Effect of Acute Administration of Eel (*Anguilla bicolor bicolor*) Oil to Hematological Parameters in Mice

Heru Sasongko
Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57126, Central Java Indonesia

Keywords: acute toxicity, eel, fish oil, hematology, omega-3

Abstract: Eel (*Anguilla bicolor bicolor*) is a fish consumed as food and found in Indonesia. The eel oil is known to contain fatty acids like omega-3. The study aims to determine the effect of acute administration of eel oil to hematological parameters in mice. A total of 24 male Swiss Webster mice were divided into four groups. Group I as the control was not administered by eel oil and administered aquadest instead, group II, III, and IV were given 0.09; 0.25 and 0.74 g/ 20 g B.W, respectively, of eel oil. The oil was administered for 14 days. On day 14th, blood sample was taken from orbital sinus. The hematological parameter consisted of the amount of erythrocyte, leukocyte, hemoglobin, hematocrit, lymphocyte, neutrophil and platelet levels were measured. The data were statistically analyzed by one way ANOVA followed by LSD test. The result is acute administration of eel oil at dose 0.74 g/20 g B.W has caused significant change of hematological parameters, except for erythrocytes and platelets level. The conclusion of this study was that at a dose of 0.74 g/20 g B.W eel oil had an effect on blood hematologic changes in mice.

1 INTRODUCTION

Anguilla bicolor bicolor is a type of fish consumed in many countries like Japan, China, Germany, and France (Sasongko *et al*., 2017). This fish has not been used optimally in Indonesia because many people do not know about it and it is more expensive than other types of fish. Eel oil is reported to contain fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Baeza *et al*., 2014; Kusharto *et al*., 2014). Eicosapentaenoic acid and docosahexaenoic acids are part of Omega-3 polyunsaturated fatty acids (Amissi *et al*., 2016). Fish contains nutrients such as proteins, fatty acids, minerals and vitamins (Vitamins A, B3, B6, B12, E, and D) and it is good for health (Suleria *et al*., 2015).

Fish oil is known as the source of polyunsaturated fatty oil and widely used for the pharmaceutical purpose and food supplement (Daiello *et al*., 2015; Suleria *et al*., 2015). Fatty acids have been used as baby food by some health agents. In previous study, eel oil was shown to have an effect of decreasing total cholesterol tested on animal models (Sasongko *et al*., 2017). Omega-3 polyunsaturated fatty acids such as EPA and DHA have been shown to protect the cardiovascular system, to protect the body from cancer, inflammatory and autoimmune diseases (Amissi *et al*., 2016; Simopoulos, 2002).

Despite the various benefit of eel oil, there is no research about the safety of eel oil consumption. Toxic effects of the eel oil might occur. This study aimed to investigate the toxic effect of acute administration of eel oil to the hematological parameters in male mice.

2 MATERIALS AND METHOD

2.1 Materials

Eel (*Anguilla bicolor bicolor*) aging between 3-4 month and weighing 100-200 gram were collected from UNAGI business department, Universitas Sebelas Maret. Studies were carried out using male Swiss Webster mice (20 – 30 g). Mice were obtained from the Faculty of Medicine Universitas Sebelas Maret, Surakarta, Indonesia. All animal handling procedures have been approved by the ethics

Sasongko, H.
Effect of Acute Administration of Eel (Anguilla bicolor bicolor) Oil to Hematological Parameters in Mice.
DOI: 10.5220/0008238500110014
In *Proceedings of the 1st Muhammadiyah International Conference on Health and Pharmaceutical Development (MICH-PhD 2018)*, pages 11-14
ISBN: 978-989-758-349-0

Table 1: Hematological parameters following 14 days observation exposure of eel oil in mice.

Groups	WBC (10^4xµL)	RBC (10^4xµL)	HGB (g/dL)	HCT (%)	PLT (10^4xµL)	LYM (10^2xµL)	NEU (10^2xµL)
Negative control	81.83 ± 17.30	779.17 ± 87.89	12.62 ± 1.42	38.15 ± 4.37	69.13 ± 24.67	85.33 ± 4.55	14.67 ± 4.55
0.09 g/20 gB.W.	55.66 ± 20.72	665.17 ± 117.42	10.05 ± 1.68	34.42 ± 4.08	69.82 ± 13.76	66.80 ± 13.67	16.53 ± 13.67
0.25 g/20 gB.W.	66.33 ± 19.19	789.00 ± 160.74	12.40 ± 2.59	40.92 ± 8.93	59.03 ± 31.62	82.18 ± 10.40	16.82 ± 10.40
0.74 g/20 gB.W.	54.16 ± 15.06*	873.33 ± 88.37	14.53 ± 1.82*	43.13 ± 3.94*	81.07 ± 34.36	59.33 ± 29.32*	26.90 ± 5.09*

Symbols represent statistical significance. *$p < 0.05$, as compared to negative control group. n = 6 animals in each group. WBC: White blood cell; RBC: Red blood cell; HGB: Hemoglobin; HCT: Hematocrit; PLT: Platelet; LYM: Lymphocyte; NEU: Neutrophile.

committee of the Faculty of Medicine, Universitas Sebelas Maret with the number 231/II/HREC/2018.

