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Antihyperlipidemic and Antiinflammatory Activity of Crude Oil Anguilla Marmorata [Q.] Gaimard in White Rats (Rattus Norvegicus)

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ABSTRACT

Fish oil contains omega-3 and omega-6 fatty acids, which have health benefits such as preventing increases in total cholesterol, triglycerides and lowering blood HDL levels, and are anti-inflammatory agents in humans. This study was conducted to determine the antihyperlipidemic and anti-inflammatory effects of eel (Anguilla marmorata [Q.] Gaimard) oil extracts in white rats (Rattus norvegicus). To test hyperlipidemia, 25 rats were divided into 5 groups and treated; 1.5% PEG 400 (negative control), 20 mg simvastatin (positive control), test doses 18, 36 and 72 mg/kg. The treatment lasted 39 days. In the anti-inflammatory study, 25 white rats were divided into 5 groups; negative control, positive control, three doses of 22, 44 and 88 mg/200 g. Blood cholesterol levels were measured and percent inflammation was determined. The results showed that the eel oil extracts led to a decreased of total cholesterol levels 49.60 %, triglycerides 31.15 %, and HDL 14.3 %. The percentage of inflammation values ranged from 31.73 to 54.48 %. The findings indicated that the most effective doses for the antihyperlipidemic effect were 72 mg/ kg, while 22 mg/200 g for the anti-inflammatory activity.

INTRODUCTION

Hyperlipidemia is an increment in add up to cholesterol, low density lipoprotein (LDL), triglycerides (TG), and a diminish in high density lipoprotein (HDL) within the blood (1). Based on the 2013 Riset Kesehatan Dasar Nasional, 35.9% of the Indonesian population aged ≥15 years and above had abnormal cholesterol levels (≥200 mg/dL). Triglyceride levels can be classified as normal (andlt; 150 mg/dL), above the limit (150-199 mg/dL) and high (200-499 mg/dL) (2). High triglyceride levels can be influenced by diet, physical activity, genetics, age, and gender (3). However, lowering the levels of triglycerides in the blood can be obtained with drugs and changes in lifestyle. These lifestyle changes include physical activity and dietary arrangements. The recommended dietary intake are reducing total energy, fat and carbohydrate intake, and increasing fiber intake by 20-30 grams (4).

One of the ingredients that can reduce cholesterol levels in the blood is fish oil (5). Fish oil contains an unsaturated fat needed by the body, such as omega-3 and omega-6. Monounsaturated fatty acid in eel fish oil also gives the advantage to the levels of blood lipids, including total cholesterol, HDL, LDL, and triglycerides. Adequate consumption of omega-3 fatty acids can reduce blood cholesterol and the risk of heart disease. The clinical blood cholesterol-lowering effect of omega-3 fatty acids is thought to be due to their effect on the mechanism

of production of lipoproteins secreted by the liver and blood. Cholesterol in the blood is basically in the form of lipoproteins (6). Monounsaturated fatty acids found in eel samples are mostly oleic acid. Oleic acid is also known as an omega-9 fatty acid, which has the protective ability to lower LDL cholesterol and raise HDL cholesterol. Monounsaturated fatty acids (MUFA) affect blood cholesterol and lower blood cholesterol more effectively than polyunsaturated fatty acids (PUFA) (7).

Several studies have shown that consumption of fish oil can lower blood cholesterol [8,9]. Fish oil or fat is composed of unsaturated fatty acid units such as omega-3 and omega-6 fatty acids. Research into the use of marine fish oil as a cholesterol lowering agent has been conducted (9). The results of these studies indicated an effect of tuna fish oil on reducing total cholesterol levels in hypercholesterolemic rats. Another study conducted focuse on identifying the omega-3 fatty acid content produced by marine fish. The researcher fed mice with a saturated fatty acid diet as an inducer of hypercholesterolemic condition.

Fish oil is widely used in nutritional supplements (10) because it contains omega-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are beneficial for health, as an anti-inflammatory agent (11). Another study found that omega-3 fatty acids, especially EPA and DHA, have anti-inflammatory properties (12). The activity of fish oil as an anti-inflammatory agent is supported by research conducted who discovered that lemuru fish (Sardinella longiceps) oil could be used as an anti-inflammatory alternative because it contains EPA and DHA which can reduce oedema in the soles of the feet of experimental animals (13).

Given that the body is unable to produce some substances, such as long-chain n-3 polyunsaturated fatty acids (n-3 LCPUFAs), which are vital for mammals, diet is a major factor in both illness prevention and health promotion (14). Docosahexaenoic acid (C22:6n-3, DHA) and eicosapentaenoic acid (C20:5n-3, EPA) are included in this category. Numerous studies demonstrate the significance of these fatty acids for good health. Among its acknowledged qualities are their anti-inflammatory qualities, favorable effects on diabetes, cancer, the development of the neurological system and cardiovascular system, improved digestibility, lower risk of Alzheimer's disease, and better digestibility (15),(16),(17). Their capacity to reduce plasma triacylglycerol (TAG) is noteworthy. These beneficial substances can be found in significant amounts in fish oils (18). Hypercholesterolemic, a lipid condition, is frequently treated with these fish oils.

This study aimed to investigate the antihyperlipidemic and anti-inflammatory effects of oil extracted from eel (Anguilla marmorata [Q.] Gaimard) in white rats (Rattus norvegicus).

METHOD

Types of research

The research included laboratory experiments, namely eel fish (Anguilla marmorata (Q.) Gaimard) in the yellow eel and silver eel phases which had been cleaned and ground, then extracted to obtain crude oil. Next, a crude fish oil emulsion was made to be administered orally to white rat (Rattus norvegicus) test animals for 14 days, then checked for hyperlipidemia and anti-inflammatory levels after being induced with high fat food. In histopathology, surgery is performed to remove organs such as the heart, liver, kidney and pancreas for histopathological examination.

