CLINICAL ANALYSIS OF CHANNA MICROPELTES FOR TREATING WOUND OF DIABETES MELLITUS

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Abstract: Toman fish contains albumin and omega-6 fatty acid that are instrumental in the healing process of diabetes mellitus (DM) wound. People with DM usually have a prolonged wound healing process. Albumin controls the osmotic pressure, the development of granulation tissue, gives the energy to re-epithelialisation process and collagen base material. Omega-6 fatty acid interrupts phagocytosis induced by neutrophil cells. It increases the action of macrophage cell, so that Toman fish can be used as an alternative in accelerating the DM wound healing process. The purpose of this research is to prove the effect of Toman fish extract at 16 mL/Kg BW rat dosage per oral on wound length and contraction on the back of wistar rat (Rattus norvegicus) with DM for 14 days. It was the experimental with a posttest only with control group design used 12 wistar rat that were divided into 3 groups; the group of Toman fish extract with dose 16 mL/Kg BW rat, positive control group using Haruan extract with dose 13.54 mL/Kg BW rat and negative control group using Comfeed BR2 feed. One-way ANOVA test result for wound length and contraction (p= 0.000). The result Post-hoc Least Significant Difference test for wound length and contraction shows that there are significant differences between the group of Toman fish extract and Haruan fish extract (p= 0.000). There is also significant difference between the group of Toman fish extract and Comfeed BR2 feed (p= 0.000). There is no significant difference between the group of Haruan fish extract and Comfeed BR2 feed (p= 0.930). The research concluded that Toman fish extract at 16 mL/Kg BW rat dosage affects the length and contraction of diabetic mellitus wound.

Keywords: Channa micropeltes, clinical analysis, diabetes mellitus, wound
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. 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. DM patients tend to have chronic wound with prolonged wound healing process due to uncontrolled hyperglycemia. South Kalimantan communities often consume toman fish and haruan fish which can accelerate wound healing process. Toman fish extract has been proven to accelerate normal wound healing process on the back of a rat at 16 mL/kg BW dosage.

Toman fish contains high albumin (approximately 5.35 g/dL) and fatty acid, especially omega-6. 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. 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. Toman fish extract has been proven to accelerate DM wound closure on the back of a rat on the eleventh day.

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

Diabetes melitus (DM) increases the formation of Reactive Oxygen Species (ROS) which has been neutralized by albumin can accelerate wound healing. Diabetes mellitus (DM) wound healing consists of several phases, including inflammatory, proliferation, and maturation phases. Acute inflammatory phase occurs on the second day and chronic inflammatory phase occurs during the forth to the eight day. Omega-6 fatty acid which has a chemical mediator in the form of prostaglandin and lipoxin, also plays a role in inflammatory phase. After inflammatory phase ends, proliferation phase occurs.

Proliferation phase begins from the eight day, in which fibroblast proliferation, neovascularization and reepithelization occur. 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.

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 diagnosed with DM when the blood glucose level reached ≥ 126 mg dL^-1. 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.
Wound contraction value was obtained by inputting the results of wound length in mm to the formula of wound contraction, which is as follows:

\[
\text{Wound contraction\% } = \frac{\text{Initial wound} - \text{Final wound}}{\text{Initial wound}} \times 100\%
\]

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).

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 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).

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

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.
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).

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).

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 sulphydryl 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 Cu2+, 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 foreign object.40 In this condition, albumin in *toman* fish extract acts to regulate osmotic pressure

This 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-Lipoxygenase) contained in neutrophils.12,44-46 Prostaglandin also acts in inflammatory phase to improve the performance of macrophages. An increase in macrophages 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-epithelialization this phase. 38,48,51 Re-epithelialization 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-epithelialization 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. 33,34 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.

