THE EFFECT OF GIANT SNAKEHEAD (*Channa micropeltes*) EXTRACT ON THE NUMBER OF MACROPHAGE IN DIABETES MELITUS WOUND HEALING
(In Vivo Study on the Back of Male Wistar Rat (*Rattus novergicus*)

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Abstract

**Background:** Giant snakehead contains albumin and omega-6 fatty acids which play an important role in diabetes mellitus wound healing. Wounds in people with diabetes mellitus have low level of albumin, thus inhibiting the macrophage function. **Objective:** To prove the effect of giant snakehead extract of 16mL/Kg dose of rats’ weight toward the number of macrophage on wistar rats’ back wounds with diabetes mellitus in day 4, 8, and 14. **Method and Materials:** This study applied pure experimental method with posttest-only control design. This study 36 wistar rats as sample which divided into 9 groups; group of giant snakehead extract with 16mL/Kg dose of rats’ weight, positive control group using haruan fish extract with 13.54mL/Kg dose of rats’ weight, and negative control group using BR2 feed. **Results:** The test result of One-way ANOVA on day 4 (p=0.022), day 8 (p=0.251) and day 14 (0.028). The test result of Post-hoc LSD showed that there were no significant differences on day 4 and 14 (p>0.05) between the groups of the giant snakehead extract and haruan fish extract. There was significant difference (p<0.05) between groups of the giant snakehead extract and BR2 feed. **Conclusion:** It can be concluded that giant snakehead extract at 16 mL / kg dose administered orally may increase the number of macrophage in wistar rat (*Rattus novergicus*) induced diabetes mellitus back wound on day 4 and decreased macrophage cell count on day 8 and day 14.

Keywords: diabetes mellitus wound healing, the number of macrophage, giant snakehead extract,

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INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by increased blood glucose levels (hyperglycemia) resulting from impaired insulin secretion, insulin work or both.¹ Basic Health Research 2013 states that the prevalence of diabetes mellitus in Indonesia reaches 12 million in 2013 and reaches 38,113 in South Kalimantan.² Diabetes mellitus has manifestations in the oral cavity such as gingivitis, periodontitis, xerostomia and slow wound healing.³, ⁴, ⁵ Slow wound healing is usually overcome by the South Kalimantan community by consuming haruan fish and giant snakehead which is believed to help the acceleration of wound healing process.⁶, ⁷ Giant snakehead extract with a dose of 16 mL / kg BW has been proven to accelerate wound healing in people without systemic disease.⁸ Giant snakehead is in one family with fish haruan. Haruan fish contains albumin and omega-6.⁹ Empirically, the patent drugs containing extracts haruan fish with albumin content has been circulating in the community.¹⁰ According to Firlianty et al (2013), the content of albumin in haruan fish is 4.53% which is equivalent to 13.45 mL / Kg of rat. Albumin in haruan fish serves as an antioxidant that has been proven to accelerate wound healing in people with diabetes mellitus.¹⁷, ²⁹ It has been proven that haruan fish extract can accelerate wound healing diabetes mellitus for 8 days.²⁹, ³⁶
The haruan fish and giant snakehead have the same content of albumin and omega-6.21 Albumin acts as a natural antioxidant that has many clusters (-SH) and plays an important role in cleaning and catching Reactive Oxygen species (ROS) in hyperglycemia.9,10,11,12 Omega-6 fatty acids which are also the content of giant snakehead play an important role in increasing the number of macrophage.13 Macrophage works as bacterial phagocytes in the inflammatory phase. In the inflammatory phase, the number of macrophage peaked on the 3rd day after the injury, whereas in the wound of diabetic macrophage cell began to decline on day 4.4,7 Macrophages function as a professional phagocytes and Antigen-presenting cell (APC) and stimulate Growth factor. The high number of macrophage cells indicates the presence of higher phagocytosis processes, so that wound healing process. Macrophages in diabetes mellitus is still very limited.

