Acute Oral Toxicity Test of Eel (Anguilla bicolor bicolor) Oil in Mice Liver and Kidney Cells

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Abstract. The present research aimed to investigate the acute toxicity effects of eel (Anguilla bicolor bicolor) oil on liver and kidney function after oral administration in mice. Eel is one of marine biodiversity and fish consumed in many countries, especially Japan, China, and Indonesia. Toxic effect symptoms were observed for 14 days. On the 15th day, the level of alanine aminotransferase (ALT), histology profile of liver, creatinine, and histology profile of kidney were measured. The current result showed the LD50 value of eel oil was > 15 g/kg b.w, which categorized practically nontoxic. Eel oil didn’t affect the toxic effect symptoms on ALT level, liver histology profile, and creatinine level. Histology profile observation showed that eel oil effects on the histological profile of mice kidney with moderate injury level (26-50%).

1. Introduction
Fish oil such as eel (Anguilla bicolor bicolor) and other types of fish are components of food supplements that are needed by humans. It is a source of unsaturated fatty acids that are required to improve the healing process and prevent pathological disorders inpatient [1-3]. Some of the fish oils have proven efficacy and safety so that many nutritional products have marketed [4]. Eel is one type of fish that is valuable and has the potential to become an export commodity in many countries such as Indonesia [5,6]. The eel exports increased by 4.0 million kg/year (2010–2012) to 6.1 million kg (2013) and expected to increase in the future in line with government policy [7].

Eel is known to contain many components of saturated and unsaturated fatty acids such as omega-3, omega-6, including EPA and DHA [5,8]. In the development of pharmaceutical products, the fatty acid content of eel oil has been proven to be effective in reducing cholesterol levels [9], analgesic, and anti-inflammatory agents [8]. As a food supplement, fish oil has been shown to reduce the risk of cardiovascular disease through preventing the development of adiposity, restoring insulin sensitivity, reducing plasma levels and liver lipids, and also preventing prothrombotic effects [1,10]. Consumption of fish oil also reduces the incidence of hematological toxicity on patients chemoradiotherapy [11]. For topical drugs, eel fish oil provides a wound-healing effect [6].

The many benefits of eel oil proven, have not been researching for safety in long-term use. Acute toxicity research to find out the interaction of fish oil with biological systems in low and high doses has never been done. In this study, we tested acute toxicity effects of eel oil in mice model.
Acute toxicity test is an initial test to determine the effect of administration a single dose on the toxic symptoms of a chemical compound [12]. The acute toxicity test aims to observe the injurious effects of a compound on the organism in short-term exposure [13].

2. Experimental

2.1. Materials

The test sample used in this study was Anguilla bicolor bicolor (Ikan Sidat) obtained from the Surakarta eel farm "UNAGI," aged 2-3 months and weighing 200-300 grams. The reagent used are: alanine aminotransferase (ALT) kit reagent, creatinine kit reagent from Biosystem®. The eel was identified and authenticated at Department Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret.

2.2. Instrumentation

Instruments used in this study were biochemistry analyzer (Biosystem® type BTS350), Gas Chromatography FID (Shimadzu® 2010), microscope (Olympus®), incubator (Memmert®), microtome (Leica® RM 1215).

2.3. Extraction and fatty acids determination

The eel was cleaned and boiled for 5 hours at 70-80 °C. Extract yield obtained in the form 2 phases (oil and the water). The oil phase were separated and then centrifuged at a speed of 3000 rpm [14]. The oil phase obtained then used as a sample for testing the fatty acid profile and activity. 0.5 mL of eel oil is added with 1.5 mL of methanolic sodium solution, then covered and heated at 60°C for 5-10 minutes while shaken and cooled. The sample was then added with 2 mL of Boron trifluoride metanoate, heated at 60°C for 5-10 minutes and cooled. The mixture was then extracted with 1 mL of Heptane and 1 mL of NaCl concentrated. The top layer of the extract were taken and put into Eppendorf. The yield (0.1 µL) as sample are injected into GC.

2.4. Animal Experimental

The animal handling procedures and standard animal experiments were approved by the ethics committee of the Moewardi General Hospital and School of Medicine, Universitas Sebelas Maret with the number 231/II/HREC/2018. For the study of acute toxicity test, it was 2–3 months old Swiss webster mice, weighing 20–30 g. The mice were obtained from the Animal Laboratory Pharmacology Universitas Sebelas Maret. The animals were bred in experimental conditions.

