Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Jan 18; 10(A):450-455.
https://doi.org/10.3889/oamjms.2022.8179
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Pharmacology*

Effects of *Channa striata* Extract on Albumin Serum and Neutrophil-to-Lymphocyte Ratio in Hyperglycemic Rats with Wound Injury: A Randomized Control Study

Nurpudji Astuti Taslim[1]*, Nur Fitriana[1], Ni Luh Eka Suprapti[1], Caroline Prisilia Marsella[1], Agussalim Bukhari[1], Haerani Rasyid[1,2], Aminuddin Aminuddin[1], Suryani As'ad[1], Andi Makbul Aman[2], Mardiana Madjid[3]

[1]Department of Clinical Nutrition, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia; [2]Department of Internal Medicine, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia; [3]Clinical Nutrition Department, Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia

Edited by: Slavica Hristomanova-Mitkovska
Citation: Taslim NA, Fitriana N, Suprapti NLE, Marsella CP, Bukhari A, Rasyid H, Aminuddin A, As'ad S, Aman AM, Madjid M. Effects of *Channa striata* Extract on Albumin Serum and Neutrophil-to-Lymphocyte Ratio in Hyperglycemic Rats with Wound Injury: A Randomized Control Study. Open Access Maced J Med Sci. 2022 Jan 18;10(A):450-455.https://doi.org/10.3889/oamjms.2022.8179
Keywords: Albumin; *Channa striata*; Hyperglycemia; Neutrophil-to-lymphocyte ratio; Streptozotocin
***Correspondence:** Nurpudji Astuti Taslim, Department of Clinical Nutrition, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia.
E-mail: pudji_taslim@yahoo.com
Received: 05-Dec-2021
Revised: 25-Dec-2021
Accepted: 08-Jan-2022

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Patients with hyperglycemia often experience slow wound healing due to poor circulation. Extract from the striped snakehead fish Channa striata has anti-inflammatory properties and a high albumin level. It has been widely used to accelerate wound healing in a post-operative setting.

AIM: This study evaluated the effect of the C. striata extract Pujimin Plus® on the albumin serum level and neutrophil-to-lymphocyte ratio (NLR) in hyperglycemic rats experiencing wound injury.

METHODS: This randomized controlled experiment was performed in 30 streptozotocin-induced hyperglycemic adult male Wistar rats (*Rattus norvegicus*). The rats were intentionally wounded, and the albumin and NLR levels were evaluated regularly. Overall, 15 rats in the intervention group were given 81 mg/day *C. striata* extract, and 15 rats in the control group received sodium-carboxymethyl cellulose as a placebo for 10 days.

RESULTS: After 10 days of intervention the albumin level in the intervention group was higher than that in the control group (2.66 ± 0.36 vs. 2.46 ± 0.13 g/dL, $p > 0.05$); the intervention group also showed a lower neutrophil level (23.22% vs. 26.98%, $p > 0.05$), a higher lymphocyte level (65.66% vs. 62.16%, $p > 0.05$), and a lower NLR value (0.37 vs. 0.45, $p > 0.05$). None of these results was statistically significant.

CONCLUSION: There was a possible positive effect of *C. striata* extract on albumin serum level and NLR value following wound injury in hyperglycemic rats.

Introduction

Hyperglycemia, a condition in which the blood glucose level increases beyond its normal limit, is one of the early signs of diabetes mellitus. Hyperglycemia could be caused by a failure of insulin production by the pancreas or a failure of insulin utilization by the human body [1], [2]. Hyperglycemic conditions can have an impact on inhibiting vascular homeostasis, increasing oxidative stress, increasing proinflammatory cytokines, and inhibiting angiogenesis, therefore, delaying the wound healing process [3]. Patients with hyperglycemia often experience slow wound healing due to poor circulation [4]. Wound healing is a complex multi-phase process, which comprises an inflammatory phase, a proliferation phase, and a maturation phase. Many nutrients are required for the wound-healing process; however, proteins are crucial as they are needed for cell growth, as components of cell structures and cell membranes, and for the formation of antibodies, hormones, and enzymes [5]. High-protein food is, therefore, important for the wound-healing process.