2.2 Methods

2.2.1 Extraction

The method of eel extraction followed the method undertaken by Sasongko *et al*., (2017).

2.2.2 Animal Experimental Design

The research using 24 male mice were divided into four groups consisting of 6 mice per group. Group I as control was given aquadest, group II, III, and IV were given 0.09; 0.25, and 0.74 g/20 g B.W, respectively, of eel oil. The oil was administered for 14 days. On day 14th, blood sample was taken from orbital sinus. Blood sample were collected to determine hematological parameters.

2.2.3 Measurement of Haematological Parameters

Hematological parameters measurement including the amount of erythrocyte (RBC), leukocyte (WBC), hemoglobin (HGB), hematocrit (HCT), lymphocyte (LYM), neutrophil (NEU) and platelet levels (PLT). Measurement of hematologic parameters followed by Guder *et al*. (2014); Pusterla and Higgins (2017).

2.3 Data Analysis

The data were statistically analyzed by one way ANOVA followed by LSD test.

3 RESULT AND DISCUSSION

The administration of eel oil for 14 days did not show mortality in test animals. The analysis of blood parameters can be used for diagnosing the organs or tissues function disorder. The hematological examination may describe the function of body organs and physiological status (Bachri *et al*., 2017). It is relevant to risk evaluation as changes in the hematological system have a higher predictive value for human toxicity (Rodeiro *et al*., 2018). The result of hematological can be seen in Table 1.

3.1 The Number of Leukocytes (WBC)

Leukocytes is involved in protecting the body as immune system cells against infectious agents or foreign invaders (Bachri *et al*., 2017). The white blood cells along with neutrophils and lymphocytes as their derivatives can prevent pathogens invasion or disease-causing. The microorganisms such as bacteria and viruses through phagocytosis process, identifying and destroying dangerous or cancerous cells (Walters *et al*., 1989).

The results shows that at dose of 0.74 g/20 gB.W eel oil have a significant effect of decreased levels of leukocytes ($p < 0.05$). This is probably related to the immunomodulatory effect on lipid-mediator generation in leukocytes from omega-3 fatty acids from eel oil (Morlion *et al*., 1996; Vedin *et al*., 2008).

3.2 The Number of Erythrocytes (RBC)

The red blood cells travel in blood carrying hemoglobin in the circulation. Their main function is carrying waste carbon dioxide back to the lungs and distributing oxygen to body tissues (Bachri *et al*., 2017; Snyder and Sheafor, 1999). The results shows that at dose of 0.74 g/20 gB.W eel oil is not significant with negative control ($p < 0.05$).

3.3 The Number of Hemoglobin (HGB)

Hemoglobin (HGB) is the protein contained in red blood cells that is responsible for delivery of oxygen to the tissues. To ensure adequate tissue oxygenation, a sufficient hemoglobin level must be maintained (Billett, 1990). The results shows that at dose of 0.74 g/20 gB.W eel oil have a significant effect on increased levels of hemoglobin ($p < 0.05$). These results suggest that fatty acids from eel oil share the property of gamma-globin gene inducibility (Liakopoulou *et al*., 1995). Gamma-globin to mediate high-level expression of hemoglobin (Arcasoy *et al*., 1997; Pestina *et al*., 2009).

3.4 The Number of Hematocrits (HCT)

The hematocrit measures the volume of red blood cells compared to the total blood volume (red blood cells and plasma) (Billett, 1990). The results shows that at dose of 0.74 g/20 gB.W eel oil have a significant effect on increased levels of hematocrit ($p < 0.05$). This is consistent with an increase in amount of red blood cells from each dose of eel oil although statistically not significantly different.

3.5 The Number of Platelet Levels (PLT)

Platelets is the blood cells that help the body in the blood clotting process to stop bleeding or process coagulation via interactions with vessel endothelial (Garraud and Cognasse, 2015). The results showed that at dose of 0.74 g/20 gB.W eel oil had not a significant difference with negative control ($p < 0.05$).

