CLINICAL ANALYSIS OF CHANNA MICROPELTES FOR TREATING WOUND OF DIABETES MELLITUS

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CLINICAL ANALYSIS OF *CHANNA MICROPELTES* FOR TREATING WOUND OF DIABETES MELLITUS

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INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders of carbohydrate, lipid and protein characterized by hyperglycemia which occurs due to insulin secretion disturbances or tissue sensitivity decrease to insulin.[1,2] The estimated prevalence of DM in Indonesia in 2030 is 21.3 million people and it will make Indonesia as the forth highest in the world.[3] DM patients tend to have chronic wound with prolonged wound healing process due to uncontrolled hyperglycemia.[4,5] South Kalimantan communities often consume *toman* fish and *haruan* fish which can accelerate wound healing proces.[6,7] *Toman* fish extract has been proven to accelerate normal wound healing process on the back of a rat at 16 mL/kg BW dosage.[8]

Toman fish contains high albumin (approximately 5.35 g/dL) and fatty acid, especially omega-6.[9,10] *Toman* fish contains high albumin protein which can be used as an alternative of Human Serum Albumin (HAS). It is an affordable source of albumin with an easy processing.[6,11] Omega-6 fatty acid also contained in *Toman* fish extract has a derivate in the form of arachidonic acid (AA) which plays a role in inflammatory phase.[10-13] *Toman* fish extract has been proven to accelerate DM wound closure on the back of a rat on the eleventh day.[14]

Toman fish belongs to the same family with *haruan* fish and has the same contents which are fatty acid and albumin.[9,15] Albumin content in *haruan* fish extract is approximately 4.53%, it is equal 13.54 mL/kg BW dosage.[9] Empirically, capsulated *haruan* fish extract has been widely distributed in the community and developed as a patent drug.[16] *Haruan* fish extract can accelerate the normal wound contraction and diabetic wound.[17,18]

Diabetes melitus (DM) increases the formation of Reactive Oxygen Species (ROS).[19] The increased number of ROS should be bound by antioxidant like albumin, which is contained in *Toman* fish extract.[9,20,21] Reactive Oxygen Species (ROS) which has been neutralized by albumin can accelerate wound healing.[22] Diabetes melitus (DM) wound healing consists of several phases, including inflammatory, proliferation, and maturation phases.[23] Acute inflammatory phase occurs on the second day and chronic inflammatory phase occurs during the forth to the eight day.[24] Omega-6 fatty acid which has a chemical mediator in the form of prostaglandin and lipoxin, also plays a role in inflammatory phase.[10,12,13] After inflammatory phase ends, proliferation phase occurs.[25]

Proliferation phase begins from the eight day, in which fibroblast proliferation, neovascularization and reepithelization occur.[24,26] Diabetes Melitus (DM) wound healing process is continued with maturation phase, in which Extracellular Matrix (ECM) synthesis and wound closure occur on the fourteenth day.[24-25]

According to the background above, the number of studies supporting the use of 16 mL/kg BW *toman* fish extract on DM wound healing is still limited. This study is aimed to prove the effect of Toman fish extract at 16 mL/kg BW oral dosage on wound length and contraction on the back of wistar rat with DM for 14 days, observed clinically.

RESEARCH METHODS

This study began by managing research permit and ethical clearance No.023/KEPKG/FKGULM/EC/VIII/2017 which was issued by The Ethic Committee of Faculty of Dentistry, Lambung Mangkurat University. This study is a true experimental study with post-test only control group design.

The sample inclusion criteria in this study were male rat, aged 2-3 months, weighed 200-250 grams and in healthy condition (active and has good appetite). Exclusion criteria include dead rat, abnormal rat (wounded or disabled), unhealthy condition (weak, has no appetite, and inactive) and has more than 10% of

weight loss after adaptation period in the laboratory. Samples used in this study were 12 male wistar rat (*Rattus norvegicus*). The samples were divided into three groups; one group given BR2 Comfeed and 16 mL/kg BW of *Toman* fish extract, positive control group which was given BR2 Comfeed and 13.54 mL/kg BW of *Haruan* fish extract, and negative control group which was given BR2 Comfeed. Each group was comprised of 4 rats. Treatment was given 2 times daily (with 2 hours interval) using a gastric tube for 14 days.