Sources of experimental animals

Eel fish (Anguila marmorata [Q.] Gaimard) in the yellow and silver phase was obtained from the Palu River, Palu, Central Sulawesi, Indonesia. The fish was cut into small pieces, weighing approximately 100 grams to facilitate the extraction process (14). Also, the male white rats (Rattus norvegicus) used for this study were purchased from Rat Farm in Palu, Central Sulawesi, Indonesia.

Ethical approval

Research ethics was taken from the Laboratory of Pathology, Anatomy and Histology, Faculty of Medicine and Health Sciences, Tadulako University, Indonesia.

Extraction of fish oil

Fish oil was extracted using the wet rendering method. A total of 1 kg of fish fillets were washed clean, cut into small cubes, and steamed for 30 minutes at 80 °C. Then, the cooked fish was pressed to extract the broth from the fish stew, which was centrifuged at 3000 rpm for 15 minutes.

Production of fat feed

High-fat feed comprising duck egg yolk (40 %), oil pork (10 %), fat pork (50 %), and pork pellets (1 kg) was made. The feed was made by mixing 50 % refined lard, 10 % lard, 40 % duck egg yolk, and 1 kg of fine pork pellets until a homogeneous solution was obtained. The dough was made into a small circle and then fried until it was a little hard. Pork oil was made by using lard in the solid form at room temperature, and then fried until it melted to produce liquid oil.

Preparation of 0.1 % propyltiouracil solution

In preparing 0.1 % propylthiouracil (PTU) solution, one PTU tablet was crushed and dissolved in a test animal's drinking bottle containing 1 L of water. The solution was shaken vigorously until it became homogeneous.

Preparation of 1.5 % polyethylene glycol carrier

A concentration of 1.5 % polyethylene glycol (PEG 400) carrier was prepared by measuring 1.5 ml of PEG 400 into a 100 ml volumetric flask. Aquadest was poured into it to the limit mark and used to dissolve it. The carrier solution was shaken vigorously until it became homogeneous.

Preparation of fenofibrate suspension

Twenty tablets of 300 mg fenofibrate were ground into powder form. Then, 40.5 mg of the powder was suspended in 12.5 ml PEG 400 to prepare the fenofibrate suspension.

Preparation of eel extract emulsion

In this study, doses of 22.68, 45.36, and 90.72 mg/ 200 g were used. Preparation of a test dosage of 22.68 mg/200 g eel fish extract was done by weighing 22.68 mg of pure oil extract, then making eel fish oil extract emulsion with 3.5 grams of tween and 1.5 g of homogenized span to form an eel fish oil extract emulsion which was used as a test preparation. A similar procedure was also used to prepare 45.36 and 90.72 mg/200 g test preparations.

Experimental grouping and treatment to investigate hyperlipidemia therapy

The experimental animals were acclimatized for 7 days. A total of 25 rats were divided into 5 treatment groups and used for this study. Each treatment group consisted of 5 rats each, which were kept in the same conditions. Oral therapy was used to administer the treatments. The negative control group was given 1.5 % PEG 400 (Group 1); the positive control group was administered with 20 mg simvastatin (Group 2); and three groups were treated with eel fish oil extract at doses of 18 mg/kg (Group 3), 36 mg/kg (Group 4) and 72 mg/kg (Group 5). The treatment was applied to each rat according to the calculated dose, and the therapy was carried out for 14 days.

Experimental grouping and treatment to investigate antihyperlipidemic effect

For the investigation of the antihyperlipidemic effect, a total of 25 rats were divided into 5 groups (each group consisted of 5 rats). Each group was placed in a different cage and acclimatized for 7 days. The cages were placed in a Laboratory at the Department of Pharmacology-Biopharmaceutical, Faculty of Pharmacy, and University of Tadulako, Indonesia. Before the start of the experiment, the rats were fasted for 16 hours, but were still allowed to drink ad libitum. The rats were fed with a high-fat diet pellet and drink, mixed with 0.01 % PTU once a day for 2 weeks. Each group was administered with 160 mg fenofibrate suspension (Group A; positive control), 1.5 % PEG 400 suspension (Group B; negative control), eel fish oil extract at doses of 22.68 mg (Group C), 45.36 mg (Group D) and 90.72 mg (Group E). The treatment was given for 2 weeks and at the end of the experiment, total cholesterol, HDL, triglyceride levels, and body weight were measured on the 14th day.

Experimental grouping and treatment to investigate the anti-inflammatory effect

At the beginning of the experiment, the mice were acclimatized for two weeks. Before treatment, the rats were fasted for 18 hours, but were still allowed to drink water. The rats were divided into 5 experimental groups and administered with 1.5 % PEG suspension (Group 1; negative control), 50 mg/kg diclofenac sodium (Group 2; positive control), eel fish oil extract at doses: 22 mg/200 g body weight (Group 3), 44 mg/200 g body weight (Group 4), and 88 mg/200 g body weight (Group 5). Each animal was weighed and marked on its feet. Then the foot volume was measured using a plethysmometer. The initial volume (V_o) was recorded, and taken as the volume of the legs before being given the drug and induced with egg white solution. Then each treatment group was induced with 0.1 ml of egg white. One hour later, each rat was administered with the test solution orally. After 60 minutes, measurements were taken with a plethysmometer. The final volume (V_t) was recorded and taken as leg volume after drug and egg white induction. Measurements were made every 60 minutes for 6 hours.

Measurement of blood cholesterol levels

At the end of the experiment, a blood sample was collected from each rat tail through the lateral vein with capillary rods and placed on a test strip. The blood samples of the experimental rats were obtained three times (before and after administration of feed fat and after administration of the treatments). A lipid strip test tool (Lipid Pro) was used to measure the levels of total cholesterol, HDL, and triglycerides.