REFERENCES
1. Guyton A.C dan Hall J.E. Fisiologi Kedokteran. Jakarta: EGC; 2007. Hal. 34
2. Arika W.M, Abdirahman Y.A, Mawia M.M, Wambua K.F, Nyamai D.M. Hypoglycemic Effect of Lippia Javanica in Alloxan Induced Diabetic Rat. J Diabetes Metab. 2015; 6(1): 2.
3. Julianto I.G.P, Elfiab U, Sofiana K.D. Pengaruh Pemberian Ekstrak Umbi Bidara Upas (Merremia mammosa (Lour)) Terhadap Proses Penyembuhan Luka dan Kadar Gula Darah pada Tikus Jantan Hiperglikemi. Artikel Ilmiah Hasil Penelitian Mahasiswa.2015; 1(1): 1
4. Soegondo S, Suwondo P, dan Subekti, I. Diagnosis dan Klasifikasi Diabetes Mellitus Terkini. Jakarta: Balai Penerbit FKUI; 2007. Hal. 18.
5. Mansur. Majalah Kesehatan Muslim ‘Diabetes Mellitus’. DI Yogyakarta: Pustaka Muslim; 2013. Hal. 31.
6. Santoso A.H. Potential of snakehead (channa stiata) extract as hepatoprotector on paracetamol induced rat. Bogor: Institute of Agriculture; 2009. Hal. 20-26.
7. Ciptanto S. TOP 10 Ikan Air Tawar. Yogyakarta: Lily Publisher; 2010. Hal. 138-143.
8. Nicodemus, Andrie M, Luliana S. Uji Efek Penyembuhan Luka Sayat Ekstrak Ikan Toman (Channa micropeltes) Secara Oral Pada Tikus Putih Jantan Wistar. Jurnal Mahasiswa Farmasi UNTAN.2014; 1(1): 1-14
9. Firlianty, Eddy S, Happy N, Hardoko, Annasari M. Chemical Composition and Amino Acid Profile of Channidae Collected From Central Kalimantan, Indonesia. IIESE International Journal of Science and Technology (IJSTE). 2013; 2 (4): 25-29.
10. Omar N.M, Nur-shahidatul A.M.D.Y, Nur’aziyah Z, Ahmad M.Z. Bioconversion of ω-Fatty Acid from Giant Snakehead (Channamicropteltes) Fish Oil. Orient. J. Chem. 2014; 30 (3): 1133.
11. Muchtadi. Technique to evaluate protein nutrient value. Bandung: Publisher Alfbeta; 2010. Hlm. 33-35.
12. Serhan C.N. Resolution Phase of Inflammation: Novel Endogenous Anti-Inflammatory and Proresolving Lipid Mediators and Pathways, Annu. Rev. Immunol. 2007; 25 (1): 101–137.
13. Diana F.M. Omega 6. Jurnal Kesehatan Masyarakat. 2012; 7 (1) : 28.
14. Murdani O.J, Andrie M, Wintari. Uji Efek Penyembuhan Luka Sayat Ekstrak Ikan Toman (Channa micropeltes) Secara Oral Pada Tikus Jantan Galuh Wistar Yang Diinduksi Streptozotocin. Jurnal Mahasiswa Farmasi UNTAN. 2016; 1 (1) : 5-6.
15. Akbar J. Potensi dan Tantangan Budi Daya Ikan Rawa (Ikan Hitam dan Ikan Putih) di Kalimantan Selatan. Banjarmasin: Universitas Lambung Mangkurat; 2014. Hal. 77-78.
16. Ghufran H.M dan Kordi K. Panduan Lengkap Memelihara Ikan Air Tawar di Kolam Terpal. Yogyakarta: Lily Publisher; 2009. Hal. 152.
17. Mustafa A, Sajuti H, Permatasari N and Widodo M.A. The Effect of Channa striata Extract on Total Amino Acid, Arginine, and Leucine Concentration in Serum of Streptozotocin Induced Diabetic Rat. International Journal of Science and Technology (IJSTE). 2014; 3 (4): 22-27.
18. Putri R.C.S. dan Agustina W. Pengaruh Pemberian Ekstrak Albumin Ikan Gabus (Channa striata) Topikal Terhadap Percepatan Kontraksi Luka Insisi Pada Tikus Putih (Rattus norvegicus) Strain Wistar. Journal of Nursing Care and Biomolecular.2016; 1(1) : 50.
19. Deindl E dan Christian K. Therapeutic Neovascularization: Quo Vodis?. Netherlands: Springer; 2007. p. 52.
20. Narwadiya S.C, Dhumne U.L, Sahare K.N, Tumane P.M, Meshram V.G, Singh V. Serum Protein Level Changes in Dots Administered Patients of Nagpur District: A Case Study. ASIAN J EXP.BIOL.SCI. 2012; 3 (1) : 251-254.
21. Suhartono E dan Djati M.S. Radikal Bebas dan Intoksikasi Kadmium. Banjarmasin: Pustaka Banua; 2014. Hal. 55-56.
22. Hameedaldeen A, Liu J, Batres A, Graves G.S, Graves D.T. FOXO1, TGF-β regulation and wound healing. Int J Mol Sci. 2014; 15(1): 16257–16269.
23. Handaya A.Y. Tepat dan Jitu Atasi Ulkus Kaki Diabetes. Yogyakarta: Rapha Publishing; 2016. Hal. 29-31.
24. Winarsih W, Winarsih I, Sutardi L.N. Aktivitas Salep Ekstrak Rimpang Kunyit dalam Proses Penyembuhan Luka pada Mencit yang Diinduksi Diabetes Melitus. Jurnal Veteriner. 2012; 13 (1):242-250.
25. Arisanty I.P. Konsep Dasar Manajemen Perawatan Luka. Jakarta: EGC; 2014. Hal. 29-32, 49-54.
26. Apriasari ML, Dachlan YP, Emrawati DS. Effect of musa acuminata stem by immunohistochemistry test in ulcer. Asian J Biochemistry 2016;11(3):135-41
27. Maharani L. Apriasari, Yusfa Ainah, Eka Febrianty and Amy N. Carabelly, 2019. Antioxidant Effect of Channa Micropeltes in Diabetic Wound of Oral Mucosa. International Journal of Pharmacology, 15: 137-143.