Diabetes mellitus wound take longer time to heal than normal wound because diabetes mellitus injury increased ROS.15,18,19 Reactive Oxygen Species can be neutralized by albumin contained in giant snakehead. Reactive Oxygen Species that have been neutralized can help macrophages in the process of phagocytosis.9,10,20 Another content of giant snakehead is omega-6 fatty acid which has arachidonic acid (AA) derivative. Arachidonic acid can produce leukotriene and prostaglandin.13,21 Prostaglandins are tissue product that arise when the inflammatory reaction plays a role to activate the macrophage work system.7,10 Macrophages are one of the cells that play an important role in the wound healing process. Macrophages function as a professional phagocytosis and Antigen-presenting cell (APC) and stimulate Growth factor. The high number of macrophage cells indicates the presence of higher phagocytosis processes, so that wound cleansing runs faster and accelerates wound healing.16,20,23

The results of research that supports the use of giant snakehead extract to accelerate wound healing in diabetes mellitus is still very limited. Research on wound healing has been widely practiced, but the effect of giant snakehead (Channa micropeltes) extract on macrophage cell numbers on wistar ratback wound with diabetes mellitus does not yet exist. Based on the description above, it is necessary to do research to determine the effect of giant snakehead extract (Channa micropeltes) with the dose of 16 mL / Kg BW orally on the number of macrophage in the wistar back wound with diabetes mellitus on the 4th, 8th and 14th day in vivo.

The general objective of this study isto demonstrate the effect of giant snakehead extract dose 16 mL / kg BW orally on the number of macrophage cells in wistar rat (Rattus norvegicus) induced diabetes mellitus on days 4, 8 and 14.

MATERIALS AND METHODS

The implementation of the research begins with taking care of the research permit and ethical clearance issued by the Ethics Committee of Faculty of Dentistry, Lambung Mangkurat no. 037 / KE PKG-FKGULM / EC / IX / 2017. This research is a true experimental research with design with complete randomized posttest-only control group design. The population of this study were wistar rat weigh of 250-300 grams, age 2 - 3 months and healthy conditions (active and have a good appetite). The researchers used experimental animals divided into 9 groups: 3 groups of negative control were diabetic rat fed with BR2 orally, 3 treatment groups were given BR2 feed plus giant snakehead extract of 16 mL / kg Kg orally in rat with diabetes mellitus and 3 positive control groups were given BR2 rat feed plus haruan fish extract 13,54 mL / kg Kg orally in rat with diabetes mellitus. Each group consisted of 4 rats sacrificed on the 4th, 8th and 14th day.

This research procedure began with the sampling of giant snakehead or haruan fish. Giant snakehead or haruan fish was collected from Traditional Market. Giant snakehead or haruan fish used in this study had total weight of 11 kg. The used portion was giant snakehead or haruan fish flesh. Each sample of giant snakehead or haruan fish that will be extracted, will be cleaned by removing its head, entrails and disposed its scale. The flesh then weigh to 9,84 kg. The flesh was put into a container and steamed in a pan for ± 30 minutes, then 750 mL pale yellow liquid that cameout of the flesh were taken and disparted. Giant snakehead or haruan fish flesh then wrapped with flannel cloth and put in a hydraulic press tool for pressing. The resulted giant snakehead or the haruan fish extract is generated to the test tube. As much as 7,5 mL resulted extract were put in the test tube and centrifuged for 15 minutes at a speed of 6000 rpm. Centrifugation results obtained 700 mL of fluid and 50 mL of sediment separated. Giant snakehead or haruan fish extracts are stored in dark glass bottles and covered with aluminum foil and clean pack.