Histopathological Testing

In histopathological testing (heart, liver, kidney, and pancreas), histopathological preparations are made using several stages, namely: fixation, trimming, dehydration, clearing, embedding, blocking, cutting, staining, and mounting. Then a histopathological examination of the organ was carried out using a microscope with 400X magnification. Mice were given an injection of 0.16 mL of ketamine as an anesthetic and the neck was dislocated. Both front and hind legs of the rat were stretched on a surgical board in a dorsal lying position. Then the entire skin and abdomen are sterilized by spraying alcohol. Make an incision in the skin from the chin to the anterior edge of the pelvic bone (pectin ossis pubis) and perform an organ necropsy. Fat and membranes attached to the organs are removed, washed using NaCl to remove blood that is still attached, then dried using tissue (36).

Statistical data analysis

Data obtained for the antihyperlipidemic effect experiment were first subjected to a normality test using the Levene Statistics for testing the value of homogeneity. The research data were normally distributed ($p>0.05$). One-way analysis of variance (ANOVA) with a confidence level of 95 % ($\alpha = 0.05$) and the Duncan test were performed. For the anti-inflammatory effect experiment, the data obtained were analyzed using the Shapiro Wilk test to observe the data distribution and the Levene test to detect the homogeneity of the data. These analyses were followed by a one-way ANOVA to determine whether the differences obtained were significant or not. Furthermore, the Kruskal – Wallis test was performed to examine the significant differences (Besral, 2010). Statistical testing was carried out using the SPSS version 22 application.

RESULTS

Levels of body lipids in experimental rats after treatments

In this study, there were 26.39 grams of eel fish oil extract obtained, which accounted for a 2.1 % yield. After the treatments, the weights of the experimental rats were monitored for 39 days and the result is presented in Figure. 1.

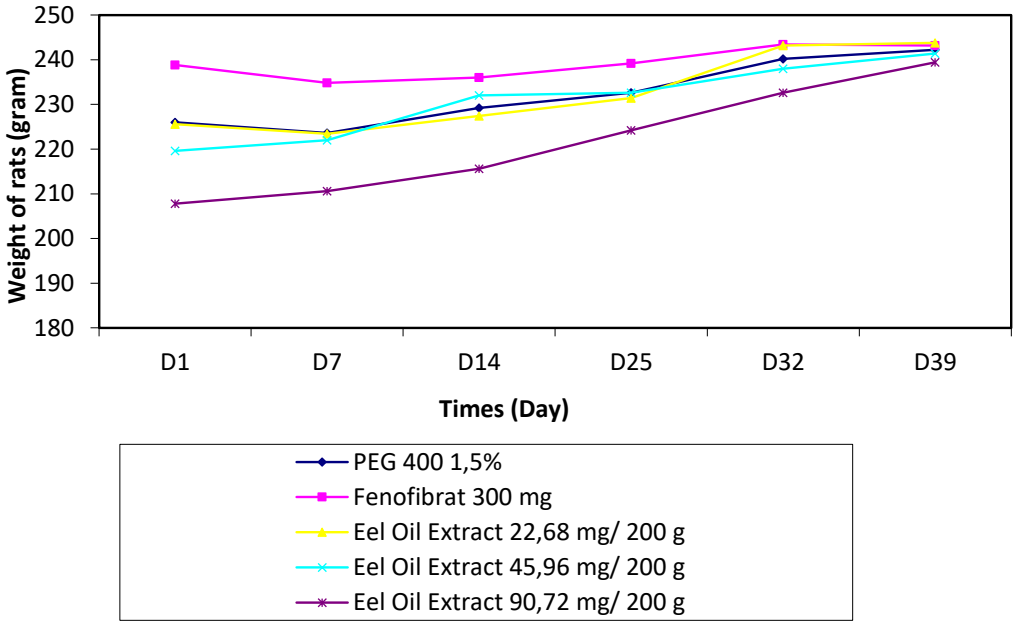

Figure 1. Weight of white rats monitored for 29 days

The results of the analysis of total cholesterol levels after therapy (Table 1) revealed that the percentage reduction in cholesterol levels on the 39th day after therapy showed a decrease in total cholesterol levels in each treatment group.

Table 1. Total cholesterol levels in white rats after hyperlipidemia therapy

Treatment group	Mean total cholesterol (H25 ± SD)	Mean total cholesterol (H39 ± SD)	% Decrease
1.5 % PEG 400 (Neg. ctrl.)	253.8 ± 46.04	233.2 ± 31.73	8.11
20 mg Simvastatin (Post. ctrl)	216 ± 34.61	111 ± 10	48.61
Eel oil extract dose 18 mg/kg	232.4 ± 35.45	171.8 ± 31.93	26.07
Eel oil extract dose 36 mg/kg	208.4 ± 36.77	138.4 ± 18.18	33.58
Eel oil extract dose 72 mg/kg	229.8 ± 50.32	115.8 ± 7.91	49.60

The lowest percentage reduction in total cholesterol level was observed in the negative control group (1.5 % PEG 400), while the highest percentage was in the eel fish oil extract treatment group with a test dose of 72 mg/kg. The trend in triglyceride levels after therapy indicated a decrease in each treatment group (Table 2).

