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Acute and Lethal Dose Toxicity Test of Pure Eel Fish (*Anguilla marmorata* [Q.] Gaimard) Oil in Mice (*Mus musculus*)

Jamaluddin Jamaluddin[1*], Ihwan Ihwan[1], Khildah Khaerati[1], Asriani Hasanuddin[2], Novalina Serdiati[3], Jusri Nilawati[3], Berniawan Wijaya[1], Yuliet Yuliet[1], Iftitah Ahmad[1]

[1]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Tadulako University, Jalan Soekarno-Hatta, Tondo, Palu City, Central Sulawesi, 94148, Indonesia

[2]Department of Animal Husbandry, Faculty of Animal Husbandry and Fishery, Tadulako University, Palu, Central Sulawesi, 94148, Indonesia

[3]Department of Aquaculture, Faculty of Animal Husbandry and Fishery, Tadulako University, Palu, Central Sulawesi, 94148, Indonesia

[*]**Corresponding Author: E-mail:** jamal_farmasi02@yahoo.co.id

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ABSTRACT

Introduction: Eel fish oil (*Anguilla marmorata* (Q.) Gaimard) highlighted its nutritional advantages, especially vitamin A, EPA (*eicosapentaenoic acid*), DHA (*docosahexaenoic acid*), and omega-3. Unsaturated fats in fish oil are known to have positive effects in the treatment of various diseases, although the potential negative impacts also need to be considered.

Methods: This research aims to focus on the cytotoxic activity of eel fish oil and carry out extraction using the soxhlation method, *Thomson and Weil,* and *Metode fixed dose*. To evaluate the toxicity of the extract, the acute toxicity test was carried out by measuring the middle lethal dose (LD_{50}), which is often used as a benchmark. Apart from that, the research also aims to determine the lethal dose 50 (LD_{50}) value of eel fish oil, namely the dose that can cause the death of 50% of the test animal population. The extraction process uses diethyl ether solvent at 60°C, followed by purification using a 3% bentonite adsorbent. The test was carried out twice with female mice and rats as test animals, divided into groups with oral doses of 5, 50, 300, 2000, and 5000 mg/kg.

Result: The results showed no deaths in the test animals, and the LD_{50} of eel fish oil extract was greater than the dose used. Clinical symptoms in mice include decreased motor activity and increased grooming frequency as the dose increases, as well as increased muscle tone and tension responses.

Conclusion: Based on the research results, it can be concluded that eel fish oil extracts up to a dose of 5000 mg/kg are still classified as "safe" for use because they do not cause death in test animals.

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INTRODUCTION

Fish oil, a valuable source of fatty acids, offers a range of potential health benefits. These fatty acids vary in carbon chain length (C12-26) and the number of double bonds (0-6). While fish oil typically comprises 15-25% saturated fatty acids, it's the 35-60% of monounsaturated and 25-40% of polyunsaturated fatty acids that garner the most attention for their positive impacts on health, potentially including reducing the risk of conditions like cancer, heart disease, and arthritis. Eel fish oil stands out with a particularly rich nutritional profile (1). Beyond its high fat content (21.35%), it boasts significant levels of vitamin A, DHA (docosahexaenoic acid), and EPA (eicosapentaenoic acid). Notably, eel surpasses other fish like salmon and mackerel in DHA and EPA content (2). Eel fish oil contains 1,337 mg/100g of DHA compared to 820 mg/100g in salmon and 748 mg/100g in mackerel. Similarly, its EPA content reaches 742 mg/100g, exceeding the 492 mg/100g found in salmon and the 409 mg/100g in mackerel.

DHA and EPA, highly potent omega-3 polyunsaturated fatty acids, hold promise in pharmaceutical applications. These fatty acids have been investigated for their potential therapeutic roles in various conditions, including atherosclerosis, cancer, heart disease, stroke, systemic lupus erythematosus (SLE), inflammation, hypertension, growth disorders, diabetes, and fungal infections. In the pharmaceutical industry, high-quality, pure fish oil is a key ingredient in products like cod liver oil (often fortified with vitamin D3), high-concentration EPA/DHA supplements, and children's fish oil emulsions. Cod liver oil is traditionally used to support brain development and may help prevent degenerative diseases. The omega-3s present in fish oil are also thought to contribute to maintaining healthy vision (3).

River fish, including eels, offer a unique profile compared to their marine counterparts. Their freshwater habitat and specific environmental conditions influence the characteristics of their oil. River fish oil may exhibit distinct fatty acid compositions and potentially different levels of certain nutrients compared to marine fish oil. For example, the types of algae and other food sources available in freshwater ecosystems can impact the accumulation of specific fatty acids in the fish. Furthermore, it is crucial to consider potential environmental contaminants present in freshwater systems, as these could accumulate in the fish and subsequently in the fish oil. Therefore, rigorous testing for heavy metals and other toxins is essential to ensure the safety and quality of river fish oil. While the high DHA and EPA content in eel oil is promising, further research is needed to fully understand the specific characteristics of river fish oil, including its potential benefits and any associated risks. This will help determine the suitability of river fish oil for various applications, including pharmaceutical and nutritional uses. The quality of river fish oil is determined by its bright yellow color, characteristic fish aroma, and compliance with safety standards based on scientific evidence. However, potential toxicity risks, such as heavy metal contamination or oxidative compounds, must be considered. Fish oil that will be used as a raw material for a product must meet the requirements where the oil is bright yellow and has a distinctive fish aroma and must meet safety aspects based on scientific evidence. To fulfill these requirements, toxicity testing can be carried out as supporting evidence for the safety of a preparation to be tested (4).

