

## ANTI INFLAMMATORY EFFECT OF TOMAN FISH (*Channa micropeltes*) EXTRACT IN WOUND HEALING PROCESS

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**Abstract:** *One phase of wound healing is the inflammatory phase. Haruan fish extract has shown to accelerate the healing process up because it has anti-inflammatory effects. Toman fish originates from the same genus as the Haruan fish, but its anti-inflammatory effect remains unknown. It was done to analyzed anti-inflammatory effects of Toman fish extract. This study was true experimental design with posttest-only control design. Twenty-seven male mice of Balb-C strain were divided into 3 groups. Incision wound of 1 cm was made along the back of the mice. Nine mice in each group were sacrificed on 3<sup>rd</sup> days, then histopathology examination was conducted with Haematoxylin eosin. There were significant differences between treatment group given Toman fish extract and positive control group given Haruan fish extract, and also compared to the negative control group in PMN cell examination. There were significant differences between treatment group given Toman fish extract and positive control group given Haruan fish extract compared to negative controls on the number of macrophage cells. Toman fish extract could lower the number of PMN cells and was able to increase the number of macrophages on the 3<sup>rd</sup> days. Toman fish has anti-inflammatory effects on the wound healing process.*

**Keywords:** *Anti-inflammatory effects, haruan fish extract, macrophages, PMN cells, toman fish extract*

## INTRODUCTION

Wound healing is a tissue response to injury, which consists of regeneration and setting of fibrosis tissue. The wound healing is very important to maintain the normal structure, the function and the survival of organisms. The wound healing is generally divided into several phases, namely inflammation, granulation tissue formation and re-epitelitation, wound contraction, formation of extra cellular matrix (ECM), and *remodeling*. The inflammatory stage is divided into two groups namely acute and chronic inflammations. Acute inflammatory process involves PMN cells (Polymorphonuclear Neutrophil) including neutrophil cells. Meanwhile, chronic inflammation is characterized by the presence of mononuclear cells, namely macrophages, lymphocytes and plasma cells.<sup>1</sup> Therapy administration to accelerate wound healing comes from both commercial drug and natural materials. One of the natural ingredients that can be used to heal wound is by eating a certain fish. Some fish have been proven to possess ingredients accelerating wound healing, i.e. *Haruan* fish (*Channa striata*).<sup>2,3</sup>

Haruan fish (*Channa striata*) is a freshwater fish originating from Kalimantan. Due to its natural habitat in the swamps, reservoirs, and rivers, it can even live in dirty water with low oxygen levels. Haruan fish protein content is higher than other food commonly known as a protein source.<sup>4</sup> Haruan fish collagen protein is also lower than the flesh of cattle, ranging between 3-5 percent of the total protein. Another advantage of Haruan fish protein is rich in albumin, a protein that makes up a half of total body protein that is equal to 62.24 g/kg. The main role of albumin in the body is to compose the structures of the cells, antibodies, enzymes, even hormones. Haruan fish is also consumed by people of the post-circumcision incision.<sup>5</sup>

Haruan fish extract contains essential compounds for the synthesis of tissue, such as albumin, zinc (Zn), copper (Cu), and iron (Fe). Albumin in Haruan fish has been proven to maintain healthy liver from damage caused by excessive consumption of paracetamol. Previous research showed that the Haruan fish extracts have anti-inflammatory effects by decreasing the number of PMN cells and increasing macrophage cells in wound healing on the 3<sup>rd</sup> day.<sup>6-9</sup>

Another commonly eaten fish among society to accelerate wound healing is Toman fish (*Channa Micropeltes*) or Giant Snakehead. Toman fish originates from Indonesia, especially Kalimantan. Toman fish has the same genus as the Haruan fish. Both Toman and Haruan fish are often consumed as a side dish by the people of South Kalimantan. Haruan fish is even recommended for consumption by post-operative patients such as mothers after childbirth, because the fish has an ability to accelerate post-operative wound healing.<sup>10-12</sup>

Based on the fore mentioned explanation, yet there is no research on the effects of Toman fish in the inflammation phase during the wound healing process. Based on assumption that both Haruan and Toman fish are classified as the same genus, it is necessary to conduct a research aiming to analyzed the anti-inflammatory effects of Toman (*Channa bicropeltes*) fish extract against PMN as acute inflammatory cells and against macrophages as chronic inflammation in the wound healing process.