28. Wibawani L, Wahyuni E.S dan Utami Y W. Pengaruh Pemberian Ekstrak Etanol Daun Melati (Jasminum sambac L.Ait) Secara Topikal terhadap Peningkatan Kontraksi Luka Bakar Derajat II A Pada Tikus Putih (Rattus norvegicus) Galur Wistar. Majalah Kesehatan UB. 2015; 2(4) : 199.

29. Fisheries and Marine Services of Central Kalimantan Tengah. Fish Resources Profile of Central Kalimantan. Ed. Ke-2. Jakarta: Fisheries Marine Services; 2009. p. 12-13.

30. Ngui W.S.Y, Nur H.H, Nadiah R, Saiful I.Z. Malaysia Snakehead Channa Striatus and Micropeltes : Physico-chemical Properties of Fillet Fish Oil and Water-soluble Extract. Chemical Engineering Transaction. 2017; 56 (1) : 61-66.

31. Collins N dan Sulewski C. Ostomy Wound Management. Netherlands: Springer; 2011. p. 10-13.

32. Mustafa A. M, Aris W, Yohannes K. Albumin and Zinc Content of Snakehead Fish (Channa striata) Extract and Its Role in Health. IESEE International Journal of Science and Technology (IISTE). 2012; 1(2) : 1-8.

33. Yadav D.P, Prakash S, Sharma S, Yadav K. Biochemical Analysis of Peroxynitrite Modified Human Serum Albumin (PN-HAS) in Rheumatoid Arthritis and Type I Diabetes. Ijppr. 2015; 4(2): 193-206.

34. Lord R.S. dan Bralley J.A. Laboratory Evaluations For Integrative And Functional Medicine. Ed. Ke-2. Canada: Metametrix Institute; 2008. p. 520.

35. Halliwel B dan Gutteridge J.M.C. Free Radical in Biology and Medicine. New York: Oxford; 2015. p. 138-143.

36. Sayuti K dan Yennina R. Antioksidan, Alami dan Sintetik. Padang: Andalas University Press; 2015. Hal. 7-38.

37. Suhartono E. Toksisitas Oksigen Reaktif dan Antioksidan di Bidang Kedokteran dan Kesehatan. Yogyakarta: Goyen Publishing; 2016. Hal. 13-82.