One method that can be used to test toxicity parameters is the acute toxicity test. The acute toxicity test is one of the pre-clinical tests. This test is carried out by measuring the degree of toxic effect of a compound that occurs within 24 hours or up to 14 days to see the effect of an extract given using a single dose (5). The benchmark most often used to state the acute toxicity dose is the middle lethal dose (LD_{50}). Toxicity tests provide information about the health hazards resulting from exposure to certain substances in the body. It is hoped that the results of this toxicity test can become a reference for the use of eel fish oil as a complementary medicine in the community to avoid excessive use, which can cause poisoning. The data obtained include clinical appearance, morphology, and mechanisms of toxic effects.

This research needs to be carried out because there is still not much-published research regarding the safety level of fish oil and the importance of knowledge about the negative impacts of consuming fish oil fat in large quantities, especially in eel fish (*Anguilla marmorata* (Q.) Gaimard) yellow eel phase from the Palu River. This research was carried out by determining the acute toxicity value (LD_{50}) of eel fish oil administered orally and determining the LD_{50} value from the toxicity category in the test preparation classification criteria table (6). These are some of the reasons behind this research.

METHODS

Types of research

This study employs two methods: pure experimental research, which involves acute toxicity testing using the fixed-dose method according to OECD guidelines and the observation of toxic symptoms, and in vivo experimental research, which includes acute toxicity testing with parameters based on the Thomson and Weil method.

Research period and location

This research was carried out in December 2021– December 2023, where eel fish oil samples were prepared **(***Anguilla marmorata* (Q.) Gaimard) in the Analytical Chemistry Laboratory and extraction was carried out in the Pharmacognosy-Phytochemical Laboratory, fish oil emulsion was made in the Pharmaceutical Laboratory, treatment of test animals was carried out in the Pharmacology-Biopharmacy Laboratory, Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Tadulako University, Palu, Central Sulawesi, Indonesia.

Research tools

The tools used in this research were a soxhletation tool, a rotary evaporator (EYELA®), a Centrifuge C2 Series tool, centrifuge tube, dropper pipette, flannel cloth, hotplate stirrer (DENVILLE®), porcelain cup, blender (Cosmos®), oven (SHEL LAB®), separating funnel, aluminum foil, measuring flask (IWAKI CTE33®), Erlenmeyer (IWAKI CTE33®), beaker glass 500 mL (IWAKI CTE33®), test tube, mortar and pestle, stir bar, brown bottle, analytical balance (Precisa XB4200C®), measuring cup (IWAKI CTE33®), mixing homogenizer, scissors, dispo (OneMed), and oral sonde.

Sources of research materials

The main ingredient used is the eel species (*Anguilla marmorata* (Q.) Gaimard). The chemicals used for testing include diethyl ether, 3% bentonite, 0.5% Na-CMC, Tween 80, and Span 80. White mice are female because they are slightly more sensitive, 2 to 4 months old, weighing 20 to 30 grams, and 25 mice were used. with an age of 2-4 months and a body weight of 180-250 grams, there are 25 individuals.

Preparation of pure eel fish oil extract

The eel fish was cleaned (the gills and entrails were removed), then washed until there was no more blood and mucus. The fish was cut into smaller pieces. Next, the fish that has been cut into small pieces is placed in the oven at 60°C for 24 hours. Then grind it using a blender until it becomes powder, and store it in a stainless-steel container (2). A total of 50 grams of sample was weighed and put into each fat cartridge. After that, fill the round bottom flask with 150 milliliters of diethyl ether solvent. After that, extraction was carried out for five hours at 60°C. In a round-bottomed flask, dry the fat and solvent mixture by evaporating it. For approximately one hour, bake at 105°C. After that, the fat and pumpkin were cooled for 30 minutes in a desiccator. Make sure the fat-laden round pumpkins are the same weight (7). The extracted fish oil is heated using a *hot plate* at a temperature of 60°C, then bentonite with a concentration of 3% by weight of fish oil was added. Then, it is mixed with an attractive stirrer for 30 minutes. After that, the pure oil was extracted using a dropper pipette and centrifuged for 30 minutes at 2600 rpm (8).

Preparation of a pure eel fish oil extract emulsion

Oil-in-water (o/w) emulsion is a type of emulsion used in the production of pure eel fish oil extract. This emulsion preparation is used in the process of making extracts. In making an emulsion preparation with a weight of 100 mL, it is divided into two phases, namely the oil phase and the water phase. In the oil phase (fish oil, span 80) and water phase (Tween 80 and distilled water 100 mL, NaCMC 0.5%), to make NaCMC, mucilage is made first. Each phase is heated first at a temperature of 70°C on a hotplate. From the oil phase to the water phase little by little while stirring using a homogenizer at a speed of 100 rpm for 15 minutes until an emulsion forms, the tool is turned off, and the emulsion is put into a brown bottle (9).