3.6 The Number of Lymphocytes (LYM)

A lymphocyte is a type of white blood cell that is part of the immune system. There are two main types of lymphocytes: B cells and T cells. The B cells produce antibodies that are used to attack invading bacteria, viruses, and toxins (Pubmed, 2018). The results showed that at dose of 0.74 g/20 gB.W eel oil had a significant effect on decreasing the levels of lymphocyte ($p < 0.05$). This is consistent with the decreased amount of white blood cells from each dose of eel oil although statistically not significantly different. This may be due to the effect of omega-3 fatty acid supplementation on cytokine production and lymphocyte proliferation (Meydani *et al*., 1991).

3.7 The Number of Neutrophils (NEU)

Neutrophils is the most commonly found in immune cells of human blood. These cells form a defence after a person got an infection (Hayashi *et al*., 2003). The results shows that at dose of 0.74 g/20 gB.W eel oil have a significant effect of increased levels of neutrophil ($p < 0.05$). Fatty acid omega-3 dan omega-6 differentially influence the plasma free fatty acid profile with impact on neutrophil functions. Lipid-based parenteral nutrition may thus exert a profound influence on sequelae and status of immunocompetence and inflammation (Mayer *et al*., 2003).

4 CONCLUSIONS

The acute administration of eel oil can cause the change of hematological parameters in the dose 0.74 g/20 g b.w, except for erythrocytes and platelets level. There is significant difference on hematological parameters of mice that treated with eel oil compared to the control group ($p < 0.05$).

ACKNOWLEDGEMENTS

The author would like to thank Universitas Sebelas Maret for funding this research with the Hibah Fundamental PNBP Grants scheme.

REFERENCES

Amissi, S., Rasul Niazi, Z., Burban, M., Kessler, R., Canuet, M., Toti, F., Monassier, L., Boehm, N., Auger, C., Meziani, F., Schini-Kerth, V.B., 2016. 0369 : The optimized omega-3 EPA: DHA 6:1 product prevents the monocrotaline-induced pulmonary

arterial hypertension and vascular remodeling in rats. Arch. Cardiovasc. Dis. Suppl., Printemps de la Cardiologie : Recherche Fondamentale et Clinique - Centre de Congrès Dijon.7-8 April 2016 8, 244.

Arcasoy, M.O., Romana, M., Fabry, M.E., Skarpidi, E., Nagel, R.L., Forget, B.G., 1997. High levels of human gamma-globin gene expression in adult mice carrying a transgene of the deletion-type hereditary persistence of fetal hemoglobin. *Mol. Cell. Biol*. 17, 2076–2089.

Bachri, M.S., Yuliani, S., Sari, A.K., 2017. Effect of subchronic administration of nutmeg (*Myristica fragrans* Houtt) ethanolic extract to hematological parameters in rat. *IOP Conf. Ser. Mater. Sci. Eng*. 259, 12009.

Baeza, R., Mazzeo, I., Vílchez, M.C., Gallego, V., Peñaranda, D.S., Pérez, L., Asturiano, J.F., 2014. Effect of thermal regime on fatty acid dynamics in male European eels (*Anguilla anguilla*) during hormonally-induced spermatogenesis. *Aquaculture* 430, 86–97.

Billett, H.H., 1990. *Hemoglobin and Hematocrit*, in Walker, H.K., Hall, W.D., Hurst, J.W. (Eds.), Clinical Methods: The History, Physical, and Laboratory Examinations. Butterworths, Boston.

Daiello, L.A., Gongvatana, A., Dunsiger, S., Cohen, R.A., Ott, B.R., 2015. Association of fish oil supplement use with preservation of brain volume and cognitive function. *Alzheimers Dement*. 11, 226–235.

Garraud, O., Cognasse, F., 2015. Are Platelets Cells? And if Yes, are They Immune Cells? *Front. Immunol*. 6.

Guder, W.G., Narayanan, S., Wisser, H., Zawta, B., 2014. *Diagnostic Samples: From the Patient to the Laboratory: The Impact of Preanalytical Variables on the Quality of Laboratory Results*. John Wiley & Sons.

Hayashi, F., Means, T.K., Luster, A.D., 2003. Toll-like receptors stimulate human neutrophil function. *Blood* 102, 2660–2669.

Kusharto, C.M., Widyasari, R.A.H.E., Budywiryawan, Wiyono, E.S., Sugengherisuseno, 2014. Nutritive Value and Fatty Acids Profile of Fresh Indonesian Eel (*Anguilla bicolor*) and Kabayaki. *J. Sains Kesihat. Malays*. Malays. J. Health Sci. 12.