The first procedure of this study was sampling of *Toman* fish and *Haruan* fish. The fishes were obtained from Martapura Traditional Market, South Kalimantan. *Toman* fish and *Haruan* fish used in this study had a total weight of 11 kg and only the meat was used. Each sample had its head and gut cleaned and descaled, then the meat was weighed at 9.84 kg. The meat was steamed in a pot for ± 30 minutes, until 750 mL of pale-yellow liquid came out from the meat and was set aside. The meat of *Toman* fish and *Haruan* fish was wrapped in flannel for pressing using hydraulic press. Produced *Toman* fish and *Haruan* fish extracts were placed into reaction tubes for 7.5 mL and centrifuged for 15 minutes at 6000 rpm. Centrifugation produced 750 mL of liquid and 50 mL of deposit which were separated to obtain *Toman* fish and *Haruan* fish extract. Those extracts were placed into dark glass bottles which were covered with aluminum foil and clean pack, then stored in the refrigerator. The extracts were taken 4 daily which were added 2.5 mL distilled water to obtain 16 mL/kg BW dose for *Toman* fish extract and 13.54 mL/kg BW dose for *Haruan* fish extract.

The rat in this study were induced with DM using Streptozotocin (STZ) with 35 mg/kg dose. Blood glucose level of the rats were measured before and after 7 days of STZ administration. The rat were iagnosed with DM when the blood glucose level reached ≥ 126 mg dL^{-1}.[27] Wounding of rat's back began by adapting the rat at laboratory environment for 1 week. The hair on the back of rat were shaved with 3 cm diameter and cleansed with 70% ethanol. The rats were given sedative using diethyl ether inhalation at 5 mL dose until the rat fell asleep. The wound was made for 1 cm width and 2 mm depth using sterile scalpel and blade, then the blood was rinsed using distilled water. Incised wound was wrapped with sterile gauze. *Toman* fish and *haruan* fish extracts were given 2 times daily (12 hours interval) using gastric tube for 14 days. After day 14, the rats were returned to the laboratorium used for other studies.

RESULTS AND DISCUSSION

The result of Saphiro-wilk normality test result for wound length and contraction in all groups showed $p > 0.05$, thus data were normally distributed. Levene's homogeneity test for wound length and contraction in all groups showed $p > 0.05$, thus the data were homogenous. Data were normally distributed and homogenous; therefore, One-way ANOVA was performed.

The results of One-way ANOVA for wound length and contraction in all groups showed $p = 0.000$ ($p < 0.05$) which means that there were significant differences between treatment groups. Therefore, post-hoc Least Significant Difference (LSD) was performed. The results of LSD for wound length and contraction showed significant difference between 16 mL/kg BW of *toman* fish extract group and 13.54 mL/kg BW of *haruan* fish extract group with p value = 0.000. There was also significant difference between 16 mL/kg BW of *Toman* fish extract and BR2 Comfeed with p value = 0.000. There was insignificant difference between 13.54 mL/kg BW of *haruan* fish extract and BR2 Comfeed with p value = 0.930.