## RESEARCH METHODS

The research was conducted in the Laboratory of Veterinary Investigation Centres (BPPV) Regional V Banjarbaru, South Kalimantan, Indonesia. Ethical examination was conducted at the Dentistry Faculty of Lambung Mangkurat University. This type of this research was a *true*

experimental by posttest-only with control design.

Biological materials used in this research were male mice (*Mus musculus*) of Balb-C strain. The total number of test animals per group was determined in this study were 27 mice. The study consisted of three groups, each which consisted of untreated 9 mice with cuts on their back of the as the negative control (P0), 9 mice with cuts on their back which were given. Before treated, the mice were adjusted to laboratory environment for one week.

The chosen Toman and Haruan fish were still alive. The fish weighed about 500 g - 1 kg. Both fish were cleared of their scales and entrails, then the meat was cut into small cross-sectional size with thickness of  $\pm 1$  cm. Toman fish extracts as much as 30 ml/kg body weight. It was given until the 3<sup>rd</sup> day as the treatment (P1), and 9 mice with cuts on their back which were given Haruan fish extracts as much as 30 ml/kg body weight. It was given until the 3<sup>rd</sup> day as the positive control (P2).

The making of Toman and Haruan fish extracts was done through a steaming process. Each of the fish meat was put in a steamer pan and was steamed for 50 minutes on a medium heat. The steamed fish then was pressed to remove their fluid until they were dry. The liquid coming out next was filtered and precipitated for about six minutes. The liquid would be divided into two layers. The liquid from the bottom layer, which was the fish extract, was taken.

The mice were divided into three groups, then they were measured on the back to perform treatment. The sedative action was done using diazepam until the mice were asleep. Aseptic action was carried out on the biopsy area with 10% Povidone iodine. Then 1 cm long incision wound was made on the back using a sterile scalpel. The emerged blood was cleaned with gauze and

sterile distilled water. This treatment was given for 3 days.

On the 3<sup>rd</sup> day all of the mice in each group were decapitated to see the number of PMN cells and macrophages by doing biopsy on the back area of the incision scar. The tissue fixation was done in 10% formalin solution and made into preparations with *Haematoxylin eosin* (HE) staining. Observations were conducted using light microscopy. Numbers of PMN and macrophage cells per ten view fields were calculated. Observations between the three groups were compared. The yield data were further tested by ANOVA statistical analysis and post Hoc Dunnet T3

## RESULTS AND DISCUSSION

Results of statistical analysis using one way ANOVA and Post Hoc Dunnet T3 indicated a significant difference ( $p < 0.05$ ) between the number of PMN cells in all treatment groups, namely the negative control, Toman and Haruan groups. In Table 1, it appeared that the average value of the highest number of PMN cells was present in the negative control group ( $1.9 \pm 0,43$  cells). The lowest mean number was in the Toman group ( $1.0 \pm 0.00$  cells).

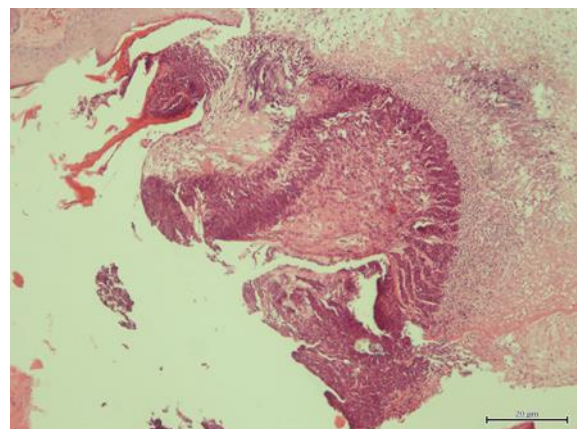


Figure 1. PMN and Macrophage Cells in Wound Healing Process on 3<sup>rd</sup> Days