Preparation of 0.5% Na-CMC

A total of 1000 ml of distilled water is heated at a temperature of ± 70°C. Add 5 grams of Na-CMC little by little while stirring using a stirrer until a homogeneous colloidal solution is formed.

Administration of pure eel fish oil

Oil-in-water (o/w) emulsion is a type of emulsion used in the production of pure eel fish oil extract. This emulsion preparation is used in the process of making extracts. In making an emulsion preparation with a weight of 100 mL, it is divided into two phases, namely the oil phase and the water phase. In the oil phase (fish oil, span 80) and water phase (Tween 80 and distilled water 100 mL, NaCMC 0.5%), to make NaCMC, mucilage is made first. Each phase is heated first at a temperature of 70°C on a hotplate. From the oil phase to the water phase little by little while stirring using a homogenizer at a speed of 100 rpm for 15 minutes until an emulsion forms, the tool is turned off, and the emulsion is put into a brown bottle (9).

Treatment of test animals

White mice are female because they are slightly more sensitive, aged 2 to 4 months, weighing 20 to 30 grams, and 25 mice were used. Before conducting the research, mice were acclimatized for one week by being given enough food and drink. The mice that will be used are mice that are healthy and visually show normal treatment. Animals used as test subjects are selected randomly and then given identification marks. Treatment of test animals.

And then, for the test animals, mice were separated into 5 groups after fasting for 3-4 hours before treatment. The following procedure was used to administer pure eel fish oil emulsion orally to each group of five mice, with the following treatment:

Group I: given pure eel fish oil emulsion at a dose of 5 mg/Kg.
Group II: given pure eel fish oil emulsion at a dose of 50 mg/Kg.
Group III: given pure eel fish oil emulsion at a dose of 300 mg/Kg.
Group IV: given pure eel fish oil emulsion at a dose of 2000 mg/Kg.
Group V: given pure eel fish oil emulsion at a dose of 5000 mg/Kg.

Observation of test animals

According to (Turner, 1965) various toxicity criteria that can be considered in clinical observations of test animals include:

Behavioral profile (behavior history)

Mice will show alertness (*awareness*). In addition, mice also experience movements such as looking for the head, spinning, biting themselves, walking backward, and licking, which showed central stimulation or depression. Mice were placed and restrained using the thumb and forefinger on the skin of the back of the neck, but the mice were allowed to walk. A normal mouse will struggle to escape. If toxicity occurs, then the mice will weaken, and their strength will decrease.

Neurological profile

Central Excitation (Arousal center)

Seeing Straub's response, tremors, and seizures from the mice.

Straub answered (tension response), a tense state of the tail. The mice's tails were observed to see whether stiffness occurred after administration of the extract or not.

Tremor (shaking), visible shaking due to stimulation of the central nervous system. Observe by placing the mice on the floor and then see whether the mice experience tremors or not when standing still or walking.

Convulsion (seizures), mice appear to convulse when stimulated by the central nervous system. Observe by placing the mice on the floor and seeing whether they experience convulsions or not.

Motoric Coordination (motor coordination)

Body position and limb position.

Thank you (staggering), the mice will be allowed to walk, and if toxicity occurs, abnormal walking will be seen.

Somersault test (reflex reaction), the mouse is placed with its back as a footing. A normal mouse will turn its body back and if there is toxicity then there will be an inability to turn itself by showing depression.

Muscle Tone (muscle formation), is done by letting the mice catch a pencil in a horizontal position. Then the grip strength and muscle tension in the mice were looked at.

Reflexes, this is done by touching the center of the earlobe (*surface)* with hair or other fine instruments. Mice that are not affected will withdraw.

Autonomic profile (autonomous profile)

Optical signs (Visual sign)

Pupil size (pupillary size), pupil dilation, or mydriasis are signs of adrenergic sympathomimetic or parasympatholytic effects, conversely, narrowing or miosis are signs of parasympathomimetics or cholinergic.

Palpebra position (palpebral position), the eyelids are usually open, if they are narrowly open or ptosis this is a sign of an effect of tranquilizer or sedation, and vice versa if the pupils are dilated, this is a sign of the effects of sympathetic stimulation.

Secretory Signs (Sign of secretion)

Urinary, excessive urine output which has muscarinic properties or irritation of the urinary tract.

Salivation, where there is a lot of saliva produced with muscarinic signs.

General Signs (General characteristics)

Writhing (squirming), the animal will press its stomach to the bottom of the platform because it feels pain.

Piloerection (piloerection), animal hair will stand up as a sign that there is cold air which indicates an adrenergic or adrenaline effect.

Skin colour (skin color), Pale skin indicates an adrenergic effect, or vasoconstriction, while redness indicates vasodilation.

Ethical clearance

Research ethics is taken up by the Medical and Health Research Ethics Committee, Faculty of Medicine, Tadulako University, to ensure scientific principles, ethics, and practices as guidelines for research No. 682/UN28.1.30/KL/2023.

