Characterization and Acute Toxicity Bioactive Compound Canning Waste of Lemuru Fish Oil as Potential Immunomodulator

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ABSTRACT

Background: Poly Unsaturated Fatty Acids have beneficial effects as an immunomodulator for periodontitis therapy. Lipid studies show that fish can be a unique source of polyunsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid. Lemuru fish (Sardinella longicep) is one of the fish that are abundant in Indonesia. Methods: characterization study was tested using capillary gas chromatography coupled method with flame ionization. Acute toxicity performed by given lemuru orally with dose 174.1-973mg/20g mouse weight for 24 hours observation and sacrificed for histopathology, meanwhile groups 96 hours observation continued. Result: Lemuru Fish oil contains 14.5% eicosapentaenoic acid and 13.2% Docosahexaenoic acid. Acute toxicity showed the percentage of death below 50%, however, liver structure start to show an effect on dose 355.3mg/20g Conclusion: Lemuru Fish oil had a component Poly Unsaturated Fatty Acids an potential immunomodulator. There was no acute toxicity found and the maximum dose without changing the microscopic structure of the liver is 174.1mg / 20g.

Key words: Lemuru fish oil, Eicosapentaenoic acid, Docosahexaenoic acid, Polyunsaturated fatty acid, Toxicity.

INTRODUCTION

Sardines are a group of small pelagic fishes which comes under the family Clupeidae. Sardinella longicep are well-known to be rich in long-chain polyunsaturated fatty acids (PUFA). Fish oil from a sardine species Sardinops melanostica has also been shown to inhibit microbial growth. Fish oil from Sardinella longicep is one type of animal oil derived from fish processing obtained in the coastal Muncar, Banyuwangi. The production is abundant, during this time the lemuru fish besides being made fish oil is also made a fish meal as livestock food. Lemuru fish oil can be used to meet energy sources in the feed because the energy contained in fat is 2.25 times greater than carbohydrate energy and protein. Lemuru fish oil contains n-3 polyunsaturated fatty acids (PUFA) namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Eicosapentaenoic and DHA have a function in reducing the mediator of prostaglandin bone resorption (PGE2) and proinflammatory cytokines (IL-1α, IL-1β, TNF-α). If there is a decrease in PGE2, IL-1 and TNF-α, it will increase osteoblast activity and inhibit osteoclast formation in alveolar bone.

Omega-3 fatty acids, also known as fish oil, have been used for hyperlipidemia, coronary heart disease, hypertension, and other conditions. Some studies have demonstrated that consumption of fish oil concentrate, n-3 polyunsaturated fatty acid (n-3 PUFA), results in cardiovascular benefits that include reductions in mortality, sudden death, nonfatal myocardial infarction, and thrombotic stroke, as well as improvement in graft patency. Omega-3 fatty acids are obtained from two dietary sources: seafood and certain nut and plant oils. Fish and fish oils contain the 20-carbon eicosapentaenoic acid (EPA) and the 22-carbon docosahexaenoic acid (DHA). However, fish do not naturally produce these oils but obtain them through the ocean food chain from the marine microorganisms that are the source of the omega-3 polyunsaturated fatty acids (-3 PUFA) found in fish oil.

Several experimental studies showed that dietary intake of n-3 PUFAs and the improvement in omega-6 and omega-3 ratio could modulate the immune and inflammatory response. The anti-inflammatory effects of fish oils are partly mediated by inhibiting the 5-lipoxygenase pathway in neutrophils and monocytes and inhibiting the leukotriene B4 (LTB4)-mediated function of leukotriene B5 (LTB5). Also, omega-3 decrease interleukins IL-1 and IL-6 inhibits inflammation. For example, rheumatoid arthritis has a strong inflammatory component, which is observed through increased interleukin 1, IL-1.