One of the determinants of disease prognosis which is accessible and sensitive is the neutrophil-to-lymphocyte (NLR) [6]. The NLR describes the absolute neutrophil to absolute lymphocyte counts in the blood, which are greatly affected by systemic inflammation. An NLR value below 4.3 predicted complete wound healing in patients with diabetic ulcers with 63% sensitivity and 71% specificity [7].

Channa striata extract has a relatively high protein content compared to those from other types of fish [8]. *C. striata* is a common freshwater fish in tropical countries such as Indonesia. It has a potential role in regulating molecular and immunological mechanisms [3]. Its extract has been widely used to accelerate the wound-healing process. The *C. striata* extract was also used to treat wounds due to its role

in plastic process of new cell tissue in the body [9]. It contains the amino acids (AAs) necessary for albumin synthesis (such as lysine, arginine, and glutamic acid) and wound healing (such as aspartic acid), and those with anti-inflammatory properties (such as arachidonic acid) that promote collagen formation and wound epithelialization [10]. Furthermore, it has antimicrobial and antinociceptive effects [11], [12]. The increase knowledge and awareness about the benefits of consuming *C. striata* have resulted in high demand and put the natural stock of this species under high pressure. The success of *C. striata* albumin in raising albumin level in hypoalbumin patients have made this particular fish albumin attracting even more attention [13].

In a RCT study by Fauzan *et al*., the albumin content of *C. striata* extract was shown to enhance tissue healing by increasing the albumin level by 0.7 g/dL for 10 days [14]. As an adjuvant therapy for chronic inflammation, *C. striata* extract was proven to increase T-cell regulator, decrease macrophage cells, and reduce proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interferon-gamma (IFN-γ), and interleukin-6 10. However, its effect on NLR is not fully known.

Thus far, there has been only one study performed in the Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia, on the influence of the *C. striata* extract Pujimin Plus® administration on total protein intake and hemoglobin value in patients with hypoalbuminemia. The current study evaluated the effect of the *C. striata* extract Pujimin Plus® on albumin and NLR levels in hyperglycemic rats experiencing wound injury.

Materials and Methods

Study design and sample

This randomized controlled study was performed in 30 adults male Wistar rats (*Rattus norvegicus*) in April 2021, in the veterinary laboratory of the Faculty of Medicine, Hasanuddin University, Makassar. Blood tests were conducted at the Hasanuddin University Hospital Clinical Pathology Laboratory. The rats were randomly divided into two equal sized groups (intervention and control) where the sample size was determined using the Federer formula.

The inclusion criteria were adult (8–10 weeks), male, white Wistar rats, weighing 150–200 g, with good appetite and activity, no visible anatomical abnormalities, and normal hair distribution and color. The exclusion criteria were the presence of abnormal exudate discharged from the eyes, mouth, anus, or genital area, or death during the adaptation period.

Design and data collection

The research tools used in this study included analytical balances to measure weight, rulers (mm scale), insulin needles, cotton, and alcohol, glucometer tools and strips, and a biopsy punch. The blood-sampling tools were disposable syringes (1 ml) and 1.5-ml tubes. The blood-sample examination equipment was a Pentra 400 autoanalyzer, Rotofix 32 A centrifuge tubes, and a volumetric pipette. The rats were housed in standard cages (40 × 30 cm) lined with paper on the bottom to ensure easy cleaning.

The laboratory animals were treated in compliance with National Institutes of Health rules, and we completed the work in compliance with the ARRIVE Guidelines for Animal Research Reporting [15]. The study was conducted in three steps: pre-intervention, intervention, and post-intervention. In the first step (pre-intervention), all rats that matched the inclusion criteria were housed in a pathogen-free environment and were adapted to the laboratory environment on a 12-h light-dark cycle for 1 week (habituation procedure). Five rats were placed in each plastic cage under standardized conditions (at room temperature 22 ± 2°C) with unlimited access to standard feed and water. The cages were cleaned weekly, a feeding bowl was placed inside each cage and water was supplied in a suspended bottle with a pipette.

