The oil phase was added to the water phase with continuous stirring until cooled to room temperature. The gel base was made by dispersing 3% HPMC gradually into distilled water temperature of 80˚C with constant stirring. Snakehead fish extract was added to the gel had been formed. The emulsions mixed into the gel base gradually and stirred until emulgel was formed. Physical-chemical characterization of emulgel consists of organoleptic test, homogeneity, pH, viscosity, and spreadability.

Animals test

White rats (*Rattus norvegicus*) were adapted for 7 days and grouped into three groups randomly. Six animals were evaluated in each group. Emulgel was applied topically 350 mg twice a day. Observations were carried out twice,

Table II. Characterization of Snakehead Fish Extract

Composition	Content (/100 g)
Total protein	9.950 g
Albumin	8.401 g
Zn	4.917 mcg
Cu	4.669 mcg
Mn	1.796 mcg

after 3 and 7 days. As a positive control was povidone-iodine 10%, while negative control or placebo was emulgel without extract.

All groups of rats were shaved on the *musculus dorsi* area of 3 cm x 2.5 cm a day before the incision. The skin was sterilized with 70% alcohol first and the rat was anesthetized using ether. The incision was 2 cm length and 0.25 cm depth in this area. The scalpel was held to form an angle of 30-40˚ to the skin in a caudal direction. Observation of wound length was carried out using calipers on the third and seventh days.

Histopathological Examination

The retrieval of rat skin tissue was carried out on day 3 (to know the inflammatory phase with parameters neutrophils and macrophage) and day 7 (to know the proliferation phase with parameters macrophage, fibroblast and collagen density). Each rat was sacrificed in each phase by ether inhalation. Specimens were taken from each incision wound on the back by excision with a diameter of about 2 cm to as deep as the muscle. Skin tissue samples were fixed in 10% buffered formalin for 24 h and routine paraffin wax embedding procedures were used and blocked. Examination of the number of macrophages, neutrophils, and the number of fibroblasts was carried out by staining hematoxylin-eosin and observed under a microscope (Bancrof, 2013). All the parameters were measured using *Adobe Photoshop 5.0* computer program.

Statistical analysis

Data were presented as mean. Statistical comparison of the groups was performed using SPSS Ver. 12.0. Non-parametric Kruskall Wallis tests were used to analyze the significant difference between groups in parameters wound length, neutrophil, macrophage, fibroblast and collagen density. P value below 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The wound healing involves some stages in the process. The acute inflammatory phase described during the third day of healing dan proliferation phase described start on the seventh days of wound healing. The inflammatory phase is characterized by granulation tissue formation, such as fibroblasts and inflammatory cells dominated by macrophages, the process takes place together with the formation of new blood capillaries embedded in extracellular loose tissue from the collagen matrix. The number of macrophage increased from third day to seventh day (Gurtner, 2007)

Characterization results of snakehead fish extract were shown in Table II. The content of albumin from snakehead fish in this study was 8,401% (w/w). In the inflammatory phase of wound healing, albumin acts to regulate osmotic pressure in the blood to 50% of plasma protein. When the skin is injured, an inflammatory reaction will occur due to a foreign object outside the body that infects an open wound. Foreign objects trigger a disturbance of hydrostatic pressure so that intracellular fluid will enter the cell due to an imbalance of concentration inside and outside the cell through an osmotic mechanism, which causes edema cells. In these conditions, albumin is needed so that edema does not worsen and the wound healing process can take place during the proliferation stage. Albumin plays a role in the process of developing granulation tissue, one of which is fibroblasts and collagen formation processes and the strength of collagen which can affect the level and quality of wound healing (Irwanda *et al.*, 2015).

In this study, snakehead fish contains total protein 9.950 g/100 ml, Zn 4.917 mcg/100 g, Cu 4.669 mcg/100 ml and Mn 1.796 mcg/100 ml more higher than other research by Mustafa *et al.* (2013), the snakehead fish extract contains Cu 0.01±0.001 mg/100 ml, , Zn 0.41±0.010 mg/100 ml, and total protein 5,524±0.020 g/100 ml.