An acute toxicity test is intended to obtain information about the symptoms of poisoning, causes of death, a sequence of death processes and the range of doses that kill the test animals (Lethal dose 50% or abbreviated LD50) of an ingredient. Should be obtained to increase the confidence in their safety to humans, particularly for use in the development of pharmaceuticals. An acute toxicity test is very important to measure and evaluate the toxic characteristics of a chemical. Effect of Lemuru fish oil as a food supplement or drug on induced
Toxicity remains unclear. Hence the present study to detect acute toxic effects so that a safety picture of lemuru fish oil can be obtained.

**MATERIALS AND METHODS**

All experiments were approved by the Faculty of dentistry Animal Care Committee komite etik penelitian kesehatan gigi (KEPKG) and performed following the guidelines of the Faculty of Dentistry, Universitas Hangtuah Council on Animal Care.

**Characterization**

Lemuru fish oil was collected from a fish canning factory, that been reproduced by the home industry for an additional supplement for duck and chicken farm food. Lemuru fish obtained from Bali strait waters, in Muncar Bayuwangi Indonesia. The catching lemuru fish in the Bali strait is the largest in the world. As much as 6 ml of lemuru fish oil are tested using the capillary gas chromatography coupled method with flame ionization detection (GC-FID).\(^7\)

The procedure was modified from Khodammi methods combine with Soria methods. GC–FID analysis of the volatile components was carried out using an Agilent 4890D instrument coupled to an ionisation flame detector (FID). Compounds were separated on an HP-5 capillary column (5% phenylmethyl polysiloxane, 25 m, 0.32 mm i.d.: 0.17µmfilm thickness) (J&W Scientific, Folsom, CA, USA). The oven temperature was set at 115°C, raised to 180°C at a rate of 8°C/min and held for 10 min and finally raised to 240°C at a rate of 8°C/min and held for 10 min. The sample size was 1 µL and flashed through with carrier gas (helium) at a rate of 1.6 ml/min. Identifications of the methyl esters were made by comparison of retention times of FAME with the standard 37 component FAME mixture.\(^8,10\)

**Acute toxicity**

This study used mice (mus musculus), male, age 3 months with an average weight of 20-30 grams which were divided into 6 groups, control group and 5 treatment groups. Groups were divided into two group observation for 48 hours and 96hours (4 days). Toxicity determination was carried out by looking at the number of death of mice in each treatment group. Lemuru fish oil was given fish oil starting at a dose of 174.1 – 917.3 mg / 20g BW at a single dose, the dose was chosen from non-toxic level until a level of dose that assumed to have a high level of toxicity 200% time level from recommended omega 3 dose. The number of deaths was observed for 24 hours. Before and after treatment mice were weighed, on first observation group after 24 hours of mice sacrificed and observed for liver microanatomy. The others group were continued observed for 96 hours, weighed and observed for the number of death.

**Histopathological analysis**

The part liver was excised quickly and samples were preserved and fixed in 10% neutral formalin for histopathological analysis using haematoxylin-eosin. The sample after being dehydrated in alcohol and subsequently cleared with xylene the tissue samples were then infiltrated with paraffin as blocks, sectioned (5 µm-thick slides) stained with haematoxylin and eosin. In the observations, dilation on sinusoid with haematoxylin-eosin. The sample after being dehydrated in alcohol and subsequently cleared with xylene and subsequently infiltrated with paraffin as blocks, sectioned (5 µm-thick slides) stained with haematoxylin and eosin. The sample after being dehydrated in alcohol and subsequently cleared with xylene the tissue samples were then infiltrated with paraffin as blocks, sectioned (5 µm-thick slides) stained with haematoxylin-eosin.

**RESULT**

Capillary gas chromatography coupled method with flame ionization detection (GC–FID) allows detecting fatty acid profiles quantitatively in lemuru fish oil waste.