The diabetic mouse model was obtained by injecting streptozotocin (STZ) in ratat a dose of 35 mg / kg. Rat were fed and then examined after day
7. Glucose level in rats were measured by glucometers before and after being given STZ. Rats declared to have diabetes when blood glucose levels ≥ 126 mg/dL. The physical condition of rats with diabetes appears lethargic, lean with inactive movement. Treatment in rats was started by adapting rats for 1 week in laboratory atmosphere, then divided into 9 treatment groups with 36 samples of rats and numbered according to its group. Rats were taken and were given sedative using diethyl ether. The cut was made by 1 cm long and 2 mm deep on the back of the rat using a sterile scalpel, the blood that comes out was cleaned with Aquadest. The wound on the back of the rat was then bandaged with gauze bandages. Treatment of each rat was given orally to each treatment group using gastric pipe and administered twice daily for 14 days. At day 4, 8 and 14 all rats in each group were sacrificed to see the healing process. The sacrifices were done with inhaled anesthetic using diethyl ether. The observed tissue was taken by biopsy using excisional biopsy technique. The biopsy was performed in the dermis area of the wistar rat back dorsal incision, 1 cm long and 2 mm deep and tissue was soaked in 10% formalin solution. The histopathologic preparation was made and then stained with Haematoxylin Eosin (HE).

RESULTS

The mean results of macrophage cell number on wound healing in diabetes mellitus can be seen in Table 1. Graph of the mean number of macrophage on the wistar rat back for 14 days in each group can be seen in Figure 1.

<table>
<thead>
<tr>
<th>Observation Day</th>
<th>Giant Snakehead Extract</th>
<th>Haruan Fish Extract</th>
<th>Feed Only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>12.25 ±1.70 (10.0-14.0 Cell)</td>
<td>11.0 ±1.63 (9.0-13.0 Cell)</td>
<td>8.5 ± 1.29 (7.0-10.0 Cell)</td>
</tr>
<tr>
<td>Day 8&lt;sup&gt;th&lt;/sup&gt;</td>
<td>10.25 ± 1.70 (8.0-12.0 Cell)</td>
<td>9.0 ± 1.70 (7.0-11.0 Cell)</td>
<td>8.25 ±1.25 (7.0-10.0 Cell)</td>
</tr>
<tr>
<td>Day 14&lt;sup&gt;th&lt;/sup&gt;</td>
<td>6.0 ± 0.82 (5.0-7.0 Cell)</td>
<td>7.25 ±0.95 (6.0-8.0 Cell)</td>
<td>8.0 ± 0.81 (7.0-9.0 Cell)</td>
</tr>
</tbody>
</table>

One-way Anova test results showed on day 4 (p = 0.022), day 8 (p = 0.251) and day 14 (p = 0.028). The result of statistical test of day 4 and 14 (p <0.05) which meant that there was significant difference in the mean of macrophage cell number in treatment of wistar rat wound with diabetes mellitus between giant snakehead extract, haruan fish extract and feed-only group. The result of Least Significance Difference (LSD) test on the 4<sup>th</sup> and 14<sup>th</sup> day showed no significant difference between giant snakehead extract group and haruan fish extract group.
extract group, but there was significant difference between giant snakehead extract group and feed group only. The mean of research result showed that macrophage cell count in giant snakehead extract group was higher than haruan fish extract and feed only group.

Day 8 has $p > 0.05$ which means that there was no significant difference in the mean of macrophage cell count after the treatment of diabetic wistar rat back injury between giant snakehead extract, haruan fish extract and feed only group.

Figure 2 Histopathology of macrophage cell counts in wistar rat back wound on giant snakehead extract (a) haruan fish extract (b) and feed only (c) group on day 4 with 400 × magnification by olympus light microscope.

Figure 3 Histopathology of macrophage cell counts in wistar rat back wound of giant snakehead extract (a) haruan fish extract (b) and feed only (c) group on day 8 with 400 × magnification using the olympus light microscope.

Figure 4 Histopathology of macrophage cell counts in wistar rat backs in giant snakehead extract (a) haruan fish extract (b) and feed only (c) group on day 14 with 400 × magnification using an olympus light microscope.