Data analysis

The data collected in this research is primary data from observations of experimental animals, both control and treatment groups. The data obtained is in the form of quantitative and qualitative data. The quantitative data that will be obtained is the number of experimental animals that died. LD_{50} data was taken from the number of dead and living mice in each group. Next, the LD value is calculated50 using the Thomson and Weil method. According to Manggung (10) this method is widely used because it does not require too many experimental animals and has a high level of confidence or*"confidence level"* which is quite high. Calculation of LD value50 using the Thomson and Weil method. The Thomson and Weil calculation table is used to determine the LD value50. LD value50 calculated with the following equation:

Formula : $\log m = \log D + d (f+1)$

Where : m = LD value50

D = The smallest dose used

d = Log of dose multiples (log R)

f = A factor in Weil's table, because of the number of certain death (r).

RESULTS

Eel fish (*Anguilla marmorata* (Q.) Gaimard) fase *yellow eel* with a wet simplicia weight of 1,400 grams. Then extracted using the soxhletation method using diethyl ether solvent at a temperature of 60℃ for approximately 5 hours then evaporated for approximately 1 hour. Results of eel fish oil extract (*Anguilla marmorata* (Q.) Gaimard) which is 29.07 grams for Thomson and Weil method and 135,21 – 157,19 for method *fixed dose*. The percent yield value obtained was 2.07% for Thomson and Weil method and 49,31% for method *fixed dose*. The yield calculation was carried out to compare the amount (quantity) of extract produced from oil extract. Based on the table 2, the yield

of eel fish oil weighing 135.21 g obtained a yield of 46.29%, the yield of eel weighing 157.19 g obtained a yield of 52.39%, thus obtaining an average value of 49.34 and a standard deviation. 4.31.

Table 1. Percent (%) yield of eel fish oil (*Anguilla marmorata* (Q.) Gaimard) in rat test animals using the Thomson and Weil method.

Weight Extract (g)	Solvent	Oil Extract Weight (g)	Yield (%)
29.07	Diethyl ether	189.31	2.07

Table 2. Percent (%) yield of eel fish oil extract (*Anguilla mamrorata* (Q.) Gaimard) in mice test animals method *fixed dose*.

Weight (g)	Solvent	Oil Extract Weight (g)	Yield %
135.21	Diethyl ether	62.60	46.29
157.19	Diethyl ether	82.36	52.39
	Mean ± SD		49.34 ± 4.31

Fish oil refining

Based on the results of refining eel fish oil (*Anguilla marmorata* (Q.) Gaimard) *yellow eel* using 3% Bentonite adsorbent can be seen in table 3 below:

Table 3. Results of Purification of eel fish oil (*Anguilla marmoratal* (Q.) Gaimard) in rat test animals using the method *Thomson and Weil*.

Method *Thomson and Weil*	Before Purification (a)	After Purification (b)	Foreign components in oil (a-b)
Volume (ml)	43,57	34,29	9,28
Weight (g)	34,21	29,07	5,14

Metode fixed dose	Treatment	Before Purification	After Purification
Volume (ml)	A	73.82	66.01
	B	97.12	87.99
	Average value		77
Weight (g)	A	62.60	55.98
	B	82.36	74.62
	Average value		65.3

Information:

A: The 1st purification

B: 2nd Purification

Method *Thomson and Weil* the adsorbent used is Bentonite, amounting to 3% of the total yield of crude eel fish oil which can be seen in table 3. With foreign components, the volume is 9.28 ml and the weight is 5.14 g. Method *fixed dose* Purification of oil is carried out to remove unpleasant tastes and odors (rancidity). In this research, 3% bentonite was used as an adsorbent. Based on table 3, the volume of the first purification was 66.01 ml and the second

purification was 87.99 ml with a weight of 55.98 g in the first purification and 74.62 g in the second purification. So the average volume value is 77 and the average weight value is 65.3.

Toxicity test results

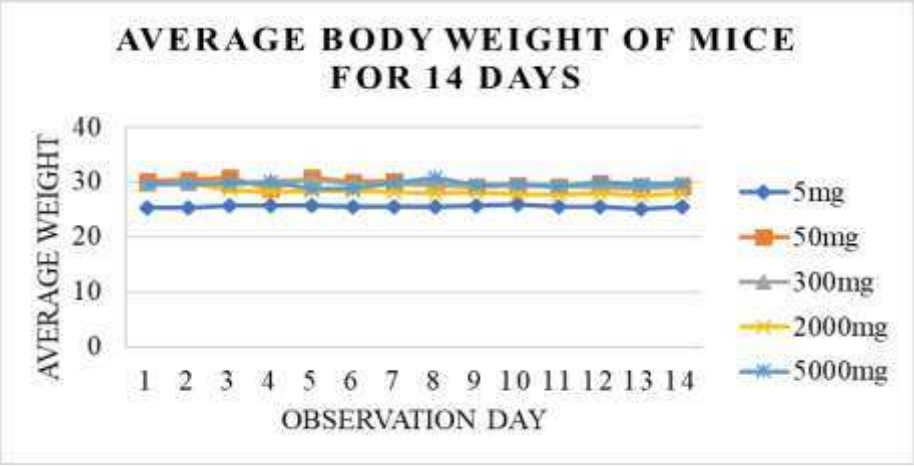
The results of the toxicity test of eel fish oil extract (*Anguilla marmorata* (Q.) Gaimard) in white mice (*Rattus norvegicus*) are presented in Table 4. From the table above, it can be seen that there were no deaths in the test animals in each test group with a predetermined dose so the LD50 value was not obtained and the LD_{50} value was declared "pseudo" LD_{50} by taking the highest dose used. Ingestion of the entire dose does not cause death or toxicological symptoms. This showed that it did not cause toxicity or death in female mice and rats at any dose.