38. Sinno H dan Prakash S. Complements And The Wound Healing Cascade : An Update Review. Hindawi Publishing Corporation. 2013; 2013 (1) : 1-3.

39. Stankov S.V. Definition of Inflammation, Cause Of Inflammation and Possible Anti- Inflammatory Strategies. The Open Inflammation Journal. 2012; 5(1) : 1.

40. Irwanda W.F, Andrie M, Luliana S. Uji Efek Penyembuhan Luka Fase Air Ekstrak Ikan Toman (Channa micropeltes) Pada Tikus Putih Jantan Wistar yang Diberi Luka Sayat. Jurnal Mahasiswa Farmasi UNTAN. 2015; 1 (1) : 10.

41. Suriadi. Manajemen Penyembuhan Luka. Pontianak: Stikep Muhammadiyah; 2007. Hal.23-25.

42. Ishida S, Hashimoto I, Seike T, Abe Y, Nakaya Y, Nakanishi H. Serum Albumin Levels Correlate With Inflammation Rather Than Nutrition Supply In Burns Patients : A Retrospective Study. The Journal of Medical Investigation. 2016; 1 (1) : 361.

43. Ganong W.F. Buku Ajar Fisiologi Kedokteran. Ed. Ke-22. Jakarta: EGC; 2013. Hal. 534-536.

44. Rosales C, Demaurex N, Clifford A, Lowell dan Uribe-Querol E. Editorial Neutrophils : Their Role in Innate and Adaptive Immunity . Journal of Immunology Research. 2016; 6 (1) : 1-2.
45. Strandling, S. Gray’s Anatomy. Ed. Ke-41. London: Elsevier; 2016. p. 70.
46. Serhan C.N, Ward P.A dan Gilroy D.W. Fundamental of Inflammation. New York USA: Cambridge University Press; 2010. p. 170.
47. Suhartono E, Triawanti, Yunanto A, Firdaus R.T, Iskandar. Chronic cadmium hepatooxidative in rats:treatment with haruan fish (Channa striata) extract. Amsterdam: Elsevier; 2012. p. 1-5.
48. Pastar I, Stojadinovic O, Yin N.C, Ramírez H, Nusbaum A.G, Sawaya A, dkk. Epitelization in wound healing: a comprehensive review. Adv Wound Care (New Rochelle). 2014; 3 (7) :445–64.
49. Agung S.S, Maksum I.P, Subroto T. Serum Otologus dan human Epidermal Growth Factor (hEGF) Mempercepat Proliferasi dan Migrasi Keratinosit pada Proses Re-Epitelisasi. MKB. 2016; 48(4) : 206.
50. Prasetyono T.O.H, Saputra D.K.A, Permadhi I, Swantari N.M, Hanafi E. 2016. Panduan Klinis Manajemen Luka. EGC, Jakarta. Hal. 3-22.
51. Corderio J.V. dan Jacinto A. 2013 The role of transcription-independent damage signals in the initiation of epithelial wound healing. *Nature Rev Mol Cell Biol*. 14 (4) :249–62.
52. Lilis M dan Marjiyanto. Hubungan Kadar Albumin dengan Penyembuhan Luka Pada Pasien Post Operasi Lapartomy di Ruang Mawar Rumah Sakit Slamet Riyadi Surakarta. Jurnal Ilmiyah. 2013; 1 (1) : 25.
53. Maharani Laillyza Apriasari and Dewi Puspitasari, 2018. Effect of *Channa micropeltes* for Increasing Lymphocyte and Fibroblast Cells in Diabetic Wound Healing. *Journal of Medical Sciences*, 18: 205-210.
54. Mercandetti M, Cohen A. Wound healing, healing and repair. Ed. Ke-1. New Yorki: McGraw-Hill Company; 2014. p.154-159.