Liakopoulou, E., Blau, C.A., Li, Q., Josephson, B., Wolf, J.A., Fournarakis, B., Raisys, V., Dover, G., Papayannopoulou, T., Stamatoyannopoulos, G., 1995. Stimulation of fetal hemoglobin production by short chain fatty acids. *Blood* 86, 3227–3235.

Mayer, K., Fegbeutel, C., Hattar, K., Sibelius, U., Krämer, H.-J., Heuer, K.-U., Temmesfeld-Wollbrück, B., Gokorsch, S., Grimminger, F., Seeger, W., 2003. ω-3 vs. ω-6 lipid emulsions exert differential influence on neutrophils in septic shock patients: impact on plasma fatty acids and lipid mediator generation. *Intensive Care Med*. 29, 1472–1481.

Meydani, S.N., Endres, S., Woods, M.M., Goldin, B.R., Soo, C., Morrill-Labrode, A., Dinarello, C.A., Gorbach, S.L., 1991. Oral (n-3) Fatty Acid Supplementation Suppresses Cytokine Production and Lymphocyte Proliferation: Comparison between Young and Older Women. *J. Nutr.* 121, 547–555.

Morlion, B.J., Torwesten, E., Lessire, H., Sturm, G., Peskar, B.M., Fürst, P., Puchstein, C., 1996. The effect of parenteral fish oil on leukocyte membrane fatty acid composition and leukotriene-synthesizing capacity in patients with postoperative trauma. *Metab. - Clin. Exp*. 45, 1208–1213.

Pestina, T.I., Hargrove, P.W., Jay, D., Gray, J.T., Boyd, K.M., Persons, D.A., 2009. Correction of Murine Sickle Cell Disease Using γ-Globin Lentiviral Vectors to Mediate High-level Expression of Fetal Hemoglobin. *Mol. Ther*. 17, 245–252.

Pubmed, 2018. Lymphocytes - National Library of Medicine. PubMed Health. URL https://www.ncbi.nlm.nih.gov/pubmedhealth/PMHT0022042/ (accessed 7.20.18).

Pusterla, N., Higgins, J., 2017. *Interpretation of Equine Laboratory Diagnostics*. John Wiley & Sons.

Rodeiro, I., Remirez, D., Flores, D., 2018. Sacha Inchi (*Plukenetia volubilis* L.) powder: acute toxicity, 90 days oral toxicity study and micronucleus assay in rodents. *J. Pharm. Pharmacogn. Res.* 6, 17–26.

Sasongko, H., Efendi, N.R., Budihardjo, A., Farida, Y., Amartiwi, T., Rahmawati, A.A., Wicaksono, A., Sugiyarto, 2017. Solvent and extraction methods effects on the quality of eel (*Anguilla bicolor*) oil. *J. Phys. Conf. Ser*. 795, 12021.

Sasongko, H., Sugiyarto, S., Budiharjo, A., Efendi, N.R., 2017. Anti-hypercholesterolemia effects and quality of eel (*Anguilla bicolor*) oil. *Int. J. Sci. Appl. Sci. Conf. Ser*. 2, 174–180.

Simopoulos, A.P., 2002. Omega-3 Fatty Acids in Inflammation and Autoimmune Diseases. *J. Am. Coll. Nutr*. 21, 495–505.

Snyder, G.K., Sheafor, B.A., 1999. Red Blood Cells: Centerpiece in the Evolution of the Vertebrate Circulatory System. *Integr. Comp. Biol*. 39, 189–198.

Suleria, H.A.R., Osborne, S., Masci, P., Gobe, G., 2015. Marine-Based Nutraceuticals: An Innovative Trend in the Food and Supplement Industries. *Mar. Drugs* 13, 6336–6351.

Vedin, I., Cederholm, T., Freund Levi, Y., Basun, H., Garlind, A., Faxén Irving, G., Jönhagen, M.E., Vessby, B., Wahlund, L.-O., Palmblad, J., 2008. Effects of docosahexaenoic acid–rich n−3 fatty acid supplementation on cytokine release from blood mononuclear leukocytes: the OmegAD study. *Am. J. Clin. Nutr.* 87, 1616–1622.

Walters, M.D.S., Matthei, I.U., Kay, R., Dillon, M.J., Barratt, T.M., 1989. The polymorphonuclear leucocyte count in childhood haemolytic uraemic syndrome. *Pediatr. Nephrol*. 3, 130–134.