Table 4: Number of Deaths of Thomson and Weil Method Mice and Method Mice *Fixed Dose.*

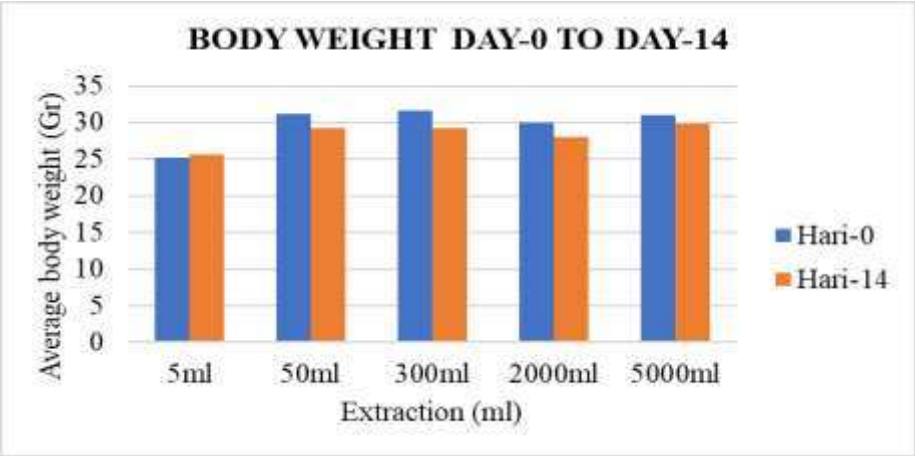
NO	Group	Number of Mice	Extract Dosage (mg/kg)	Number of Deaths
1	I	5	5	0
2	II	5	50	0
3	III	5	300	0
4	IV	5	2000	0
5	IN	5	5000	0

Weight observation

Observations of mice test animals obtained an average body weight for 14 days after Administration of eel fish oil extract in the 5 mg/kg, 50 mg/kg, 300 mg/kg, 2000 mg/kg, and 5000 mg/kg dose groups experienced relative weight loss but increased on certain days. However, there was no significant difference in weight gain between all treatment groups. This can be seen in graphic 1 and 2 below:



Graph 1: Observation of mice body weight for 14 days.



Graph 2: Difference between day 0 and day 14 in mice

Difference between Day 0 and Day 14

To assess variations in body weight in test animals, graph 1 and 2 compares body weight observations on days 0 and 14. At a dose of 5 mg/kg, the body weight of test animals increased by 0.4 g, and at a dose of 50 mg/kg, 300 mg/kg, 2000 mg/kg, and 5000 mg/kg decreased respectively by 2 gr, 2.4 gr, 2 gr, and 1.2 gr. This is in line with the declining level of toxicity when increasing the extraction dose. Thus, it can be stated that the administration of eel fish oil extract did not affect the growth and weight development of mice during the 14 days of observation after administration of the test material.

Administration of eel fish oil extract (*Anguilla marmorata* (Q.) Gaimard) studied for 6 hours to 24 hours on the first day of administration did not show data on the number of deaths in test animals, according to the study. The findings are shown in Table 3, this is because the extract dose is still within the maximum dose limit and the length of time is still relatively small to cause toxic symptoms. According to Nugroho *et al.* (11) The amount of time a chemical is exposed to a material (also known as "duration") is a significant component that influences toxicity. Longer exposure will harm the organism more chronically. However, from the results of observations testing the toxicity parameters of eel fish oil extract (*Anguilla marmorata* (Q.) Gaimard) several clinical symptoms occurred, the clinical symptoms that occurred were due to the response that emerged from the administration of the test material. The dose-response connection is the relationship between the quality of exposure and various consequences. The chemical administered is what causes the reaction, and the dose impacts the reaction. There must be a quantitative approach to precisely measure the toxicity of chemicals when using dose-response. Based on Titiek (12) correlation relationships known as dose-response relationships are shaped by exposure features and the range of consequences. The chemical administered is the cause of the reaction, and the dose influences the reaction. These include treatment, ptosis, mydriasis, saliva, diuretics, feces, and motor activity. At the maximum dose of 5000 mg/kg BW, most clinical signs and toxic consequences are seen. After six hours of observation, clinical symptoms and toxic effects occurred more frequently, but after 24 hours of administering the extract, the symptoms began to decrease. Behavioral and performance changes were recorded throughout the observation period. The mice exhibited alertness at lower doses but showed signs of neurological depression at higher doses. Some test subjects displayed signs of tremors and Straub's tail response, suggesting central excitation. Motor impairments, such as staggering movements and difficulty in maintaining coordination, were observed, particularly at higher doses. The somersault test indicated that some mice experienced reduced reflexes, struggling to right themselves when placed on their backs. Additionally, weakened grip strength in the muscle tone test suggests reduced neuromuscular function.Autonomic responses were also observed, with changes in pupil dilation (mydriasis) and ptosis being notable at higher doses. Increased salivation and urination were evident in some test subjects, correlating with muscarinic stimulation. General signs such as piloerection and writhing behavior were also documented, indicating physiological stress responses. After six hours of administration, these symptoms were more pronounced, but they gradually subsided within 24 hours as the drug concentration decreased. Based on research conducted by Nugroho *et al.* (13) the inability to reverse itself indicates