Albumin synergizes with Zn for cell development and the formation of new cell tissue. Zn involved in the hemostatic process which interacts with platelets plays a role for antibody production and immune cell function. Zn plays an important role in the proliferation process of inflammatory cells and modulates inflammation to limit membrane damage caused by free radicals

Table III. Evaluation of Snakehead Fish Extract Emulgel

Characteristics	Snakehead fish extract emulgel
Colours and odor	White and slightly odor
Homogenity	Homogen
pH	5,41 ± 0,2
Spreadability	5,0 – 6,1 cm
Viskosity	191.000 ± 300cps

Table IV. Parameters of incision wounds healing

Parameters	Formulas	Day 3	Day 7
Wound length (cm)	Control (-)	$1{,}113^{b} \pm 0{,}091$	$0{,}05^{a} \pm 0{,}09$
	Control (+)	$1{,}213^{b} \pm 0{,}150$	$0{,}00^{a} \pm 0{,}00$
	Snakehead fish extract emulgel	$0{,}81^{a} \pm 0{,}17$	$0{,}00^{a} \pm 0{,}00$
Number of neutrophil	Control (-)	$5{,}74^{a} \pm 1{,}68$	$1{,}40^{a} \pm 0{,}62$
	Control (+)	$6{,}67^{a} \pm 1{,}86$	$4{,}33^{b} \pm 0{,}58$
	Snakehead fish extract emulgel	$6{,}99^{a} \pm 2{,}89$	$4{,}83^{b} \pm 1{,}57$
Number of macrophage	Control (-)	$0{,}82^{a} \pm 0{,}43$	$6{,}24^{a} \pm 1{,}299$
	Control (+)	$2{,}09^{a} \pm 1{,}37$	$5{,}99^{a} \pm 0{,}32$
	Snakehead fish extract emulgel	$1{,}57^{a} \pm 0{,}67$	$14{,}15^{b} \pm 3{,}67$
Number of fibroblast	Control (-)	$10.66^{c} \pm 0.87$	$8.67^{b} \pm 0.00$
	Control (+)	$17.89^{a} \pm 1.35$	$11.22^{a} \pm 0.95$
	Snakehead fish extract emulgel	$12.89^{b} \pm 0.38$	$9.22^{a} \pm 1.64$
Density of collagen	Control (-)	$245.57^{a} \pm 15.46$	$232.89^{b} \pm 2.55$
	Control (+)	$247.72^{a} \pm 1.90$	$246.27^{a} \pm 3.32$
	Snakehead fish extract emulgel	$247.74^{a} \pm 1.03$	$247.52^{a} \pm 1.957$

*Superscribe mean of significantly different ($p<0{,}05$)

during inflammation. During the proliferation and maturation phase, Zn is needed for collagen synthesis. The Zn element is also needed for the proliferation of fibroblasts and keratin cells and to accelerate the process of re-epitalization (Mustafa *et al.*, 2012)

Results of the characterization of Snakehead fish extract emulgel were shown in Table III. Based on this evaluation, the emulgel is appropriate so it can be used for this research. Compare to the previous study by Sinambela *et al*. (2012), emulgel preparation give the more slightly odor than ointment preparation because water was an outer phase. Unfortunately, our study using different parameters with the previous study to explain the effectivity, so it not be compared.

The result of parameters of incision wounds healing is presented in Table IV and Figure 1. After third day, snakehead fish extract had the smallest wound length with a significant difference ($p<0{,}05$) compare to negative and positive control, but after seventh day had no significant difference.

The number of neutrophil cells on the third day in all treatment groups did not have a significant difference ($p>0.05$), whereas on the seventh-day snakehead fish extract had no significant difference with positive controls, but had a significant difference with the negative control. The number of macrophage n the third day in all treatment groups did not have a significant difference ($p>0.05$), while on the seventh day, Snakehead fish extract was given a much greater than either the positive or negative control.

In this study, the number of neutrophil cells decreased while the number of macrophage cells increased from third to the seventh day. Of the three treatment groups, the number of neutrophil cells decreased along with the healing process of the wound due to several inflammatory mediators that had been released by neutrophils such as histamine lysozyme enzymes and platelet activation factors. This shows that neutrophil cells did their task as defense cells only at the beginning of post-injury because their task would be replaced by macrophage cells as the second cellular defense cell. Macrophages are one of the main phagocyte cell types which have a longer life force compared to neutrophils. Phagocytosis by macrophages on dead cells is one way to remove damaged cells. The existence of macrophage and neutrophil cells are interconnected in the process of wound healing. Neutrophil cells are the first cellular defense in