In the second step (intervention), rats belonging to the intervention group were given 81 mg/day of *C. striata* extract (Pujimin Plus®) (Table 1) through a tube for 3 days, whereas the control group rats were given sodium-carboxymethyl cellulose (Na-CMC) as a placebo. On the 3rd day of *C. striata* extract consumption, the rats were injected with streptozotocin (STZ) to induce hyperglycemia. STZ was given intraperitoneally at a dose of 35–65 mg/kg body weight (BW) or 8.5 mg/200 g BW. The blood glucose target level was >200 mg/dL and was evaluated 24 h post STZ induction [16], [17], [18], [19].

Table 1: The composition of *C. striata* extract (Pujimin Plus®)/ capsule (750 mg)

Parameter	*C. striata* extract	Unit
Protein level	78.99	%
Albumin	39.34	%
Water level	3.00	%
Aspartate	62,191	ppm
Glutamate	109,447	ppm
Serine	35,678	ppm
Glycine	51,839	ppm
Histidine	23,596	ppm
Arginine	59,775	ppm
Threonine	43,552	ppm
Alanine	43,525	ppm
Proline	28,364	ppm
Valine	39,261	ppm
Tyrosine	36,890	ppm
Isoleucine	35,792	ppm
Leucine	64,527	ppm
Phenylalanine	46,993	ppm
Lysine	72,948	ppm
Cystine	2,581	ppm
Methionine	29,967	ppm
Zinc	29	ppm
Iron	43	ppm
Magnesium	1,041	ppm
Calcium	4,112	ppm

C. striata: Channa striata. PPM: Parts per million.

The rats' back hair was shaved with Veet gel® and disinfected with 70% alcohol solution, and then left to dry. The rats were anesthetized with ketamine intraperitoneally (80–100 mg/kg BW) [20]. Next, an 8-mm diameter punch biopsy was performed. The wound was as deep as the fascia profunda. After wounding, the intervention group continued to receive the same dose of *C. striata* extract and the control group received placebo for the next 10 days.

The third step (post-intervention) comprised 1-ml blood-sample collections performed on days 0, 3, and 10. The independent variable in this study was the *C. striata* extract, and the dependent variables were albumin and NLR levels.

Statistical analysis

All data were processed using the SPSS 25 program. The accepted level of statistical significance was $p \leq 0.05$. The independent t-test and Mann-Whitney test were used to investigate significant differences between the two groups. Repeated ANOVA test was used to compare the repeated measurements on days 0, 3, and 10.

Ethical clearance

Ethical approval was obtained from the Research Ethic Commission of the Hasanuddin University Faculty of Medicine, Makassar (no. 209/UN4.6.4.5.31/PP36/2021).

Results

In total, 30 adult male Wistar rats were included in the study. Rats in both groups had comparable weights (control group 173.2 ± 7.47 g vs. intervention group 171.6 ± 6.85 g, p > 0.05) and blood glucose levels (control group 251 ± 22.14 mg/dL vs. 238.2 ± 27.16 mg/dL, p > 0.05). Table 2 displays these results.

Table 2: Basic characteristics of rats

	Control group	Intervention group	p-value
Body weight (g)	173.20 ± 7.47	171.60 ± 6.85	0.786
Blood glucose level 24 h post-induction+ (mg/dL)	251.00 ± 22.14	238.20 ± 27.16	0.439

+Blood glucose level was measured 24 h after injection of STZ.