depression of the central nervous system. the inability to right itself suggests central nervous system depression. This motor dysfunction may lead to decreased motor efficiency, further disrupting normal activity levels.

DISCUSSION

In this study, the sample used was the eel fish species (*Anguilla mmarmorata* (Q.) Gaimard) combined adult phase (*yellow eel* and *silver eel)* This type is the type of fish most consumed by the public which can be obtained from the Palu River. This research is experimental in vivo to determine the toxic properties of eel fish oil and also determine the toxicity category of eel fish oil extract. According to Mustapa *et al.* (14) it is important to know the degree of efficiency, safety, and various effects of a compound to provide information to consider its use. There has been a lot of research regarding the pharmacological activity of fish oil. This is supported by Alifa (15) who stated that the calculation results showed that the anti-inflammatory power at the 1st dose was 34.35%. 2nd, namely 35.132%, and 3rd, namely 40.28% and by Kusumastuti (16) regarding "Testing the Analgesic Activity of Eel Fish Oil (*bicolor eel*) in Male Mice with Acetic Acid Induction" states that fish oil has an analgesic effect at a dose of 2.34 mg/20 g; 4.68 mg/20 g; and 7.02 mg/20 g and administration of fish oil 7.02 mg/20 g provides an analgesic power of more than 50%. Therefore, this research is based on previous research guided by the effective dose in research on the pharmacological activity of eel fish oil extract *(Anguilla marmorata* (Q.) Gaimard) which can reduce triglyceride levels in white mice at a dose of 90.72 mg/ 200g BW (17).

The oil-in-water (o/w) emulsion was chosen for the formulation of pure eel fish oil extract due to its stability, ease of absorption, and suitability for oral administration. This type of emulsion allows for the effective dispersion of hydrophobic fish oil in an aqueous medium, improving bioavailability and preventing phase separation. The selection of Span 80 and Tween 80 as emulsifiers was based on their HLB (Hydrophilic-Lipophilic Balance) values, which are ideal for stabilizing oil-in-water emulsions. Span 80 (HLB ~4.3) stabilizes the oil phase, while Tween 80 (HLB ~15) stabilizes the water phase, ensuring a well-balanced emulsion. The combination of these surfactants helps reduce interfacial tension and prevent coalescence of oil droplets. NaCMC (0.5%) was included as a suspending agent to enhance emulsion stability and viscosity, preventing phase separation during storage. Heating both phases to 70°C facilitates emulsification by reducing viscosity and improving the dispersion of oil droplets. The homogenization step at 100 rpm for 15 minutes ensures uniform droplet size, which is crucial for maintaining emulsion stability over time. The mice test animals were divided into 5 test groups to see the number of deaths and clinical symptoms that appeared in the test animals after administration of eel fish oil extract (*Anguilla marmorata* (Q.) Gaimard) with several doses. The doses used are 5, 50, 300, 2000, and 5000 mg/kg based on BPOM regulations (2022). The high dose is also by research that has been conducted Herza *et al.* (18) where increasing the dose given shows symptoms of toxicity. The basis of dose is based on the orientation used in determining the dose of a compound in acute toxicity testing which is taken from the effectiveness of the dose from the results of pharmacological tests with test animals and the same route of administration (19).

The chosen doses follow the standard approach used in acute toxicity testing, where a logarithmic dose scale is applied to assess toxicity thresholds systematically. The lower doses (5 and 50 mg/kg) represent potential therapeutic levels, while the higher doses (300, 2000, and 5000 mg/kg) serve to determine toxicity limits and lethal effects. The highest dose of 5000 mg/kg is consistent with OECD guidelines for acute toxicity testing, which classify substances based on their LD50 values. Additionally, the dose selection was guided by the pharmacological effectiveness of similar extracts in previous studies. The administration route and test species were kept consistent to ensure reliable toxicity evaluation. This approach allows for a comprehensive assessment of the extract's safety profile, ensuring that both therapeutic and toxic effects can be identified systematically.

This research was carried out to know the condition of the test animals after administering the test material at a predetermined dose. The most common sign that an animal is in pain or suffering is when its body weight loses more than 20% or more than 25% for seven days or more. Changes in body weight in test animals after extract treatment indicate disease or illness (20). However, during the 14 days of observation, not a single test animal experienced pain or discomfort as a result of consuming the extract, which caused the test animals to gain weight.