Table 2. Triglyceride levels in white rats after treatments with eel fish oil extracts

Treatment group	Average TG (H21 ± SD)	Mean TG (H35 ± SD)	% Decrease
1.5 % PEG 400 (Neg. ctrl.)	137.6 ± 16.19	129 ± 14.04	6.25
5.4 mg Fenofibrate (Post. Ctrl.)	136 ± 9.24	92 ± 19.17	32.35
Eel oil extract dose 22.68 mg/200 g	159.6 ± 55.74	117.2 ± 23.76	26.56
Eel oil extract dose 45.36 mg/200 g	137.2 ± 42.10	106.8 ± 25.37	22.15
Eel oil extract dose 90.72 mg/200 g	138 ± 19.71	95 ± 9.13	31.15

TG: Triglyceride

The lowest percentage decrease in triglycerides was observed in the negative control group (1.5 % PEG 400), while the highest was in the positive control group (5.4 mg Fenofibrate). Table 3 shows the result of the HDL levels obtained after treatment in each group. HDL levels decreased until the detection limit of the lipid measuring instrument was reached (Low/ under range), which indicated that it was below 25 mg/dl.

Table 3. High-density lipoprotein levels in white rats after therapy

Treatment group	Initial HDL level (mg/dl)	HDL level after feeding (mg/dl)	Final HDL level (mg/dl)
1.5 % PEG 400 (Neg. ctrl.)	38.9	27	<25
300 mg Fenofibrate (Post. ctrl.)	42.7	25	<25
Eel oil extract dose 22.68 mg/200 g	44.7	28	<25
Eel oil extract dose 45.36 mg/200 g	43.2	28	<25
Eel oil extract dose 90.72 mg/200 g	46.5	28	<25

HDL: High-density lipoprotein; <25 mg/dl: Lo (Low/ under range)

Organ histopathological examinations

Histopathological examination of the heart of white rats with hyperlipidemia after treatment (Table 4) revealed local hydropic degeneration and even degeneration of local fat. Also, there was uneven bleeding and an increase in necrosis.

Table 4. Histological examination of heart of white rats with hyperlipidemia

Treatment group	Sample	/1000 cells		Qualitative analysis		
		Normal	Necrosis	Hydropic degeneration	Fat degeneration	Bleeding
1.5 % PEG 400 (Neg. ctrl.)	Rat 1	952	48	Local	Local	Local
	Rat 2	948	52	Local	Local	Local
	Rat 3	956	44	Local	Local	Local
	Rat 4	958	42	Local	Local	Local
	Rat 5	952	48	Local	Local	Local
Eel oil extract dose (18 mg/kg)	Rat 1	937	63	Local	Local	Local
	Rat 2	928	72	Local	Local	Local
	Rat 3	935	65	Local	Local	Local
	Rat 4	934	66	Local	Local	Local
	Rat 5	932	68	Local	Local	Local
Eel oil extract dose (36 mg/kg)	Rat 1	925	75	Local	Local	Local
	Rat 2	921	79	Local	Local	Local
	Rat 3	917	83	Equally	Local	Local
	Rat 4	918	82	Local	Local	Local
	Rat 5	923	77	Local	Local	Local
Eel oil extract dose (72 mg/kg)	Rat 1	911	89	Local	Local	Local
	Rat 2	916	84	Local	Local	Local
	Rat 3	904	96	Equally	Local	Local
	Rat 4	903	97	Equally	Local	Equally
	Rat 5	912	88	Local	Local	Local

Table 5 shows the results of liver histopathological examination of hyperlipidemic white rats after treatment in each group. Localized and evenly distributed hydropic degeneration, localized and even fat degeneration, bleeding, and increased necrosis as displayed in Table 5. More so, the centralis and sinusoid veins appeared normal except in the treatment group which was administered with eel fish oil extract at a dose of 72 mg/kg.

Table 5. Histological examination of liver of white rats with hyperlipidemia

Treatment Group	Sample	Necrosis/ 1000 cells	Qualitative analysis				
			Hydropic degeneration	Fat degeneration	Bleeding	Centralis vein	Sinusoid
	Rat 1	73	Local	Local	Local	Normal	Normal
	Rat 2	64	Local	Local	Local	Normal	Normal

1.5 % PEG 400 (Neg. ctrl.)	Rat 3	68	Local	Local	Local	Normal	Normal
	Rat 4	77	Local	Local	Local	Normal	Normal
	Rat 5	61	Local	Local	Local	Normal	Normal
Eel fish oil extract, dose 22.68 mg/200 g	Rat 1	94	Local	Local	Local	Normal	Normal
	Rat 2	104	Equally	Local	Local	Normal	Normal
	Rat 3	101	Local	Local	Local	Normal	Normal
	Rat 4	110	Local	Local	Local	Normal	Normal
	Rat 5	108	Local	Local	Local	Normal	Normal
Eel fish oil extract, dose 45.96 mg/200 g	Rat 1	132	Equally	Local	Equally	Normal	Widened
	Rat 2	127	Local	Local	Local	Normal	Normal
	Rat 3	130	Local	Equally	Equally	Normal	Normal
	Rat 4	124	Local	Local	Local	Normal	Normal
	Rat 5	126	Local	Local	Local	Normal	Normal
Eel fish oil extract, dose 90.72 mg/200 g	Rat 1	146	Equally	Local	Local	Normal	Normal
	Rat 2	152	Equally	Local	Local	Lesions	Widened
	Rat 3	144	Local	Local	Local	Normal	Normal
	Rat 4	148	Equally	Local	Local	Normal	Normal
	Rat 5	157	Equally	Equally	Equally	Lesions	Narrowing

The results of kidney histopathological examination of hyperlipidemic white rats after treatment in each group showed localized and evenly distributed hydropic degeneration, localized and evenly distributed fat degeneration, bleeding, and necrosis as shown in Table 6.