Table 1. Means and Standard Deviation of PMN and macrophage cells

Groups	Means $\pm$ SD scoring (cell)	
	PMN	Macrophages
Negative control group	1,9 $\pm$ 0,43 <sup>a</sup> (1,5-2,7)	1 $\pm$ 0,00 <sup>a</sup> (1-1)
Toman group	1,0 $\pm$ 0,00 <sup>b</sup> (1-1)	1,4 $\pm$ 0,42 <sup>b</sup> (1-2,1)
Haruan group	1,1 $\pm$ 0,08 <sup>c</sup> (1-1,2)	1,8 $\pm$ 0,35 <sup>bc</sup> (1,4-2,3)

Notes : \* significant with  $\alpha=0,05$ .

<sup>abcd</sup> same superscripts indicated no difference between groups

Based on the total score of macrophage cells, there was a significant difference in the negative control group when compared to treatment group of Toman fish extract and positive control group with Haruan fish extract. In Table 2, there was no significant difference between the number of macrophages in the treatment group of

Toman fish extract and positive control group of Haruan fish extract. The highest average value of score of macrophage cells existed in the Haruan fish extract group (1.8  $\pm$  0.35 cells) and the lowest one was in in the negative control group without treatment (1  $\pm$  0,00 cells).

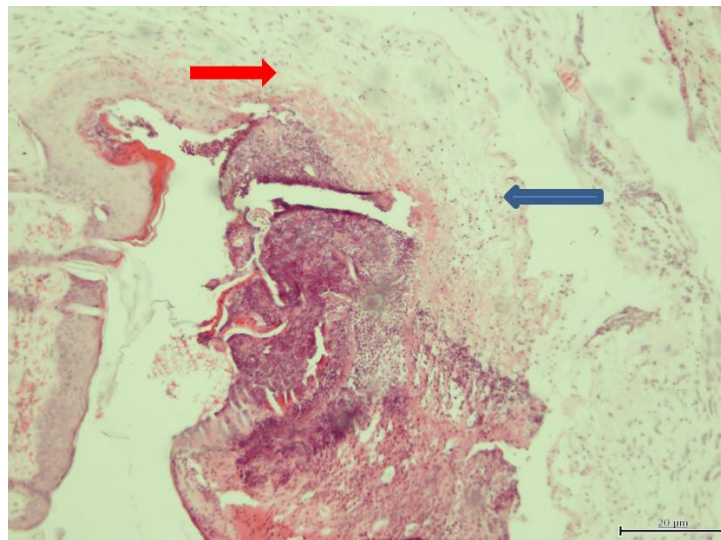


Figure 2. PMN and macrophages cells in wound healing proces on 3<sup>rd</sup> days



 : machrophage cell  
 : PMN cell

Table 2. Significant value of each group

Groups	PMN			Macrophage		
	negative	toman	haruan	negative	Toman	haruan
Negative control group	-	0,001*	0,001*	-	0,049*	0,00*
Toman group	-	-	0,026*	-	-	0,127
Haruan group	-	-	-	-	-	-

Notes : \* significant with  $\alpha=0,05$

Skin wound healing process begins at the inflammation phase serving to address the damage, removing the stimuli which causes the wound, and starting the extra-cellular matrix (ECM) precipitation in injury spot. The inflammatory phase is divided into two stages namely acute and chronic inflammations.<sup>13</sup> Acute inflammatory process involves Polymorphonuclear Neutrophil (PMN) cells. PMN cells reach the maximum number in the first 24-48 hours in the scar tissue. Acute inflammatory phase immediately turns into a chronic inflammatory phase. In the phase of chronic inflammatory, the cells indicate chronic inflammation characterized by the presence of mononuclear cells, including macrophages, lymphocytes and plasma cells. On the 3<sup>rd</sup> day, monocytes are recruited in a large number to replace PMN. The monocytes will be activated into macrophages.<sup>14</sup>

Activated macrophages are capable of presenting antigen and releasing cytokines that can stimulate the growth and function of T lymphocytes. Macrophages also stimulate the process of Re-epithelialization, create granulation tissue and accelerate angiogenesis.<sup>14,15</sup> The activated macrophages release *growth factors* that stimulate the proliferation of fibroblasts, fibroblast migration to the wound spot, and stimulate the synthesis of *Glycosaminoglycans*, *proteoglycan* and collagen to form a new extracellular matrix.<sup>16</sup>