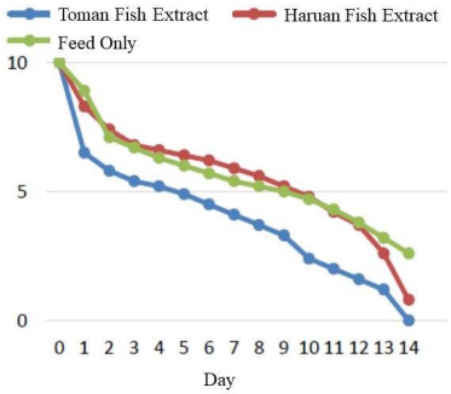
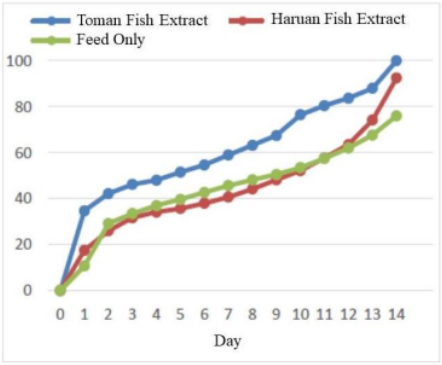


Figure 1. Diagram of Average Wound Length (mm) on the Back of Wistar Rat for 14 Days in Each Group

Wound length on the back of wistar rat were obtained by measuring wound length every day until 14 days using caliper. Wound closure was different in each group for 14 days. The smaller the wound length, the better the wound closure. The fastest wound closure among the three groups in respective order were *toman* fish extract, *haruan* fish extract, and BR2 Comfeed (Figure 1).



Figurer 2. Diagram of Average Wound Contraction (%) on the Back of Wistar Rat for 14 Days in Each Group

Wound contraction value was obtained by inputting the results of wound length in mm to the formula of wound contraction, which is as follows:[28]

$$Wound\ contraction\%: \frac{Initial\ wound - Final\ wound}{Initial\ wound}\ x\ 100\%$$

Wound contraction on the back of wistar rat were different in each group for 14 days. Higher wound contraction means better wound healing. The highest wound contraction value among the three groups in respective order were toman fish extract, haruan fish extract, and BR2 Comfeed, respectively (Figure 2).

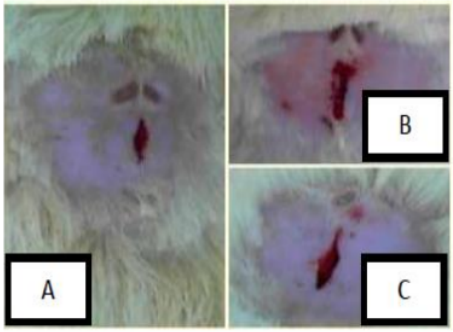


Figure 3. Wound Healing on the Back of Wistar Rat in Toman Fish Extract (A), Haruan Fish Extract (B) and BR2 Comfeed (C) on Day 0.

Clinical cross-section of wound length on the back of wistar rat given toman fish extract, haruan fish extract, and BR2 Comfeed observed on day 0.The groups given toman fish extract, haruan fish extract, and BR2 Comfeed showed wound length of 1 cm wide and 2 mm depth (Figure 3).

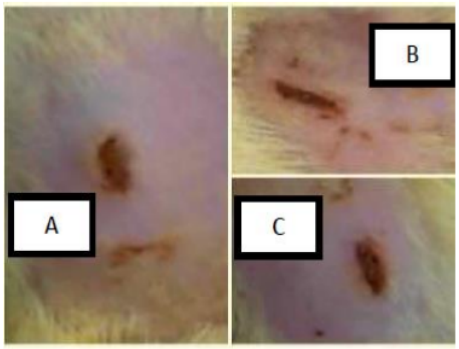


Figure 4. Wound Healing on the Back of Wistar Rat in Toman Fish Extract (A), Haruan Fish Extract (B) and BR2 Comfeed (C) on Day 2.

Clinical cross-section of wound length on the back of wistar rat given Toman fish extract, Haruan fish extract, and BR2 Comfeed observed on day 2. The group given Toman fish extract showed faster wound closure compared to the groups given Haruan fish extract and BR2 Comfeed with average wound length of 5.8 mm. The group given Haruan fish extract showed wound closure of 7.4 mm. The group given BR2 Comfeed also showed wound closure of 7.2 mm (Figure 4).