Table 3 and Figure 1 show the mean albumin values from the control and intervention groups, measured on days 0, 3, and 10. The intervention group had a significantly lower albumin level compared to the control group on the first day of examination (day 0) (2.66 ± 0.15 vs. 2.9 ± 0.12 g/dL, p < 0.05). Both groups experienced a decrease of albumin level on the 3rd day, but the albumin level of the intervention group was higher than that of the control group (2.42 ± 0.08 vs. 2.38 ± 0.16 g/dL, p > 0.05), although this was not statistically significant. Similarly, although the intervention group had a higher albumin level at the end of the experiment (2. ± 0.36 vs. 2.46 ± 0.13 g/dL, p > 0.05), this difference was also not statistically significant. Differences in the mean albumin values within groups at each time point were tested using the Independent t-test and Mann-Whitney test and the control group showed significant changes (2.9 ± 0.12 vs. 2.38 ± 0.16 vs. 2.46 ± 0.13 g/dL, respectively, p < 0.01), whereas the intervention group did not (2.66 ± 0.15 vs. 2.42 ± 0.08 vs. 2.66 ± 0.36 g/dL, respectively, p > 0.05).

Table 3: Mean albumin levels (g/dL) of control and intervention groups on days 0, 3, and 10

	Plasma albumin level (g/dL)						p-value***
	Day 0		Day 3		Day 10		
	Mean	SD	Mean	SD	Mean	SD	
Control group	2.9	0.12	2.38	0.16	2.46	0.13	0.007
Intervention group	2.66	0.15	2.42	0.08	2.66	0.36	1.000
p-value	0.025*		0.911**		0.283*		

*Independent t-test, **Mann-Whitney test, *** Repeated Anova Test.

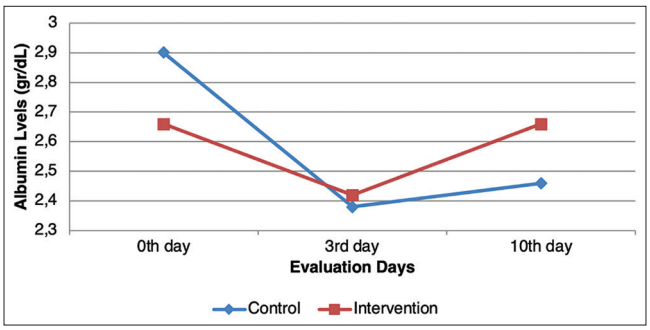

Figure 1: Average albumin levels on the evaluation days for control and intervention groups. The blue line represents the control group, and the red line represents the intervention group. The albumin level was measured in g/dL

The differences of albumin levels between days 0 and 3 (control group –0.52 ± 0.29 vs. intervention group –0.24 ± 0.23 g/dL), and days 3 and 10 (control group 0.08 ± 0.19 vs. intervention group –0.24 ± 0.39 g/dL), were not statistically significant (p > 0.05 for both), although it was clear that the intervention group lost less albumin and had higher albumin values. When comparing day 0 with day 10, it was also clear that the albumin value in the intervention group had not changed, whereas that in the control group was –0.44 g/dL lower, although this result was not statistically significant (p > 0.05) (Table 4).

Table 4: Comparison of differences between albumin level(g/dL) between evaluation days

	Plasma albumin level (g/dL)					
	Day 0 versus 3		Day 0 versus 10		Day 3 versus 10	
	Mean	SD	Mean	SD	Mean	SD
Control	–0.52	0.29	–0.44	0.19	0.08	0.19
Intervention	–0.24	0.23	0	0.42	0.24	0.39
p-value	0.127*		0.068*		0.436*	

*Independent t-test.

Table 5 presents the neutrophil percentage of both groups on days 0, 3, and 10. The two groups displayed opposite responses: the neutrophil percentages in the control group increased (20.28% vs. 25.86% vs. 26.98%) whereas the values in the intervention group increased (27.70% vs. 27.72%

Table 5: Neutrophil (%) level of control and intervention groups on days 0, 3, and 10

	Plasma albumin level (g/dL)						p-value***
	Day 0		Day 3		Day 10		
	Mean	SD	Mean	SD	Mean	SD	
Control group	20.28	4.54	25.86	10.03	26.98	6.67	0.163
Intervention group	27.70	8.15	27.72	4.54	23.22	6.7	0.415
p-value	0.113*		0.720**		0.400*		

*Independent t-test,**Mann-Whitney test, ***Repeated Anova Test.

vs. 23.22%). However, these differences were not statistically significant when compared within or between groups (p > 0.05 for both).