DISCUSSION

The results showed that giant snakehead extract gave better effect on wound healing of diabetes mellitus than haruan fish extract and feed only group. The result of statistical test on the 4th day of average macrophage number on diabetic wistar rat wound showed that there was no significant difference in giant snakehead extract group, but there was significant difference compared to feed only group. This is because giant snakehead extracts and haruan fish extracts have the same content of albumin and omega 6 fatty acids. Omega-6 fatty acids have a prostaglandin derivative that activate macrophage which increase the inflammatory phase. In the normal inflammatory phase, normal macrophage number reaches its peak on the 3rd day after injury, whereas in diabetic wound the increase of macrophage number occurs on the 4th day. Macrophages act as antigen-presenting cells (APC) and professional phagocytosis. If there is a disturbance, it will result in infection by non-phagocytic pathogens from macrophages thereby increasing the wound healing time. This can be overcome by using giant snakehead extracts. It is
capable in activating and mobilizing macrophage cells in the process of phagocytosis. Other function of macrophages is phagocytosis, which can also synthesize various kinds of mediators. The macrophage-synthesized mediators include growth factor, interleukin, protease, interferon and TNF. The mediator is related to the biological activity of macrophages, namely phagocytosis. Phagocytosis by macrophages is done by cleansing bacteria, damaged tissues and phagocytic remnants of neutrophil cells.  

Macrophage will continue to work until the wound-healing proliferation phase of diabetes mellitus.  

In diabetic wound, there is an increase of Reactive Oxygen Species (ROS) 9 Increased Reactive Oxygen Species cause cell damage that inhibit wound healing. 29 This can be overcome by albumin providing with containing giant snakehead extract and haruan fish extract. Albumin acts as an antioxidant and protein in the body that has a hydride sulfur bond and a thiol group that functions in binding ROS rapidly. 12,22 Albumin works by binding to metal ions involved in ROS formation through copper bonds Cu2+, vanadium ions, cobalt and nickel with high affinity. 10,22 Albumin also plays a role in the termination of oxidation reactions in the process of ROS formation. 30 In addition to cutoxidation reactions and bindmetal ions, albumin as a secondary antioxidant is also capable of destroying ROS which were made from oxidation process capturing oxygen, decomposing hydrogen peroxide intron radical, so ROS can be neutralized. 10,30 Reactive Oxygen Species that have been neutralized by albumin help macrophage in phagocytic process. 9,31,32,33  

The result of statistical test in the study of macrophage number on diabetic wistar rat back injury on the 8th day showed that showed that there was no significant difference between the group given giant snakehead extract, haruan fish extract and feed only. This is because the wound healing process has entered the proliferation phase marked by a decrease in the number of macrophage cells. Research of Mutiara et al (2015) showed that macrophages numbers will increase in the inflammatory phase and will decrease in the proliferation phase. The normal proliferation phase is starting on the 4th day until the 21st day after the injury, whereas in the wound of diabetes mellitus the proliferation phase begins on the 8th day. 27,28 This is due to this is due to the growth factor stimulant disturbance experienced by macrophages in chronic inflammatory phase which resulting in a slower diabetes mellitus wound healing in the proliferative phase. 27,34,35  

On day 14, the number of macrophages decrease in each treatment group. The decrease in the average number of macrophage was highest in the giant snakehead group, this is because the active substance of albumin in giant snakehead extract which is higher than the haruan fish extract. The content of albumin in giant snakehead was 5.35%, while haruan fish have only 4.53% concentration. 17  

Albumin is able to decrease the number of macrophage by increasing T lymphocyte cell activation. T lymphocyte cells are capable of triggering cytokines that can inhibit the function of effector cells directly such as TGF beta, II.10 and IL 35 produced by macrophages. In addition, T reg lymphocyte activation serves as a cytotoxic cell that secretes cell granymes, granymes a and b as well as several other types of direct apoptotic effector cells. 27,34,35 Macrophages produce fibroblasts in conjunction with lymphocytes. Fibroblasts play a role in the formation of tissue and produce large amounts of collagen so that the wound can quickly encloses. 14,16 In this study, macrophages were still observed on the 14th day of diabetes mellitus wound healing. This is in line with the study of Mutiara et al, (2015) which states that the macrophages were still discovered on day 12 and began to gradually decline by day 14 in diabetes mellitus wounds. 28 It can be concluded that giant snakehead extract at 16 mL / kg dose administered orally may increase the number of macrophage in wistar rat (Rattus novergicus) induced diabetes mellitus back on day 4 and decreased macrophage count at day 8 and day 14.  

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