2.5. Study of acute toxicity

Twenty-four mice were distributed in 4 groups (6 animals each). The dose administration of eel oil to animals test in this study was from omega-3 component the eel for anti-inflammation. Omega-3 therapeutic dose commonly used in humans and converted to mice doses. The dose is determined as the lowest dose and is tripled to the second and third dose. The animals were weighed, and the eel oil doses were given base on their body weight. Group 1 was given 1 ml aquadest as control. The tree next groups received single doses of eel oil at 0.09, 0.25, and 0.74 g/20g respectively. The animals were observed for any toxicity within the 1st four hours after the administration, and after that, throughout 24 h [15]. Surviving animals were weighted and observed once in a day for behavior changes, physical appearance, and signs of toxicity for 14 days. The blood was collected and centrifuged at 3000 rpm for 10 min. Serum was separated to determination of alanine aminotransferase (ALT) and creatinin. Liver and kidney tissue samples were taken for histological analysis.

2.6. Data Analysis

Quantitative data (serum of alanine aminotransferase, creatinin and necrosis cell of liver and kidney) were statistically evaluated. Levene's test of the homogeneity of variance was performed: if the variances were homogeneous, the single factor analysis of variance was performed for inter-group
comparison; if Analysis of Variance (ANOVA) showed significant differences, Least Significance Different (LSD) test was performed.

3. Result and discussion
3.1. Fatty Acids Profile

The development of fish oil-based food supplements has been done in many research, but not all types of fish oil. Oil or fat consists of fatty acid units, based on saturation of fatty acids, that is saturated fatty acids (SFA), mono unsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). The difference laid on the chemical bond, whereas the saturated fatty acid does not has a double bond [5]. Chemical compound of eel oil showed the presence of fatty acid profile (Table 1).

| Fatty acyd profile                        | Concentration (%relative) | Fatty acyd profile                        | Concentration (%relative) |
|------------------------------------------|--------------------------|------------------------------------------|--------------------------|
| Saturated fatty acid                     |                          | Poli unsaturated fatty acid              |                          |
| M laurate                                | 0.24                     | M linoleate                              |                          |
| M tetradecanoate                         | 5.63                     | Linoleaidic acid                         | 0.5                      |
| Myristoleic acid methyl ester            | 0.18                     | Cis-11,14-eicosadienoic                  | 4.73                     |
| M pentadecanoate                         | 0.63                     | M docosanoate                            | 0.33                     |
| M palmitate                              | 25.04                    | gamma-linolenic acid                     | 0.1                      |
| M Heptadecanoate                         | 0.53                     | M cis-5-8-11-14-                        | 0.27                     |
| cis-10-pentadecenoic acid                | 0.11                     | eicosatetraenoic                         | 1.32                     |
| Methyl tricosanoate                      | 0.68                     | cis-8,11,14-eicosatrienoic acid          |                          |
| Methyl lignocer ate                      | 0.91                     | M cis-5-8-11-14-17-                     | 0.38                     |
|                                          |                          | eicosapentaenoate                        |                          |
| Total SFA                                | 33.95                    | Cis-4,7,10,13,16,19-docosahexenoate      | 4.17                     |
| Mono unsaturated fatty acid              |                          | Total PUFA                               | 11.96                    |
| M Palmitoleate                           | 8.46                     |                                          |                          |
| Cis 10-heptadecenoic acid                | 0.48                     |                                          |                          |
| M Octadecanoate                          | 3.21                     |                                          |                          |
| Cis-9-oleic methyl ester                 | 2.96                     |                                          |                          |
| Methyl cis-11-eicosenoate                | 0.97                     |                                          |                          |
| M nervonate                              | 0.53                     |                                          |                          |
| trans-9-elaidic acid                     | 36.66                    |                                          |                          |
| Total MUFA                               | 53.27                    |                                          |                          |