Similar results were obtained for the lymphocyte percentages. The values on days 0, 3, and 10 for the intervention (61.94% vs. 57.04% vs. 65.66%) and control (62.28% vs. 60.08% vs. 62.16%) groups were comparable within and between groups (p > 0.05 for both) (Table 6).

Table 6: Lymphocyte (%) level of control and intervention groups on days 0, 3, and 10

	Lymphocyte (%)						p-value***
	Day 0		Day 3		Day 10		
	Mean	SD	Mean	SD	Mean	SD	
Control group	68.28	5.00	60.08	10.54	62.16	7.51	0.247
Intervention group	61.94	9.01	57.04	1.63	65.66	8.79	0.563
p-value	0.206*		0.690**		0.518*		

*Independent t-test, **Mann-Whitney test, ***Repeated Anova Test.

At the end of the study, the NLR values for the intervention group (0.45 vs. 0.49 vs. 0.37) showed a greater reduction compared to the control group (0.30 vs. 0.46 vs. 0.45); however, these results were not statistically significant when compared within or between groups (p > 0.05 for both). These results are displayed in Table 7 and Figure 2.

Table 7: NLR of the control and intervention groups on days 0, 3, and 10

	NLR						p-value***
	Day 0		Day 3		Day 10		
	Mean	SD	Mean	SD	Mean	SD	
Control group	0.30	0.09	0.46	0.23	0.45	0.16	0.168
Intervention group	0.47	0.22	0.49	0.09	0.37	0.17	0.491
p-value	0.222**		0.841*		0.473*		

NLR: Neutrophil-to-lymphocyte ratio. *Independent t-test, **Mann-Whitney test, ***Repeated Anova Test.

Table 1 shows the composition of a 750-mg capsule of *C. striata* extract.

Table 2 displays the basic characteristic of the rats in the control and intervention groups. Rat BW

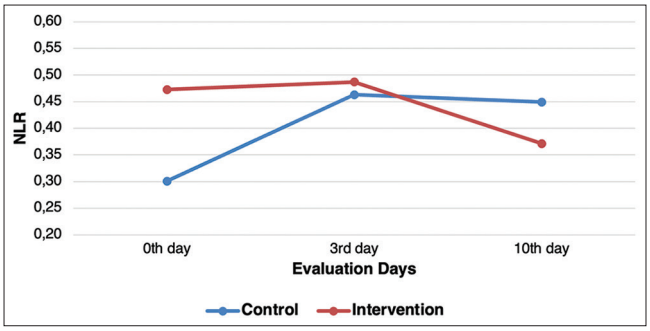

Figure 2: NLR on each evaluation day for the control (blue line) and intervention (red line) groups

was measured in g. Blood glucose level after 24 h was measured in mg/dL. The accepted p-value for statistical significance was <0.05.

Table 3 presents the means and standard deviations (SDs) of the albumin values of the control and intervention groups on days 0, 3, and 10. The albumin level was measured in g/dL. The accepted p-value for statistical significance was <0.05.

Table 4 compares the differences between albumin levels on each evaluation day. Albumin levels were measured in (g/dL) and displayed as the mean and SD. The accepted p-value for statistical significance was p < 0.05.

Table 5 presents the mean and SD neutrophil levels (%) of the control and intervention groups on days 0, 3, and 10. The accepted p-value for statistical significance was p < 0.05.

Table 6 presents the mean and SD of the lymphocyte (%) values for the control and intervention groups on days 0, 3, and 10. The accepted p-value for statistical significance was p < 0.05.

Table 7 presents the means and SDs of the NLR values of the control and intervention groups on days 0, 3, and 10. The accepted p-value for statistical significance was p<0.05.