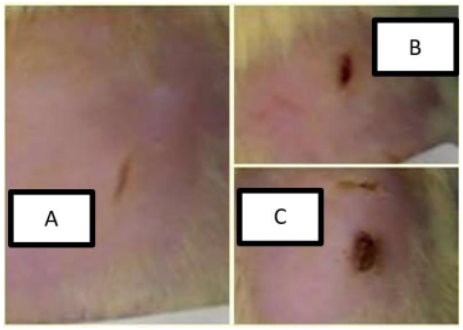


Figure 5. Wound Healing on the Back of Wistar Rat in *Toman* Fish Extract (A), *Haruan* Fish Extract (B) and BR2 Comfeed (C) on Day 8.

Clinical cross-section of wound healing on the back of wistar rat given *toman* fish extract, *haruan* fish extract, and BR2 Comfeed observed on day 8. The group given *toman* fish extract showed faster wound closure compared to the groups given *Haruan* fish extract and BR2 Comfeed with average wound length of 3.7 mm. The picture showed that the group given *Haruan* fish extract had wound closure of 5.6 mm. The group given BR2 Comfeed also showed closure of 5.2 mm (Figure 5).

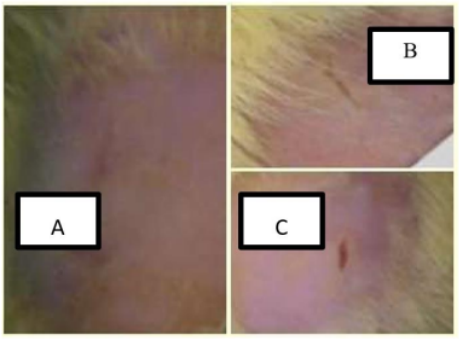


Figure 6. Wound Healing on the Back of Wistar Rat in *Toman* Fish Extract (A), *Haruan* Fish Extract (B) and BR2 Comfeed (C) on Day 14

Clinical cross-section of wound length on the back of wistar rat given *Toman* fish extract, *Haruan* fish extract, and BR2 Comfeed observed on day 14. The group given *Toman* fish extract showed complete wound closure on day 14. The group given *Haruan* fish extract showed incomplete wound closure on day 14 with average wound length of 0.8 mm. The group given BR2 Comfeed also showed incomplete wound closure on day 14 with average wound length of 2.6 mm (Figure 6).

The results of this study revealed that *toman* fish extract had an effect to the length and contraction of diabetic wound on the back of the rats. The effect demonstrated by the administration of toman fish extract was faster when compared to *haruan* fish extract and BR2 Comfeed. The group given *Toman* fish extract had a faster healing time compared to *Haruan* fish because of a difference in the content of omega-6 fatty acid and albumin between the two extracts.[29,30] According to Ngui et al (2017), the

content of omega-6 fatty acid in *toman* fish was 7.2 mg higher than *haruan* fish which contained only 3.7 mg. Omega-6 fatty acid has a derivate in the form of arachidonic acid (AA) and chemical mediators of lipoxin and prostaglandin.[12,31]

The content of albumin is also different between both extracts.[29] According to Firlianty et al (2013), the content of albumin in *toman* fish extract was 5.35% while the content of albumin in *haruan* fish extract was only 4.53%. Albumin in both *toman* fish and *haruan* fish extracts acts as antioxidant, which is important in diabetic wound healing process.[14-15,20,32]

Toman fish extract group also had faster wound healing process compared to BR2 Comfeed group due to albumin and fatty acid content in *toman* fish extract which has potential in accelerating DM wound healing process.[14,29] The results of this study was in line with Murdani et al (2016) who claimed that rat, which induced with STZ and then given *toman* fish extract per orally, had faster wound healing effect compared to STZ induced group that was only given distilled water.