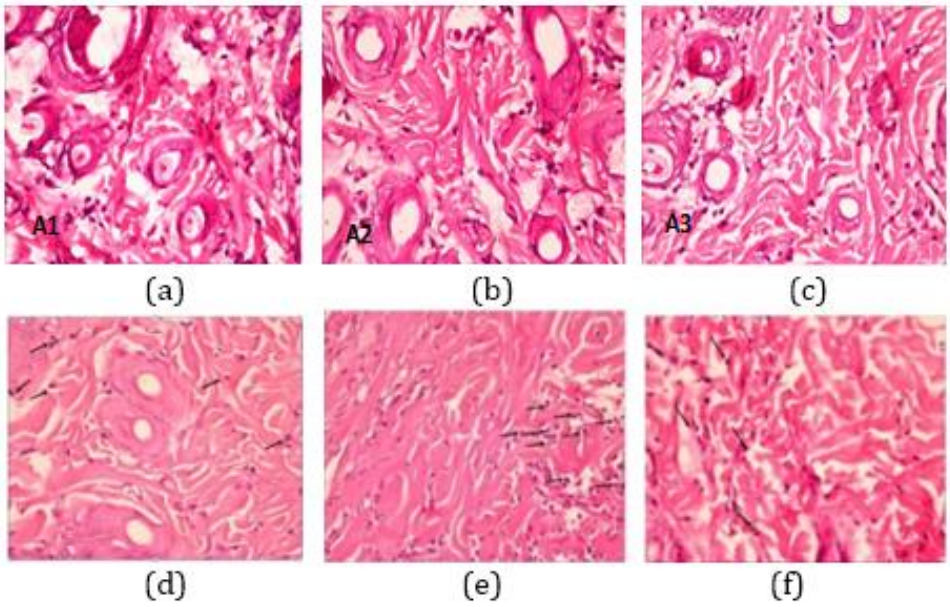

Figure 1. Microscopic observations of (a) third day of control negative, (b) third day of control positive, (c) third day of snakehead fish extract emulgel, (d) seventh day of control negative, (e) seventh day of control positive, (f) seventh day of snakehead fish extract emulgel.

which numbers will increase at the beginning of post-injury. Foreign objects that are not having phagocytosis by neutrophil cells will be forwarded by macrophage cells as the second cellular defense. Macrophages have the ability to phagocytosis which is greater than neutrophils, even capable of performing phagocytosis of 100 bacteria (Guyton and Hall, 2006). Active macrophages, in addition to performing phagocyte functions, also release some active ingredients that are important for the inflammation and wound repair process. Active ingredients released by macrophages are plasma proteins, *platelet activating factor* (PAF), chemotactic factors, cytokines, and growth factors, therefore the presence of high macrophages in the inflammatory phase will increase more growth factors in which also increase the number of new cells and faster formation of granulation tissue so that the wound healing process will run faster (Martini *et al.*, 2012).

The number of fibroblast cells on the third day, the three groups treatments had a significant difference ($p<0.05$), with the highest number of fibroblasts in the positive control group, snakehead fish extract emulgel, and negative controls respectively. On the seventh day, there was no significant difference between the positive control and the snakehead fish extract emulgel group ($p>0.05$) but had significant differences with negative controls.

The number of fibroblast cells in each group to mark the progress of wound healing was through the formation of granulation tissue. Granulation tissue in the wound healing process was formed on the third day to replace the provisional blood clot matrix. Fibroblasts were originated from stem cells found in skin lamina propia which underwent a process of migration and proliferation where transforming growth factor-β (TGF-β), basic fibroblast growth factor (bFGF), platelets, and macrophages infiltrate cytokines and growth factors in the process. The secretion of bFGF macrophages and fibroblasts increased on days 7 to 14 days after injury in line with the number of fibroblasts that increased in that period (Yun *et al.*, 2010).

Collagen density on the third day showed no significant difference between the three groups ($p>0.05$), whereas on the seventh day there was no significant difference between the snakehead fish extract emulgel and the positive control ($p<0.05$) but both differ significantly from negative controls. This finding showed that snakehead fish extract emulgel had an equal effect on wound healing with positive controls.

Collagen is the main protein component to compile extracellular matrix components and is the highest protein found in the human body. In the maturation phase, albumin serves as the main ingredient through the body's catabolic remodeling to form collagen. Collagen is composed of a triple helix from the α-polypeptide chain. The forming of collagen fibers is mediated by IL-4 (Inter Leukin–*4*) from macrophage cells and was

seen on day 3 on the edge of the wound after injury. The proliferation process of collagen fibers is initiated by fibroblasts stimulated by TGF-β from macrophage cells and fibroblasts themselves, especially collagen which is type III in the form of fibers. Collagen deposition seen in this phase is arranged randomly. Reshaping the collagen arrangement and cross-linking collagen occurs in the remodeling phase which will provide new tissue strength and density. This remodeling depends on the process of continuous synthesis and collagen catabolism (Johnston and Gillis, 2017).

CONCLUSION

Snakehead fish (*Canna striata*) extract emulgel can accelerate wound healing: increase the number of neutrophil cells and macrophages, increase the average number of fibroblasts and increase collagen density in incision wounds with experimental white rats. In general, the Snakehead fish extract emulgel has no significant difference compare to the 10% povidone-iodine and significantly different from the negative control.

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