Discussion

This study evaluated the effects of *C. striata* extract on albumin and NLR levels in hyperglycemic rats that were experiencing wound injury. It was performed using a rat model, which allowed relatively large sample sizes and fast response times, was relatively cheap and was representative of similar events in humans. The *C. striata* extract Pujimin Plus® was chosen because it had higher albumin, AA, and mineral levels compared to the regular Pujimin® extract.

The different values of albumin, lymphocytes, and neutrophils on day 0 between both groups could have been externally influenced by environmental factors such as the cleanliness of the food containers or cages.

The albumin and neutrophil levels on day 3 showed that inflammation was occurring as a result of the injury. The oxidative stress and inflammation produced by the stressors triggered acute-phase protein secretion and reduced albumin synthesis [21]. This result was expected, as it is common for albumin levels to fall within the first 3–7 days of acute injury, to remain low or become lower as the inflammatory response continues for 48–72 h, and then to slowly rise as the body recovers [22]. At the same time, neutrophils are the first leukocytes to be released during inflammation [23], hence, there was an increase in the neutrophil values in both groups when the injury began.

When observing the trends in the intervention group on day 10, there was a reduction in the neutrophil

percentage, which indicated that the inflammation had subsided. A reduction in the neutrophil percentage is possible only when there is a decreased signal from the proinflammatory cytokineIL-17, which suppress their production in the bone marrow [24]. In addition, the albumin and lymphocyte values had increased on day 10. This provided further support that the wound had begun to enter the proliferation phase, which was characterized by fibroblast migration, formation of new extracellular matrices, replacement of damaged tissue with fibrin and fibronectin, and collagen formation that provided a foundation for the intracellular matrix [25]. The lymphocyte levels increased as they have a crucial role in the wound-healing process. The lymphocytes release lymphokines that affect macrophage aggregation and the production of fibroblast growth factors that influence the wound-healing process [26], [27], [28]. Furthermore, the intervention group displayed increased albumin levels, which indicated that the body could resume its physiologic functions to synthesize albumin as the inflammation diminished [29], [30].

Although these trends were not statistically significant, the intervention group had better laboratory outcomes compared to the control group. The intervention group was supplied with better nutrients to combat inflammatory processes, to support the immune system, and enable the development of granulated tissue [31]. This phenomenon could be attributed to the effect of the *C. striata* extract supplementation. Many components of the *C. striata* extract, such as zinc, iron, copper, and essential fatty acids, have immunomodulating properties. The anti-inflammatory property of albumin in *C. striata* could inhibit the formation of pro-inflammatory cytokines such as TNF-α and IFN-γ. This inhibition created another inhibitory cascade that prevented polymorphonuclear leukocyte recruitment, release of reactive oxygen species and other proteases, and tissue damage [32]. Moreover, *C. striata* contains almost 63% albumin, and so could potentially increase the albumin level. However, these data must be interpreted with caution, because albumin production is also influenced by the function of the liver and an adequate supply of AAs [33]. The limitations of this study suggest that further research should be done with a greater number of samples over a longer duration to achieve a more significant result.

Conclusion

Although most of the results were not statistically significant, the present study indicated a positive effect of *C. striata* extract on albumin and NLR levels following wound injury in hyperglycemic rats. *C. striata* extract should be considered as a reliable method to prevent hypoalbuminemia and reduced inflammation in wound injury.