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Table 1: Result Characterization of Lemuru Fish Oil.

| Measurand                                      | %    |
|-----------------------------------------------|------|
| **Saturated Fatty Acid**                      |      |
| Capric Acid (C6:0)                            | 0    |
| Caprylic Acid (C8:0)                          | 0    |
| Capric Acid (C10:0)                           | 0    |
| Undecanoic Acid (C11:0)                       | 0    |
| Lauric Acid (C12:0)                           | 0.146|
| Myristic Acid (C14:0)*                        | 13.4 |
| Pentadecanoic Acid (C15:0)                    | 0.825|
| Palmitic Acid (C16:0)                         | 22.4 |
| Margaric Acid (C17:0)                         | 0.794|
| Stearic Acid (C18:0)                          | 4.71 |
| Arachidic Acid (C20:0)                        | 0.403|
| Heneicosanoic Acid (C21:0)                    | 0.339|
| Behenic Acid (C22:0)                          | 0.281|
| Lignoceric Acid (C24:0)                       | 0    |
| Unsaturated Fatty Acid                        | 56.7 |
| **Mono Unsaturated Fatty Acid**               |      |
| Myristoleic Acid (C14:1)                      | 0    |
| Cis-10-Pentadecanoic Acid (C15:1)             | 0    |
| Palmitoleic Acid (C16:1)                      | 11.0 |
| Cis-10-Heptadecanoic Acid (C17:1)             | 0.211|
| Oleic Acid (C18:1n9c)                         | 5.99 |
| Cis-11-Ericosenoic Acid (C20:1)               | 1.81 |
| Erucic Acid (C22:1n9)                         | 3.31 |
| Nervoic Acid (C24:1)                          | 0.647|
| **Poly Unsaturated Fatty Acid**               | 33.7 |
| Linoleic Acid (C18:2n6c)                      | 1.63 |
| Linolenic Acid (C18:3n3)                      | 0.767|
| Y-Linolenic Acid (C18:3n6)                    | 0.358|
| Cis-11,14-Eicosadienoic Acid (C20:2)          | 0.266|
| Cis-11,14,17-Eicosaatrienoic Acid (C20:3n3)   | 0    |
| Cis-8,11,14-Eicosaatrienoic Acid (C20:3n6)    | 2.97 |
| Arachidonic Acid (C20:4n6)                    | 0    |
| Cis-5,8,11,14,17-Eicosapentaenoic Acid (C20:5n3)* | 14.5 |
| Cis-13,16-Docosahexenoic Acid (C22:2)         | 0    |
| Cis-4,7,10,13,16,19-Docosahexaenoic Acid (C22:6n3)* | 13.2 |
| Trans Fatty Acid                              | 0    |
| Elaidic Acid (C18:1n9)                        | 0    |
| Linolelaidic Acid (C18:2n6t)                  | 0.0  |
Each day Mus musculus was weighed and observed, from graphic there were decrease on mostly group after 72 hours observed, this condition due to several mice was died in several groups.

**HISTOPATHOLOGY EXAMINATION**

Cell damage and sinusoid dilation were measured after 24 hours supplementation of lemuru fish oil, to know the effect in the cellular level of the liver.

The histological picture in the control group and the dose group of 174.1mg / 20g showed that there was cell damage in the form of picnotic, cariorexis and cell cariolysis less than 25%, while in the 355.3mg / 20g dose group, it began to show cell changes in the form of picnotic and cariolyis of more than 50%. The group dose of 590.3mg, 755.0mg and 917.3mg / 20gr began to show signs of sinusoid dilation, one sign of toxicity that occurs in the liver is indicated by enlargement of the sinuses.

Cell damage was found increased start in group 4, and sinusoid dilatation was found in group 4, 5 and 6. These results show that in the cellular level lemuru fish oil was found to be effected in the microscopic structure liver.

**DISCUSSION**

Lemuru fish oil derived from lemuru fish (Sardinella longicep) is widely found on the coast of Indonesia, especially Java. The production is abundant, so the price is very cheap. Based on this research we found that lemuru fish oil contains n-3 polyunsaturated fatty acids (PUFA), eicosapentaenoic acid 14,5% (EPA) and docohexasonoic acid (DHA) 13,2%.