Table 6. Histological examination of kidney of white rats with hyperlipidemia

Sample		/1000 cells		Qualitative analysis				
		Normal	**Necrosis**	**Hydropic degeneration**	**Fat degeneration**	**Bleeding**	**Glomerulus**	**Bowman's capsule**
1.5 % PEG 400 (Neg. ctrl.)	Rat 1	948	52	Local	Local	Local	Normal	Normal
	Rat 2	945	55	Local	Local	Local	Normal	Normal
	Rat 3	953	47	Local	Local	Local	Normal	Normal
	Rat 4	950	50	Local	Local	Local	Normal	Normal
	Rat 5	949	51	Local	Local	Local	Normal	Normal
Eel fish oil extract, dose 22.68 mg/200 g	Rat 1	937	63	Local	Local	Local	Normal	Normal
	Rat 2	922	78	Local	Local	Local	Normal	Normal
	Rat 3	932	68	Local	Local	Local	Normal	Normal
	Rat 4	927	73	Local	Local	Local	Normal	Normal
	Rat 5	924	76	Equally	Local	Local	Normal	Normal
Eel fish oil extract, dose 45.96 mg/200 g	Rat 1	921	79	Local	Local	Local	Normal	Normal
	Rat 2	916	84	Local	Local	Local	Normal	Normal
	Rat 3	912	88	Local	Local	Local	Normal	Normal
	Rat 4	919	81	Local	Local	Local	Normal	Normal
	Rat 5	907	93	Equally	Equally	Equally	Normal	Normal
	Rat 1	897	103	Equally	Equally	Equally	Wrinkled	Normal

Eel fish oil extract, dose 90.72 mg/200 g	Rat 2	911	89	Local	Local	Local	Normal	Normal
	Rat 3	903	97	Local	Local	Local	Normal	Normal
	Rat 4	895	105	Equally	Equally	Equally	Wrinkled	Narrowing
	Rat 5	899	101	Equally	Local	Local	Normal	Normal

The wrinkled glomerulus and narrowed bowman capsule were observed in the treatment group administered with eel fish oil extract at a dose of 72 mg/kg. The results of liver histopathological examination of hyperlipidemic rats after treatment in each group indicated localized and evenly distributed hydropic degeneration, localized and evenly degenerated fat, bleeding, and necrosis (Table 7).

Table 7. Histological examination of pancreas of white rats with hyperlipidemia

Sample		/1000 cells		Qualitative analysis		
		Normal	**Necrosis**	**Hydropic degeneration**	**Fat degeneration**	**Bleeding**
1.5 % PEG 400 (Neg. ctrl.)	Rat 1	956	44	Local	Local	Local
	Rat 2	953	47	Local	Local	Local
	Rat 3	961	39	Local	Local	Local
	Rat 4	950	50	Local	Local	Local
	Rat 5	954	46	Local	Local	Local
Eel fish oil extract, dose 22.68 mg/200 g	Rat 1	926	74	Local	Local	Local
	Rat 2	932	68	Equally	Local	Local
	Rat 3	934	66	Local	Local	Local
	Rat 4	942	58	Local	Local	Local
	Rat 5	938	62	Local	Local	Local
Eel fish oil extract, dose 45.96 mg/200 g	Rat 1	914	86	Equally	Local	Equally
	Rat 2	926	74	Local	Local	Local
	Rat 3	921	79	Local	Local	Local
	Rat 4	917	83	Local	Local	Local
	Rat 5	919	81	Local	Local	Local
Eel fish oil extract, dose 90.72 mg/200 g	Rat 1	899	101	Equally	Local	Equally
	Rat 2	911	89	Local	Local	Local
	Rat 3	906	94	Local	Local	Local
	Rat 4	903	97	Equally	Local	Equally
	Rat 5	909	91	Local	Local	Local

Analyses of cell necrosis and anti-inflammatory effect

The mean values of cell necrosis in hyperlipidemic white rats after the various treatments increased with increasing doses of eel fish oil extract in the organs analysed (Fig. 2).

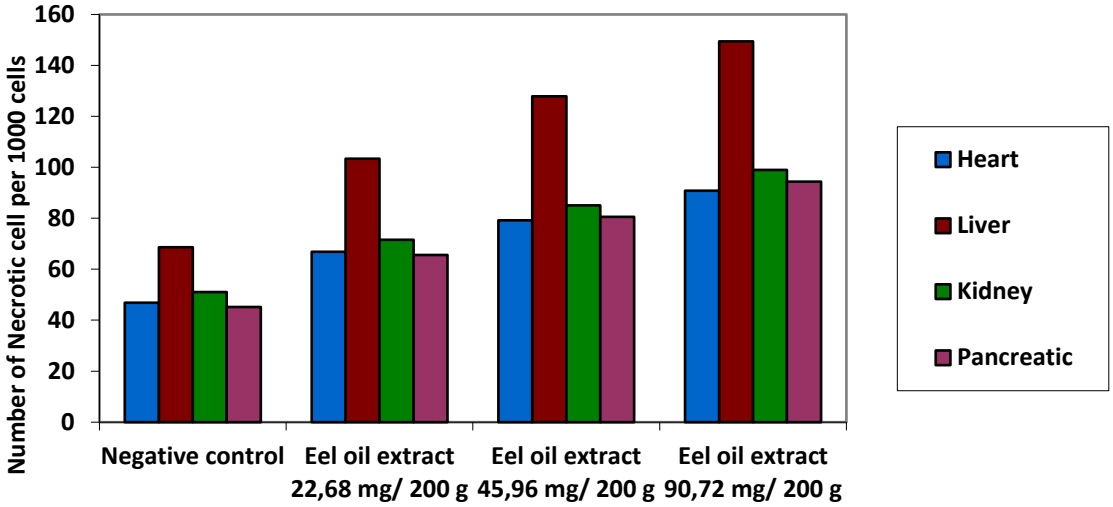

Figure 2. Cell necrosis in white rats after treatment

The results of the volume analysis of rat foot inflammation (Table 8) revealed that there was no effect in decreasing the volume of inflammation in the negative control compared with the other treatment groups.