References

1. Nugroho RH. Determinants of severity in patients with diabetes mellitus. J of Public Health. 2019;3:193-203.
2. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2009;32 Suppl 1:S62-7. https://doi.org/10.2337/dc09-S062
   PMid:19118289
3. Wijaya I, Taslim NA, Natzir R, Aman AM, Hatta M, Suhudi B, *et al*. Molecular and immunological mechanisms of *Channa striata* in diabetic wound healing. Int J Pharm Res. 2020;2:279-89.
4. Syauta D, Mulawardi M, Prihantono P, Hendarto J, Mariana N, Sulmiati S, *et al*. Risk factors affecting the degree of diabetic foot ulcers according to Wagner classification in diabetic foot patients. Med Clín Práct. 2021;4:100231.
5. Putri RC, Agustina W. Effect of topical fish albumin extract (*Channa striata*) on contraction accelerationincision wound in white rat (*Rattus norvegicus*) wistar the strain effect of albumin extract Channa striata topical administration toward the acceleration. J Nurs Care Biomol. 2017;1(1):45.
6. Balta S, Demirkol S, Unlu M, Arslan Z, Celik T. Neutrophil to lymphocyte ratio may be predict of mortality in all conditions. Br J Cancer. 2013;109(12):3125-6.
7. Vatankhah N, Jahangiri Y, Landry GJ, McLafferty RB, Alkayed NJ, Moneta GL, *et al*. Predictive value of neutrophil-to-lymphocyte ratio in diabetic wound healing. J Vasc Surg. 2017;65(2):478-83. https://doi.org/10.1016/j.jvs.2016.08.108
   PMid:27887858
8. Karina N, Wagiyo W, Elisa U. Effect of snakehead fish extract on perineal wound healing in post partum mothers at BPM Bonangrejo Demak. J Midwifery Nursing Science. 2016;1:1-16.
9. Fatma N, Nurilmala M, Metusalach M, Nurfaidah K, Astuti N. Low economic benefits of fish as source of fish albumin besides cork fish. Adv Econ Bus Manage Res. 2020;141:56-9.
10. Nicholson JP, Wolmarans MR, Park GR. The role of albumin in critical illness. Br J Anaesth. 2000;85(4):599-610. https://doi.org/10.1093/bja/85.4.599
    PMid:11064620
11. Sahid NA, Hayati F, Rao CV, Ramely R, Sani I, Dzulkarnaen A, *et al*. Snakehead consumption enhances wound healing? From tradition to modern clinical practice: A prospective randomized controlled trial. Evid Based Complement Alternat Med. 2018;2018:3032790. https://doi.org/10.1155/2018/3032790
    PMid:30538757
12. Ma'rufi I, Ali K, Sedemen IA, Purwanto P, Khoiri A. *Channa striata* (Ikan Gabus) extract and the acceleration of tuberculosis treatment: A true experimental study. Interdiscip Perspect Infect Dis. 2019;2019:8013959. https://doi.org/10.1155/2019/8013959
    PMid:31015831
13. Fatma N, Metusalach M, Taslim NA, Nurilmala M. The protein and albumin contents in some species of marine and brackishwater fish of South Sulawesi, Indonesia. AACL Bioflux. 2020;13(4):1976-85.
14. Fauzan MR, Dahlan CK, Taslim NA, Syam A. The effect of giving fish extract (Pujimin Plus) on intake of protein and hemoglobin hypoalbuminemic patients. Enferm Clin. 2020;30:452-5.
15. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: The ARRIVE guidelines

for reporting animal research. PLoS Biol. 2010;8(6):e1000412. https://doi.org/10.1371/journal.pbio.1000412
PMid:20613859