**Myristic acid**

Myristic acid (C14:0)in lemuru fish oil is about 13.4% among saturated FA in lemuru total 43.3% one of the highest components in lemuru. Myristic acid given 10% of total energy, consumed food with myristic...
acid also results in a higher HDL-cholesterol concentration within 24 h.\textsuperscript{12} Evidence from controlled clinical studies has shown that can be affected lowering total and LDL cholesterol levels, protecting against thrombogenesis, reducing LDL susceptibility to oxidation, and producing a more favorable glycemic profile, but these fatty acids have hypercholesterolemic characteristics which increase the risk of cardiac diseases.\textsuperscript{13,14}

**Polyunsaturated fatty acids**

Polyunsaturated fatty acids (PUFA) in lemuru fish oil, based on the study was about 33.7%. PUFA have been studied extensively for their benefits in some inflammatory-related diseases. a three-week daily supplementation with 3.2 g of EPA and 2.0 g of DHA reduced eicosanoids and pro-inflammatory cytokines concentration in the sputum of asthmatic patients.\textsuperscript{6} Omega-3 (n-3 PUFAs) are responsible for numerous cellular functions, such as signalling, cell membrane fluidity, and structural maintenance. They also regulate the nervous system, blood pressure, hematic clotting, glucose tolerance, and inflammatory processes, which may be useful in all inflammatory conditions.\textsuperscript{5,11}

**Eicosapentaenoic acid (EPA)**

Eicosapentaenoic acid (C20:5n3) an omega-3 polyunsaturated fatty acid with anti-inflammatory properties, and is present in fish and fish oils, including lemuru as much as 14.5% of total PUFA. Fish oil consumption appears to improve arterial compliance and endothelial function; it is not yet clear as to whether differences exist between EPA and DHA in their vascular effects. In contrast, the beneficial effect of fish oils on inflammation and insulin sensitivity observed in vitro and animal studies.\textsuperscript{16,17} Another study concerning muscle recovery and soreness after performing eccentric biceps curls displayed that seven days of 3 g/day n-3 PUFA supplementation decreased post-exercise muscle damage and soreness.\textsuperscript{7}

**Docosahexaenoic acid (DHA)**

Docosahexaenoic acid (DHA) is a long-chain, omega-3 fatty acid that is normally found in red blood cell (RBC) membranes and outer segments of rod photoreceptors. Lemuru fish oil proof to have DHA (C22:6n3) 13.2% from total PUFA. Besides, DHA seems to increase lipid oxidation and insulin sensitivity in skeletal muscle and it can stimulate glycolytic capacity in myocytes.\textsuperscript{16} DHA (doses recommended by the American Heart Association of two servings per week of fatty fish i.e. salmon, mackerel, herring, albacore tuna, etc. would be sufficient) may be as or more potent in influencing periodontitis an inflammation condition in oral health.\textsuperscript{13}

Conventional therapy for periodontitis combination with omega-3 as host modulation, especially in the presence of DHA and EPA content is recommended for cheaper and safer diet therapy for the prevention and treatment of periodontitis. Given the evidence showing the role of omega-3 in other chronic inflammatory conditions, it is possible that treating periodontitis with Omega-3 as host modulation therapy can added benefit of preventing other chronic diseases associated with inflammation because including DHA and EPA provide dramatic protection against tissue triggered by inflammation, and bone loss associated with periodontitis in the experimental model.\textsuperscript{3,18}

**Acute toxicity**

Observation 24 hours indicate the number of death below 50% of the sample, continued with the observation on 120 hours shown some death is bellow 50% of the sample. But there was decrease weight in all group on 96 hours due to several mice was die in several groups. Supplementation with lemuru fish oil was given once a day with range 174.1 g/20gr- 917.3 g/20gr mice dose. From data, we found that the lowest death of mice found in group dose 355.3 g/20gr and highest death was found in group dose 917.3 g/20gr. According to the American heart