Based on (**tables 1 and 2**), the extract yield value is needed in the extraction process because it can be used as a reference and comparison for how much extract can be produced from a sample. This is also related to the amount of bioactive content contained, because the greater the yield it can be assumed that the content of bioactive compounds contained in the sample is greater, this is in line with what was reported by Nurhayati & Permatawati (21) that a high

soak value indicates the large number of bioactive components contained therein. The value obtained is useful as an orientation to describe the amount of fish oil that can be produced, however, a higher percent yield does not indicate a better quality of fish oil. Based on research Tirpitz *et al.* (22) percent yield of the final product is used as a comparison with the main ingredients. The more you get, the more fish oil you get. The oil resulting from the extraction process has a quality that is inversely proportional to the percentage yield value, so it can be assumed that the value obtained does not affect the quality of the fish oil content, and therefore the refining process must also be carried out to improve the quality of the fish oil.

To measure the harul eel fish test it carefully, then put it in a fat sleeve. After that, fill the round bottom flask with 150 milliliters of diethyl ether solvent. After that, extraction was carried out for five hours at a temperature of 60°C. In a round-bottomed flask, dry the fat and solvent mixture by evaporating it. For approximately three hours, bake at 105°C. After that, the fat and pumpkin were cooled for 30 minutes in a desiccator. Make sure the fat-laden round pumpkins are the same weight (23). The % yield results can be seen in (**tables 1 and 2**), the extract yield value is needed in the extraction process because it can be used as a reference and comparison of how much extract can be produced from a sample. This is also related to the amount of bioactive content contained, because the greater the yield it can be assumed that the content of bioactive compounds contained in the sample is greater, this is in line with what was reported by Nurhayati & Permatawati (21) that a high soak value indicates the large number of bioactive components contained therein. The value obtained is useful as an orientation to describe the amount of fish oil that can be produced, however, a higher percent yield does not indicate a better quality of fish oil. Based on research Tirpitz *et al.* (22) percent yield of the final product is used as a comparison with the main ingredient. The more you get, the more fish oil you get. The oil resulting from the extraction process has a quality that is inversely proportional to the percentage yield value, so it can be assumed that the value obtained does not affect the quality of the fish oil content, and therefore the refining process must also be carried out to improve the quality of the fish oil.

The resulting eel fish oil extract is then purified using adsorbents. The purification process aims to increase the quality value of the oil, especially the fatty acid content in the oil, and slow down the process of damage to the oil. Based on Ayu (24) the purification process for fish oil aims to remove impurities and components that do not come from fish oil to improve the quality of the oil. The adsorbent used is Bentonite, amounting to 3% of the total yield of crude eel fish oil which can be seen in (**table 3**). The use of bentonite as an adsorbent in purification is based on the explanation Rusli (25) that bentonite is the best adsorbent compared to activated charcoal and zeolite.

The results of the research can be seen in **table 4**, this table shows that the administration of eel fish oil extract (*Anguilla marmorata* (Q.) Gaimard) which was observed for 6 hours - 24 hours on the first day of administration showed that there was no data on the number of deaths in the test animals, this was because the extract dose was still within the maximum dose limit and the period was still relatively small to cause toxic symptoms. According to Kurniawidjaja *et al.* (26) Toxic effects in biological systems will not occur if the chemical does not reach the appropriate location in the body at a concentration and for a long time sufficient to produce toxic manifestations. On research Sartika *et al.* (27) consuming large amounts of fat or omega-3 compounds can affect high blood cholesterol which can trigger toxic effects on the body such as degenerative diseases such as stroke and coronary heart disease. Observation results of testing the toxicity parameters of eel fish oil extract (*Anguilla marmorata* (Q.) Gaimard) Several symptoms occurred, the clinical symptoms that occurred were due to the response that emerged from the administration of the test material. Based on Aspari *et al.* (28) Exposure characteristics and the spectrum of effects together form a correlation relationship known as the dose-response relationship. The response arises due to the chemical being administered and the response is dose related. Among them are motor activity, ptosis, mydriasis, salivation, diuresis, defecation, and grooming. The most clinical symptoms and toxic effects were seen at the highest dose, namely 2400 mg/200g BW. Clinical symptoms and toxic effects increased in frequency at 6 hours of observation, however at 24 hours of extract administration, clinical symptoms and toxic effects appeared to begin to decrease. The increases and decreases that occur are influenced by the time the drug concentration decreases over time. The motor activity that occurs is influenced by the presence of obstacles or motor disorders which manifest as symptoms of impaired motor coordination, such as the rat's ability to turn itself over when placed on its back as a platform. Based on research conducted by Wahyu *et al.* (29) the inability to reverse itself indicates depression in the central nervous system. This motor disorder can cause a decrease in motor function which becomes less effective, which can cause motor activity to be disrupted.