Table 8. Volume of inflammation in the soles of rats' feet after treatment

Time (Hour)	Mean ± SD volume (ml) inflammation				
	K(-)	K(+)	D22	D44	D88
1	1.14 ± 0.16	1.05 ± 0.10	1.16 ± 0.08	1.06 ± 0.06	1.20 ± 0.10
2	1.13 ± 0.11	0.91 ± 0.09	1.10 ± 0.12	0.98 ± 0.16	1.21 ± 0.05
3	1.14 ± 0.15	0.86 ± 0.11	1.04 ± 0.10	0.89 ± 0.16	1.15 ± 0.07
4	1.14 ± 0.14	0.94 ± 0.13	0.98 ± 0.11	0.74 ± 0.38	1.08 ± 0.12
5	1.15 ± 0.17	0.84 ± 0.08	0.93 ± 0.16	0.84 ± 0.17	0.98 ± 0.19
6	1.16 ± 0.15	0.79 ± 0.03	0.89 ± 0.12	0.79 ± 0.18	0.96 ± 0.17

K(-): Negative control; K(+) = Positive control; D22: Dose of 22 mg/200 g; D44: Dose of 44 mg/200 g; D88: Dose of 88 mg/200 g. The test was carried out for 39 days, days 1-25 were given high fat feed, days 25-39 were given eel fish oil extract every day.

There was a reduction in the volume of inflammation in the positive control group, eel fish oil extract at a dose of 22 mg/ 200 g, 44 mg/200 g, and 88 mg/ 200 g (Fig. 3).

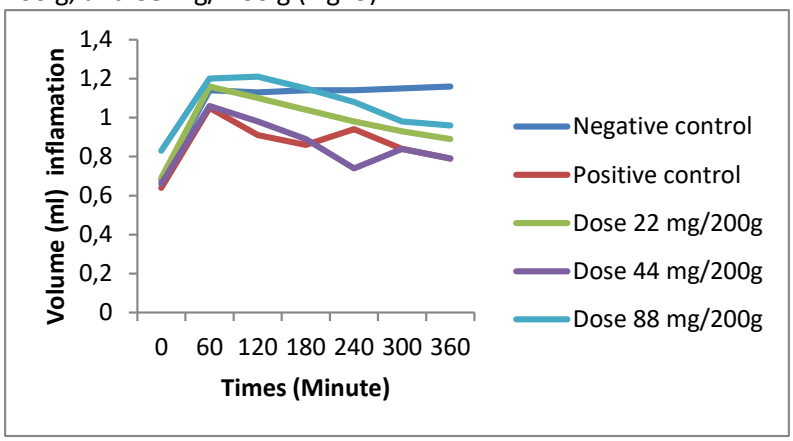

Figure 3. Average inflammation volume of rats' feets after treatment

DISCUSSION

Fish oil contains unsaturated fatty acids, especially omega-3 and omega-6 fatty acids, which the body needs. Also, monounsaturated fatty acids such as total cholesterol, HDL, LDL, and triglycerides in eel fish oil regulate the lipid levels in the blood. Fish oil is also widely used in nutritional supplements (10). This is because fish oil contains omega-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid and docosahexaenoic acid, which are beneficial for health and act as anti-inflammatory agents (11). Adequate consumption of omega-3 fatty acids can reduce blood cholesterol and the risk of heart disease. The clinical effect of omega-3 fatty acids in lowering blood cholesterol is thought to be due to their effect on the production mechanism of lipoprotein transport in the liver and its secretion into the blood. Cholesterol in the blood is basically in the form of lipoproteins (6). Monounsaturated fatty acids found in eel samples are mostly oleic acid. Oleic acid is also known as an omega-9 fatty acid, which has the protective ability to lower LDL cholesterol and raise HDL cholesterol.

The test sample used in this study was eel fish oil from the Palu River, Central Sulawesi. The phase used was the yellow eel phase, where the pigmentation process is completed, and marked by the change in the eel's skin to yellowish gray. This phase is the longest phase in the life cycle of an eel fish and is the most consumed. The yellow eel phase contains fatty acids in the form of EPA (27 mg/100 g), DHA (156 mg/100 g), and oleic acid (3.421 mg/100), which can increase HDL levels (15).

This research was conducted with 5 experimental groups. The negative control group was set up using 1.5 % PEG 400 because polyethylene glycol (PEG) 400 is a non-ionic surfactant that is widely used in drug formulation and preparation due to its stable nature, ease in mixing with other components. Non-toxic, non-irritating, and effective in a wide pH range. The positive control group was made of a 300 mg dose of fenofibrate suspension. The mechanism of action of fenofibrate is to activate the lipoprotein lipase enzyme, which breaks down triglycerides and increases HDL cholesterol levels, which is thought to be through an increase in apoproteins AI and A-II.

Administration of high-fat feed was aimed at reducing the levels of HDL and fats in the blood. The composition of high-fat feed uses lard because it contains high saturated fatty acids. Saturated fatty acids cause an increase in triglyceride levels and a decrease in HDL. The 0.01 % PTU solution that was administered as drinking water was aimed at increasing cholesterol levels by inhibiting thyroid hormone synthesis. The mechanism of action of PTU as an anti-thyroid, which inhibits thyroid cells in mice from producing thyroid, is a direct effect of hypothyroidism on lipoprotein metabolism to increase cholesterol levels in the blood. The relationship between the inhibition of thyroid hormone and cholesterol is as a result of a thyroid stimulatory hormone (TSH) which plays an important role in regulating secretion from the thyroid gland. TSH works as an antagonist of the thyroid hormone; when the thyroid hormone is present in low amounts (hypothyroidism), TSH will be produced in large quantities in the body. High TSH levels suppress HDL levels. This observation is because when TSH levels increase, the free thyroid (FT4) levels in the thyroid gland decrease. So that metabolism and fat burning decrease, total cholesterol levels increase and HDL tends to decrease.