### Table 2: Bioanalysis, number of death after 24 hours oral administration of Lemuru fish oil.

| Group | Dose (Mg/20gbw) | Weight Before Treatment (G) | Weight After Treatment (G) | Number Of Death |
|-------|----------------|-----------------------------|-----------------------------|-----------------|
| 1     | Control        | 24.8                        | 24.4                        | 0               |
| 2     | 174.1          | 26.2                        | 27                          | 0               |
| 3     | 355.3          | 26.6                        | 22.2                        | 1               |
| 4     | 590.3          | 28.2                        | 26                          | 0               |
| 5     | 755.0          | 29.4                        | 28.8                        | 0               |
| 6     | 917.3          | 28.8                        | 29                          | 0               |

### Table 3: Bioanalysis, number of death after 96 hours oral administration of Lemuru fish oil.

| Group | Dose (Mg/20gbw) | Number Of Death |
|-------|----------------|-----------------|
| 1     | Control        | 0               |
| 2     | 174.1          | 0               |
| 3     | 355.3          | 1               |
| 4     | 590.3          | 0               |
| 5     | 755.0          | 1               |
| 6     | 917.3          | 2               |

### Table 4: Effect of lemuru fish oil in microscopic structure liver of Mus musculus after 24 hours.

| Parameters | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 |
|------------|---------|---------|---------|---------|---------|---------|
| Picnotic   | 1       | 1       | 2       | 1       | 2       | 2       |
| Caryolysis | 1       | 1       | 2       | 1       | 1       | 3       |
| Cariorcexs | 1       | 1       | 1       | 2       | 2       | 3       |
| Sinusoid   | -       | -       | -       | Dilatation | Dilatation | Dilatation |
association consumption of omega 3 fish oil that recommended was 500mg-1000mg/day, this dose proof is given best result in protecting heart but no toxic effect.1,10 Those doses if conversed with mice dose about the 1.3mg-2.6mg/20gr weight of mice. In this research we multiplied the dose to know the toxicity dose, from the result we found that the maximum dose used in this study was not toxic. This shows that giving oral doses of lemuru fish-oil up to the maximum dose that can technically be given to test animals (20g /kg BW) or around 134 times the dose commonly used in humans, does not cause death in experimental animals.11 From observation 24 hours and 96hours, there was no death found 50% of the sample.

The increase in the mean bodyweight of the control group and the treatment group from the start of the treatment to the 3rd day is thought to be due to the administration of lemuru oil, giving supplementation with hypercholesterolemic characteristics can cause an increase in body weight.11 However, on day 4, the average weight loss was found in group 3, group 5 and group 6, this happened because there were rats that died so that it affected the average body weight of the mice. However, from the histopathology in liver in 24 hours supplementation was found damage cell that increases start from dose 353.3 mg/20gr and the cell damage increase with dose level. Based on the data from the results of this study, it can be seen that after administration of lemuru oil group 3, group 4, group 5, and group 6 showed damage to the microanatomic structure of the liver, which is characterized by changes in the cell nucleus (pnicnotic nucleus, karyolysis, carioiexsis) and sinusoid dilation were found in group 4 (dose 590.3 mg/20 gr), group 5 (dose 755.0 mg/20 gr), and group 6 (dose 917.3 mg/20 gr). From this study, it was found that at a dose of 355.6 mg /20 gr had caused microscopic damage to the liver, even though the number of animal deaths in trials was below 50%. It can be concluded that the maximum dose that can be given to mice without changing the microscopic structure of the liver is 174.1mg /20gr.

CONCLUSION

Lemuru fish oil contains rich in fatty lipid acid-like PUFA, including EPA and DHA that can be used as a potential immunomodulator. Eicopentasanoic acid (EPA) and DHA are known for their benefits in controlling inflammation, supplementation lemuru fish oil can be useful to decrease disease related to inflammation such periodontitis in dental disease. Lemuru fish oil proof have no acute toxicity and maximum dose that recommended without changing the microscopic structure of the liver is 174.1mg /20g.

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