Observation of the test animal's body weight for 14 days to know the condition of the test animal after administering the test material at a predetermined dose. Body weight examination in the acute toxicity test is carried out because it is the most sensitive indicator of the condition of the test animal. Conditions that can indicate an animal is experiencing pain or suffering are generally when body weight has decreased by more than 20% or body weight has decreased by more than 25% over 7 days or more. Test animals' fluctuating changes in body weight indicate illness or suffering after administration of the extract (30). However, in observations carried out for 14 days, no test animals were found to experience illness or suffering after administration of the extract, this was indicated by an increase in the body weight of the test animals. Eel fish oil extract is considered safe for use because it does not cause significant pain or suffering to the body weight of the test animals. There was no significant weight loss due to the absence of influence between clinical symptoms and the toxic effects of the sample on the mice's body weight, as indicated by 14 days of observation of the body weight of the mice which did not experience significant weight loss. The increase or decrease in body weight that occurs in mice is not necessarily influenced by the administration of the test compound but can be due to factors resulting from stress or depression experienced by the mice and can influence the mice's eating patterns, resulting in changes in the mice's body weight, as well as the environment can be a problem. one factor that can cause stress levels in mice to increase. Weight gain also occurs due to the content of eel fish oil compounds which have chemical properties and pharmacological effects as supplements to increase appetite (31). Therefore, in this study, observing and caring for mice was very important in observing the growth and development of mice for 14 days.

The body weight of the test animals was monitored for 14 days in **Graph 1** of the main body weight changes caused by the use of the extract. The onset and cessation of toxic symptoms, as well as the time of death, were all considered observations. According to Idris (32) Acute toxicity tests attempt to identify the dangerous effects of a substance that can manifest quickly after being administered at a certain dose. Observations of the body weight of the test animals with the average value can be seen in Appendix 10 which shows that the body weight of the mice for 14 days relatively decreased, but at certain doses and days it also increased. In **graph 2** statistical analysis of the data reveals that there is no significant difference in the rate of weight growth across treatment groups. This shows that giving a single dose of the five extract doses with the highest dose at a dose of 5000 mg/kg had no effect on the growth and development of body weight in mice during 14 days of observation after administering the test material and the mice's diet was still normal because nothing affected it. This is in line with research conducted (19).

Observing the body weight of the test animals for 14 days in the 5mg/kg dose group gave rise to symptoms of toxicity *awareness, straub, tremor, muscle tone, pupil size, writhing,* and *piloerection* after treatment and 24 hours after treatment, but symptoms of *ataxia* toxicity and *salivation* only occurs during treatment. This happened because the mice's bodies responded due to the administration of the dose and the body responded to symptoms of poisoning, then after 24 hours after treatment the mice's bodies had adapted.

This research was carried out to know the condition of the test animals after administering the test material at a predetermined dose. The most common sign that an animal is in pain or suffering is when its body weight loses more than 20% or more than 25% for seven days or more. Changes in body weight in test animals after extract treatment indicate disease or illness (21). However, during the 14 days of observation, not a single test animal experienced pain or discomfort as a result of consuming the extract, which caused the test animals to gain weight.

Eel fish oil extract is classified as safe for health because it does not cause pain or significant pressure on the body weight of the test animals. There was no significant weight loss because there was no relationship between clinical symptoms and the toxic effects of the samples on the mice's body weight. After 14 days of weight observation, the mice did not lose significant weight. However, the weight loss that occurs in mice is caused by stress factors on top of treatment using extracts, media, food intake, and the type and method of preparing food for mice. Meanwhile, the increase in mice body weight was caused by Vitamins A, B1, B2, zinc (Zn), unsaturated fatty acids including *docosahexaenoic acid* (DHA), *eicosapentaenoic acid* (EPA), and anti-oxidants are all present in eel marrow and contribute to its nutritional value. DHA is an unsaturated lipid that can reduce a person's blood fat levels. EPA is an omega-3 saturated fatty acid, and antioxidants can increase the development of immune cells, increase immunological activity, improve immune function, and provide free radicals in cells (24).

Limitations and Cautions

This research is limited to studying and finding out whether the pure oil extract of eel (*Anguilla marmorata* (Q.) Gaimard) has toxic properties after giving high doses and knowing the dose limits that can be used as parameters for the toxicity category of pure eel oil (*Anguilla marmorata* (Q.) Gaimard).

Recommendations for Future Research

It is necessary to conduct toxicity testing with high doses or with different methods to find out what dose can cause death and the maximum dose of eel oil (*Anguilla marmorata* (Q.) Gaimard). and also It is also necessary to conduct toxicity testing of eel oil (*Anguilla marmorata* (Q.) Gaimard) with a longer period of time so that the long-term toxicity of eel oil extract (*Anguilla marmorata* (Q.) Gaimard) can be known.

CONCLUSION

Based on the results of research conducted, there are several clinical symptoms produced by eel (*Anguilla marmorata* (Q.) Gaimard) fish oil extract. Symptoms observed in the study sample involved decreased motor activity, tension response (*straub*), narrowing of the eyelids (*ptosis*), pupil dilation (*mydriasis*), increased urine volume (*urination*), excretion of digestive waste (d*efecation*), and increased grooming effect which increases with dose. In addition, the LD50 value of eel fish oil extract (*Anguilla marmorata* (Q.) Gaimard) was considered "false" by taking the highest dose of 5000 mg/kg BW, which did not cause death in test animals. Therefore, it can be concluded that this extract is still classified in the 'Safe' category up to a dose of 5000 mg/kgBB.

AUTHOR'S CONTRIBUTION STATEMENTS

The authors declare that the researchers contributed fully to the research conducted, and in accordance with the agreed roles.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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