16. Qinna NA, Badwan AA. Impact of streptozotocin on altering normal glucose homeostasis during insulin testing in diabetic rats compared to normoglycemic rats. Drug Des Devel Ther. 2015;9:2515-25. https://doi.org/10.2147/DDDT.S79885
PMid:26005328
17. Agustin H, Massi MN, Djaharuddin I, Susanto AD, Islam AA, Hatta M, *et al*. Analysis of CD4 and CD8 expression in multidrug-resistant tuberculosis infection with diabetes mellitus: An experimental study in mice. Ann Med Surg (Lond). 2021;68:102596. https://doi.org/10.1016/j.amsu.2021.102596
PMid:34401121
18. King AJ. The use of animal models in diabetes research. Br J Pharmacol. 2012;166(3):877-94. https://doi.org/10.1111/j.1476-5381.2012.01911.x
PMid:22352879
19. Di Filippo C, Marfella R, Cuzzocrea S, Piegari E, Petronella P, Giugliano D, *et al*. Hyperglycemia in streptozotocin-induced diabetic rat increases infarct size associated with low levels of myocardial H0-1 during ischemia/reperfusion. Diabetes. 2005;54(3):803-10. https://doi.org/10.2337/diabetes.54.3.803
PMid:15734859
20. Masoko P, Picard J, Eloff JN. The use of a rat model to evaluate the *in vivo* toxicity and wound healing activity of selected combretum and terminalia (combretaceae) species extracts. Onderstepoort J Vet Res. 2010;77(1):E1-7. https://doi.org/10.4102/ojvr.v77i1.2
PMid:23327158
21. Syamsiatun NH, Siswati T. Giving extra egg white juice on albumin and Hb levels in hypoalbuminemic patients. J Nutrition Clinic Indonesia. 2015;12(2):54-61.
22. Soeters PB, Wolfe RR, Shenkin A. Hypoalbuminemia: Pathogenesis and clinical significance. JPEN J Parenter Enteral Nutr. 2019;43(2):181-93. https://doi.org/10.1002/jpen.1451
PMid:30288759
23. Agustin R, Dewi N, Rahardja SD. The effectiveness of haruan fish extract (*Channa striata*) and ibuprofen on the number of neutrophil cells in the wound healing process in vivo studies on the buccal mucosa of wistar rats (*Rattus norvegicus*). J Dentist. 2016;1(1):68-74.
24. de Jager CP, van Wijk PT, Mathoera RB, de Jongh-Leuvenink J, van der Poll T, Wever PC. Lymphocytopenia and neutrophil-lymphocyte count ratio predict bacteremia better than conventional infection markers in an emergency care unit. Crit Care. 2010;14(5):R192. https://doi.org/10.1186/cc9309
PMid:21034463
25. Velnar T, Bailey T, Smrkolj V. The wound healing process: An overview of the cellular and molecular mechanisms. J Int Med Res. 2009;37(5):1528-42. https://doi.org/10.1177/147323000903700531
PMid:19930861
26. Çekici Y, Yılmaz M, Seçen Ö. New inflammatory indicators: Association of high eosinophil-to-lymphocyte ratio and low lymphocyte-to-monocyte ratio with smoking. J Int Med Res. 2019;47(9):4292-303. https://doi.org/10.1177/0300060519862077
PMid:31319727
27. Qing C. The molecular biology in wound healing and non-healing wound. Chin J Traumatol. 2017;20(4):189-93. https://doi.org/10.1016/j.cjtee.2017.06.001
PMid:28712679
28. Laillyza AM, Puspitasar D. Effect of *Channa micropeltes* for increasing lymphocyte and fibroblast cells in diabetic wound healing. J Med Sci. 2018;18(4):205-10.
29. Chien SC, Chen CY, Lin CF, Yeh HI. Critical appraisal of the role of serum albumin in cardiovascular disease. Biomark Res. 2017;5(1):31. https://doi.org/10.1186/s40364-017-0111-x
PMid:29152305
30. Putri AA, Yuliet Y, Jamaluddin J. Analysis of albumin levels in eel (*Anguilla marmorata and Anguilla bicolor*) and test of wound healing activity in rabbits (*Oryctolagus cuniculus*). J Farm Galen. 2016;2(2):90-5.
31. Gray D, Cooper P. Nutrition and wound healing: What is the link? J Wound Care. 2001;10(3):86-9. https://doi.org/10.12968/jowc.2001.10.3.26058
PMid:11924358
32. Maharani Nurul M. Aktivitas Antiinflamasi Kombinasi Ekstrak Etanol Lempuyang Gajah (*Zingiber zerumbet*) Dan Serbuk Ikan Gabus (*Channa striata*) Terhadap Udem Telapak Kaki Tikus Putih Jantan Galur Wistar, 5th Urecol Proceeding; 2017. p. 152-60.
33. Said S, Taslim NA, Bahar B. Nutrition and Wound Healing. Makassar: Indonesia Academic Publishing; 2012. p. 108.