It was observed in the measurement of total cholesterol after the administration of a high-fat diet (Table 1) that the normal total cholesterol levels of the rats ranged from 121.90 to 163.81 mg/dl. Meanwhile, the total cholesterol value obtained after feeding the rats with high-fat feeds ranged from 166 to 313 mg/dl. This observation was significantly different from the normal total cholesterol levels of the rats. An increase in the total cholesterol levels in the blood of rats was as a result of the high-fat feed due to an exogenous inducer, where the content of saturated fatty acids and cholesterol was observed to be 38 – 43 % in lard (16). Also, the observation could be partly due to the administration of 0.01 % propyltiouration (PTU) as an endogenous inducer that acts as a thyroid hormone antagonist. Under normal conditions, thyroid hormone can increase lipid metabolism by increasing the formation of LDL receptors in hepatocytes, which causes rapid transfer of LDL from plasma and secretion of cholesterol lipoproteins from hepatocytes (17).

The percentage reduction in cholesterol levels on the 39th day after therapy (Table 1) showed a decrease in total cholesterol in each treatment group. The highest percentage reduction in cholesterol levels was in the test group with a dose of 72 mg/kg. There was a decrease in cholesterol level by 49.60 %. Meanwhile, the lowest percentage reduction in cholesterol levels was observed in the negative control group, where cholesterol level was decreased by 8.11 %. Based on the one-way ANOVA, the results were significantly different ($p < 0.05$) in the positive

control group, 36 and 72 mg treatment doses, while the results were different but not significantly (p> 0.05) in the negative control group and the treatment group of 18 mg. The largest decrease of cholesterol level was in the test dose of 72 mg/kg, while the least decrease was recorded for the negative control. The decrease in total cholesterol levels in the fish oil eel treatment group occurred because the meat of eel in the yellow eel phase has a fatty acid content of monounsaturated fatty acid (MUFA) and plural polyunsaturated fatty acids (PUFA), including omega-3 and omega-6 which have the highest concentration. Meanwhile, when the yellow eel phase eel meat has monounsaturated fatty acids as much as 4.029 g/100 g and plural unsaturated fatty acids of 0.643 g/100g [19], the most effective dose is at a dose of 72 mg/kg.

The decrease in cholesterol in the positive control group was due to the mechanism of action of the statin drug simvastatin, which inhibits HMG-CoA reductase to prevent cholesterol formation. Cholesterol levels also decreased in the negative control group due to the physiological characteristics of the animal and the body without being affected by the treatment group.

Measurement of triglyceride levels in the experimental animals (male white rats) was carried out three times during the course of the study: the 1st day after acclimatization procedure, the 21st day after induction of weight gain with high-fat feed, and the 35th day after administering drugs and extracts. On the 1st day of measurement, the first triglyceride level was considered a normal level for rats as reported that the normal level for rat triglycerides was 40.00 - 130.00 mg/dL (18). There was an increase in the triglyceride levels (71–244 mg/dl) on the 21st day of measuring the second triglyceride level. This increase was caused by the 100 g of lard containing 38 – 43 % saturated fat used for the experiment. Also, the inclusion of duck egg yolk containing a higher fat content (13.77 %) compared to chicken (11.15 %), quail (11.09 %), and goose eggs (13.27 %) may be partly responsible for the increase in triglyceride levels (19). Saturated fsat results in increased cholesterol levels in the blood because it is a precursor to cholesterol. Consumption of saturated fat can also reduce HDL in the blood, thereby increasing cholesterol levels as a result of an increase in ApoB cholesterol so that the function of LDL receptors is reduced(20). On the 35th day of measuring the third triglyceride level, there was a decrease in the range of 66 - 189 mg/dl.

The percentage reduction in triglyceride levels at day 35 post-treatment (Table 2) showed a reduction in triglyceride levels in each treatment group. The highest percentage decrease in triglyceride levels was observed in the positive control group (32.35%). At the same time, the percentage reduction of triglycerides was the lowest in the negative control group (6.23%). The one-way ANOVA $p = 0.038$ ($p < 0.05$) shows that the results were significantly different. The percentage reduction was in a descending order: the positive fenofibrate suspension treatment group > eel oil extract dose of 90.72 mg/200g treatment group > eel oil extract dose of 22.68 mg/200g treatment group > eel oil extract dose of 45.36 mg/200 g treatment group > negative control group. A reduction in triglyceride levels was observed for eel oil extract treatment at a dose of 90.72 mg/ 200 g. This observation was due to the ability of the dose to mimic the concentration of the active substance of the fenofibrate drug, thereby reducing triglyceride levels. A non-significant reduction effect was obtained for doses of 22.68 and 45.36 mg/ 200 g. Also, there was a decrease in the triglyceride levels in the negative control group; an observation which indicated that the decrease in triglyceride levels was due to the physiological characteristics of the animal's body without being influenced by the treatment group. However, the most effective dose that resulted in a decrease in the triglycerides was 90.72 mg/ 200g.

Fish oil is known to contain omega-3 unsaturated fatty acids. Consuming sufficient amounts of omega-3 fatty acids can reduce blood cholesterol levels by affecting the transport mechanism of lipoproteins secreted into the blood in the liver (6). The mechanism that lowers blood cholesterol and LDL levels through the consumption of unsaturated fatty acids occurs through three mechanisms. The first strategy involves suppression of the expression of SREBP-1 (sterol regulatory element-binding protein-1) which can reduce the lipogenesis process and reduce VLDL secretion. Also, an increase in the purification of liver lipoproteins through increased LPL expression has been reported as the second strategy, while the last is decreased levels of ApoC-III, as well as increased cholesterol transport to the liver (21).