3.2. Acute Toxicity Symptoms

The animal in all dose groups did not show acute toxicity symptoms like changes in autonomic nerves, central nervous system, hair loss, and amount of food consumption. Acute toxicity symptoms observation continued until the 14th day to determine the presence of delayed toxic effects. After the 14th day of observing, mice in all groups still showed no changes in autonomic nerves, the central nervous system, hair condition, and the amount of feed consumption. Mice in all groups consume 15 grams / 24 hours of feed (Fig 1B). The body weight increased of mice before and after eel oil administration was not significantly different in all dose (p > 0.05) (Figure 1A).
Statistical analysis showed that ALT and creatinine levels from mice after eel oil administration were not significantly different than the control group (p> 0.05) (Fig 1C and 1D). These results indicate that eel oil does not have toxic effects on liver and kidney function. The liver and kidneys are important in the process of metabolism and excretion so that it becomes a vital organ that has the most possibility to be influenced by drugs or other compounds in toxic effects [16-17]. ALT is specific biochemical enzymes in the blood serum can be used as an indicator when the liver injury [18]. The activity of this enzyme in the serum will increase when liver injury occurs, which causes this intracellular enzyme to be released into the blood [19]. Creatinine is a strong indicator for observing kidney function. It is a yield of the process of creatine phosphate catabolism in skeletal muscle. Creatinine is excreted by the kidneys through glomerular filtration. If kidney damage occurs, the filtration rate will decrease and the creatinine excretion also decreases so that creatinine levels in the blood increase [20].

3.3. Histological Profile

Histology observation of liver and kidney of mice was carried out to determine the cell injury caused by eel oil. Histological examination of liver and kidney showed a significant difference after being statistically analyzed (p <0.05). Histological observations of liver and kidney can be seen in Table 2, Table 3 and Fig 2.

Table 2. The average of liver cell lysis after eel oil administration. Symbols represent statistical significance. *p < 0.05, as compared to control group. n = 6 animals in each group.

| Groups          | Picnotic ± SD (unit) | Carioerixis ± SD (unit) | Kariyolysis ± SD (unit) | Total necrosis ± SD (unit) |
|-----------------|----------------------|-------------------------|-------------------------|---------------------------|
| Control         | 17.50 ± 1.73         | 2.50 ± 1.29             | 2.00 ± 0.81             | 22.00 ± 0.82              |
| Eel oil 0.09 g/ 20g bw | 33.75 ± 1.26         | 9.25 ± 0.96             | 3.25 ± 0.50             | 46.25 ± 1.26*             |
| Groups          | Picnotic ± SD (unit) | Carioerixis ± SD (unit) | Kariyolysis ± SD (unit) | Total necrosis ± SD (unit) |
|-----------------|----------------------|-------------------------|-------------------------|---------------------------|
| Control         | 5.25 ± 0.50          | 7.00 ± 0.82             | 4.25 ± 0.50             | 16.50 ± 1.29              |
| Eel oil 0.09 g/20g bw | 14.50 ± 0.58        | 29.00 ± 0.82            | 3.75 ± 0.57             | 47.25 ± 1.70*             |
| Eel oil 0.25 g/20g bw | 13.25 ± 0.96        | 10.00 ± 1.29            | 5.75 ± 0.82             | 29.00 ± 1.41*             |
| Eel oil 0.74 g/20g bw | 14.25 ± 0.50        | 5.00 ± 0.50             | 10.25 ± 0.96            | 29.50 ± 1.73*             |

Table 3. The average of kidney cell lysis after eel oil administration. Symbols represent statistical significance. *p < 0.05, as compared to control group. n = 6 animals in each group.

Figure 2. Histology profile of liver and kidney organ of mice after were given eel oil. Symbols represent A. liver organ B. Kidney organ. (1) control group, (2) eel oil 0.09 g/ 20g bw group, (3) eel oil 0.25 g/ 20g bw group, and (4) eel oil 0.74 g/ 20g bw group. (a) normal cell, (b) picnotic cell (c) carioerixis cell (d) kariyolysis cell.

The histological profile of liver and kidney damage in mice was a significant difference compared to the control group. This is caused by the high concentration of fatty acids in eel oil. Fatty acids are the main compound in eel oil. The high fatty acids were given to mice increases the absorption of fatty acids in the intestine and improve the content in the blood. The presence of more fatty acid delivers causes accumulation in the liver. The amount of fatty acids in the liver cell membrane increases the sensitivity of the membrane to the activity of free radicals that are autocatalytic [21]. This free radical activity produces lipid peroxide, which induces liver cell injury [22]. Besides, fatty acids need ATP to be oxidized and metabolized. Energy for the oxidation activity of fatty acids is mostly produced by proximal tubules in the kidneys. Under normal circumstances, between consumption of fatty acids in the body, metabolic activity, and oxidative activity are in a balanced condition. When the fatty acids consumed have high levels while the energy produced by the proximal tubules is insufficient there will be an accumulation of fatty acids in the epithelium of the kidney tubules which causes damage to cells in the kidneys. The severity of kidney cell damage can be seen from the nature of the accident. The
amount of cells that have irreversible damage is directly proportional to the severity of damage suffered by kidney cells [23].