Both Toman and Haruan fish also possess 14 amino acids that are the basic elements for wound healing, i.e. glycine, alanine, leucine, isoleucine, methione, tryptophan, lycine, histidine, oxyproline, tyrosine, theonine, serine, aspartate and glutamate acid. Glycine is one of main components producing human skin collagen. Along with the other essential amino acids such as alanine, proline, arginine, serine, isoleucine and phenyl alanine is a polypeptide which boosts regeneration and tissue healing.<sup>8,16</sup>

This research has proven that Toman fish extract (treatment group) can reduce the number of PMN cells, when compared to the untreated (negative control group) and Haruan fish extract (positive control group). On the 3<sup>rd</sup> day there is a reduction in the number of PMN cells in the acute phase of inflammatory. This means most of the PMN cell functions have been replaced by macrophage cells. It seemed that the number of macrophages in the group treated with Toman fish extract and positive control group treated with Haruan fish extract were more than the number in the negative control group without treatment. This can happen because the Haruan and Toman fish contain high level of fatty and amino acids. One of the fatty acids contained in the fish is arachidonic acid (AA). Hence AA is the precursor of thromboxane and prostaglandin in the wound healing process during the stage of hemostasis.<sup>12</sup> Moreover AA also acts as an activator of neutrophil functional response such as aggregation and adhesion

of leukocytes to the blood vessel's endothelium.<sup>3</sup>

In the process of biochemical reactions, there is AA in the body which produces Leukotriene and prostaglandins serving to cause inflammation. In the process of inflammation, AA is also converted into lipoxin compounds which acts to prevent the occurrence of protractive inflammation. At the same time, PMN secretes chemical mediators as a signal to recruit more neutrophil and leukocyte cells to destroy foreign substances. This process is called phagocytosis. The action of neutrophils should be hindered at some stages, because agents and enzymes released by neutrophils can damage cells and cell tissues. This causes the inflammatory phase to become longer. It occurs when the chemical mediators of (leukotriene) is stopped and switched to the biosynthesis of anti-inflammatory chemical mediators (lipoxins). All of these biosyntheses occur in neutrophil cells. The biosynthesis transition from pro-inflammatory mediator to anti-inflammatory mediator occurs by regulating the 15-LO enzyme (15-Lipooxygenase) contained in neutrophil cells. The ability of the 15-LO enzyme can enzymatically convert from AA which becomes leukotriene, then switched to produce lipoxins. The emergence of neutrophils and the formation of abscess indicate the transition from pro-inflammatory mediators and restrictions as well as the migration prevention of neutrophil cells from the blood vessels into the wound area. Anti-inflammatory mediators (lipoxins, Resolvins, and protections) will further mobilize macrophages and clear the debris of phagocytosis.<sup>16,17</sup>

Lipoxin's function is to block the infiltration of neutrophil or PMN cells heading to excessive inflammation, so that the inflammatory phase can be prevented timely and unsustainable which could

jeopardize the normal work process of cells and cell tissues. The process of returning to normal where the blood vessel permeability can be maintained against the neutrophil secretion from blood vessels is called homeostasis. Lipoxins involve in the process of recruiting mononuclear cells (monocytes) derived from the blood vessels, and then they change their function as macrophages which consume PMN cells. This process ends the inflammatory phase.<sup>14,17,18,19</sup>

## CONCLUSION

The study has shown that the administration of Toman fish extract can reduce the number of PMN cells in acute inflammation so that it is lower than the administration of Haruan fish extract. Administration of Toman fish extract is able to increase the number of macrophage cells to be equivalent to the administration of Haruan fish extract. It can be seen from the histopathology description of the wound healing process on the 3<sup>rd</sup> day (Figure 1 and Figure 2). It can be concluded that Toman fish has anti-inflammatory effects on the wound healing process. This is essential so that there will not be excessive inflammatory cells, therefore it will accelerate the wound healing process with the presence of the short inflammatory phase.

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