Toman fish extract plays a role in DM wound healing process.[14] Wounds in DM patients are identic with chronic wound with prolonged healing time due to hyperglycemic condition.[4-5] Hyperglycemia will escalate ROS level.[33] Increased ROS should be neutralized by antioxidants.[21] The effect of antioxidant can be found in albumin contained in *toman* fish extract.[9,20] Albumin as a protein in the body has sulfhydryl group and thiol compound which quickly binds ROS.[21,34-35] Albumin decreases ROS by cutting chained oxidative reaction in the process of ROS formation.[36]

Albumin can also binds metal ions involved in the formation of ROS through Cu^{2+}, vanadium, cobalt and nickel bond with high affinity.[21,37] Albumin as secondary antioxidant can also catch oxygen, processing hydrogen peroxide into non-radical compound and eliminating ROS which made from oxidation process.[21,36] Reactive Oxygen Species (ROS) decreased by albumin can accelerate wound healing.[22] The results of this study was in accordance with Nicodemus et al (2014) and Murdani et al (2016) which stated that *toman* fish extract with albumin and omega-6 fatty acid may accelerate normal wound healing and DM wound healing on the back of rat.[8,14]

Wound healing phases consist of inflammatory, proliferation, and maturation phase.[38] Acute inflammatory phase occurs on day 2 and chronic inflammatory process on day 4.[24] Inflammatory phase has five cardinal signs, with edema as the most specific macroscopic sign.[39] Edema or swelling is caused by imbalance in fluid inside and outside cells through osmotic path due to foregin object.[40] In this condition, albumin in *toman* fish extract acts to regulate osmotic pressureThis action prevents edema from getting worse.[41,42]

During the inflammatory phase, mediator cells such as neutrophils and macrophages also play a role.[25] Neutrophils acts in phagocytosis of foreign compounds. The phagocytosis should be halted because enzymes released by neutrophils can damage tissue and cells.[12,43,44] This can be prevented by the derivative of omega-6 fatty acid, which is AA by changing leukotriene (pro-inflammatory) to lipoxin (anti-inflammatory) by regulating enzyme 15-LO (15-Lipooxygenase) contained in neutrophils.[12,44-46] Prostaglandin also acts in inflammatory phase to improve the performance of macrophages. An increase in machrophages number showed a transition of wound healing process from inflammatory phase to proliferation phase.[25,47] Wound healing process continues to proliferation phase which occurs on the eight day.[24] Proliferation phase is characterized by the formation of granulation tissue.[38] Formation of granulation tissue is stimulated by growth

factor (GF) such as Epidermal Growth Factor (EGF) secreted by macrophages, platelets, and fibroblasts.48-49 Albumin in toman fish in the process of granulation tissue thus creating matrices for the base of dermis structure. [40,50] Epidermal Growth Factor (EGF) also acts in stimulating migration and proliferation keratinocytes in the process of re-epithelization this phase.[38,48,51] Re-epithelization process requires energy for epithelial proliferation to create new tissue.[52] Albumin acts in the process of energy formation in epithelial cell proliferation process by bringing oxygen and other substances required such as bilirubin, fatty acid, ions, hormones, and drugs.[52-53] After re-epithelization and granulation tissue formation, wound healing process continues to maturation phase.[38]

The main activity in maturation phase is reinforcement of scar tissue and collagen remodeling.[25] Collagen formation is triggered by GF such as fibroblast growth factor (FGF), FGF-2, insulin growth factor (IGF), keratinocyte growth factor (KGF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-α and TGF-β1,2,3 activated by macrophages, fibroblasts, and endothelial cells.[50] The main ingredient of collagen formation is albumin which is formed by the formation of body catabolism, thus collagen can quickly develops and became the main factor in matrix formation.[53,54] Continuous collagen remodeling activity causes collagen fibers, which was previously randomly distributed, to form crossed and aggregated fibers into fibril bundles.[54] Formed fibril bundles cause tissue healing and maximum wound contraction, thus the wound margins will merge and wound closure will occur on day 14.[24,25] It can be concluded that 16 mL/kg BW of *toman* fish extract show a faster accelerating effect in wound length and contraction on the back of wistar rat compared to 13.54 mL/kg BW of *haruan* fish extract and BR2 Comfeed and the wound was healed on day 14.

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