4. Conclusion
The results of the acute toxicity study of eel oil have LD50 values> 15 g / kg bw with practically non-toxic categories. Eel oil also does not show toxic effects on toxicological symptoms and biochemical parameters such as ALT and creatinine levels in mice. In histological observation, eel oil significantly affected liver and kidney histology injury at each dose compared to the control group (p <0.05) with moderate damage (26-50%). For further research, observations can be made for more than 30 days (sub-chronic toxicity tests) to provide more information on the long-term use of eel oil.

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References
[1] Haimeur A, Meskini N, Mimouni V, Ulmann L, Messaouri H, Pineau-Vincent F, et al. 2019 Nutrition. 1.57 32–9.
[2] Ristić-Medić D, Takić M, Radjen S. 2018 Therapeutic Foods 329–72.
[3] Zheng S, Chen TC. 2016 Academic Press 249–62.
[4] Mengelberg A, Leatham J, Podd J. 2018 Complement Ther Clin Pract. 1.33 118–23.
[5] Kusharto CM, Widyasari RAHE, Budywiryawan, Wiyono ES, Sugengherisuseno. 2014 Malays J Health Sci 12(1).
[6] Sasonoko H, Saputro BA, Hidayati RWN, S. RSW, Sekarjati TA. 2018 AIP Conf Proc. 2019.1 5006.
[7] Nijman V. CITES-listings, 2015 EU Mar Policy. 1.58 36–41.
[8] Sasonoko H, Kusumastuti NI, Alifa GR, Rahmawati AA. 2019 Pharmaciana. 23. 9(1) 59–70.
[9] Sasonoko H, Sugiyarto S, Budiharjo A, Efendi NR. 2017 Int J Sci Appl Sci Conf Ser. 10.2(1) 174–80.
[10] Ward R, Hintze K. 2016 Fish and Fish Oil in Health and Disease Prevention Academic Press; 217–29.
[11] Chitapanarux I, Traisathit P, Chitapanarux T, Jiratrachu R, Chottaweesak P, Chakrabandhu S, Rasio W, Pisprasert V, Sripinan P. 2020 Current Problems in Cancer. 1.44(1) 100482.
[12] Prior H, Casey W, Kimber I, Whelan M, Sewell F. 2019 Regulatory Toxicology and Pharmacology. 1.102 30-3.
[13] Walum E. 1998 Environmental health perspectives. 106.2 497-503.
[14] Sasonoko H, Efendi NR, Budihardjo A, Farida Y, Amartiwi T, Rahmawati AA, et al. 2017 J Phys Conf Ser. 795.1 12021.
[15] Nghonjuyi NW, Tiambo CK, Taïwe GS, Toukala JP, Lisita F, Juliano RS, Kimbi HK. 2016. Journal of ethnopharmacology. 3.178 40-9.
[16] Andjelkovic M, Buha Djordjevic A, Antonijevic E, Antonijevic B, Stanic M, Kotur-Stevuljevic J, Spasojevic-Kalimanovska V, Jovanovic M, Boricic N, Wallace D, Bulat Z. 2019 International Journal of Environmental Research and Public Health. 16.2 274.
[17] Peter AI, Naidu EC, Akang E, Ogedengbe OO, Offor U, Rambharose S, Kalhapure R, Chuturgoon A, Govender T, Azu OO. 2018 Toxicological research. 34.3 221-9.
[18] Sasonoko H, Pratiwi D, Amartiwi T, Efendi NR, Sugiyarto. 2019 Sciexpress 66–70.
[19] Limdi JK, Hyde GM. 2003 Postgraduate medical journal. 1.79 (932):307-12.
[20] Moledina DG, Hall IE, Thiessen-Philbrook H, Reese PP, Weng FL, Schröppel B, Doshi MD, Wilson FP, Coca SG, Parikh CR. 2017 American Journal of Kidney Diseases. 1.70(6) 807-16.
[21] Clark JM. Chapter 23 - Oxygen Toxicity. In: Neuman TS, Thom SR, editors. Physiology and Medicine of Hyperbaric Oxygen Therapy. Philadelphia: W.B. Saunders; 2008. p. 527–63.

[22] Jia R, Li Y, Cao L, Du J, Zheng T, Qian H, Gu Z, Jeney G, Xu P, Yin G. 2019 *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 1.215 56-66.

[23] Kang HM, Ahn SH, Choi P, Ko YA, Han SH, Chinga F, Park AS, Tao J, Sharma K, Pullman J, Bottinger EP. 2015 *Nature medicine* 21.1 37-46.