Measurement of HDL levels was also carried out 3 times; the 1st, 25th, and 39th day, similar to triglyceride measurement. The results obtained for the determination of HDL after 14 days of eel oil extract therapy showed a decrease in HDL levels in the experimental white rats. Based on the outcome of HDL level measurement after

treatments with eel oil extract and fenofibrate (Table 3), it was observed that the level of HDL decreased below the detectable limit of the lipid measuring instrument, which was considered <25 mg/dl. This observation indicated that eel oil extract treatment doses of 22.68, 45.36, or 90.72 mg, and with fenofibrate did not increase the HDL levels of the rats, but instead decreased them.

The eel fish oil extract contains EPA and DHA, which were expected to increase the HDL levels. Conversely, the HDL levels were not increased due to several factors. According to the Guidelines for the Management of Dyslipidemia (22), the pharmacological dose of omega-3 derived from fish consumption is > 2 grams/ day and this can increase HDL levels by 1-3 %. The effect of HDL levels on rat body's weight has been reported to increase rat's body weight or lead to overweight. If an organism is overweight, there will be an excess energy that is stored in enlarged fat cells, causing triglycerides to increase, thereby affecting other lipoproteins. When triglycerides, LDL, and HDL undergo lipolysis, they become small dense LDL and HDL, and these abnormalities are typically characterized by low HDL cholesterol levels. There was no increase in HDL levels using fenofibrate when the therapy was administered for 14 days in the positive control. To be effective, an interval of 2 months is required for this drug.

The results of the histological examination of the organs of rats administered with eel fish oil extract revealed that there were several abnormalities in the test organs which were caused by an increase in the eel fish oil extract administered. Some of the damage included bleeding, hydropic degeneration, fatty degeneration, and necrosis. The effect of eel fish oil in each treatment group resulted in different changes. This difference is due to differences in the dose of eel fish oil extract administered at the time of treatment. Cell damage can be permanent or temporary. Degeneration is a type of damage that can return to normal if the stimuli that caused the injury was stopped. The causes of cell degeneration include metabolic disorders, toxins, and trauma. If cell degeneration continues, it can cause cell death or necrosis.

The results of the heart histopathological examinations of rats administered with eel fish oil extract (Table 4) revealed various changes such as visible bleeding, hydropic degeneration, fatty degeneration, and necrosis in each treatment group. It was observed that the histopathological examination of the heart showed that cell death or necrosis increased with increasing doses in the treatment groups (Figure 3). This observation indicated that there was an increase in cell death or necrosis with increasing doses of the eel fish oil extract. The percentage of normal heart muscle cells in each treatment group decreased with both an increase in cell damage and dose of treatment. The increase in necrosis of heart cells is reversible in as much as the stimuli that cause the injury are stopped, the cells will reverse back to normal. Conversely, when the causative stimuli persist at high doses, necrosis, or cell death becomes irreversible (23). The value of the percentage of damage to heart muscle cells was categorized into minor damage which is characterized by the number of necrotic cells, <25 %. According the determination of the level of damage (24) to the histological structure of the heart is based on the percentage number of cardiac picnotic cells, which is referred to as a minor damage. In this case, there is a picnotic nucleus between normal cells or picnotic cells that are <25 % of the entire field of view. A moderate damage is considered to be when the picnotic core is 25-50 % of the entire visual field, and the damage is severe, when the picnotic core is > 50 % of the entire visual field. This finding is in accordance with the research conducted, where he reported fatty acid diet has an influence on the heart, liver, and kidneys which shows the presence of hypertrophy that is observed microscopically (6).

The liver histopathological analysis of hyperlipidemic rats after eel fish oil extract administration (Table 5) revealed several histological changes in each treatment group. There was a damage to the liver tissues in the form of hydropic degeneration, fat degeneration, and bleeding. Local degeneration can be represented as the observed frequency of cells experiencing degeneration or necrosis between 1-25 % of the microscope field of view and/ or the number of cells experiencing degeneration and necrosis of 1-25 cells/1000 cells. Meanwhile, evenly distributed degeneration can be interpreted as the observed frequency of cells experiencing degeneration or necrosis >25 %, but <50 % in the field of view on the microscope. The central veins and sinusoids appeared normal, except for the 90.72 mg/200 g dose treatment group, where lesions appeared in the central veins and sinusoids that widened and narrowed in the liver of the experimental white rats. Furthermore, the results of Kruskal Wallis' analysis of liver cell damage showed a significant value of $p = 0.067$ ($p > 0.05$), which indicated that there was no significant difference in the rat liver cell damage to the applied treatment. Hydropic degeneration and fat degeneration are reversible.

There was an increase in cell necrosis following the increase in the test dose (Figure 3). Research findings revealed that the effect of a high-fat diet in mice led to abnormalities such as sinusoidal dilatation and necrosis in the liver (25). This observation is consistent with the findings who observed the microanatomic structure of the liver of white rats administered with lemuru and palm oil which showed that there was increased liver cell damage (liver necrosis) with increasing doses(26).

The results of kidney histopathological examination of hyperlipidemia rats (Table 6) after eel fish oil extract administration showed histological changes in each treatment group. From the results obtained, there was a damage to the kidney in the form of hydropic degeneration, fat degeneration, and bleeding. The glomerulus and capsule bowman seemed normal for the eel fish oil extract treatment group at a dose of 90.72 mg/200 g, glomerular seemed wrinkle and capsule bowman seemed narrowing of the kidney rats. Meanwhile, there was an increase in cell necrosis following an increase in the test dose administered in the rat kidneys. Histopathological examination of the kidney (Figure 3) revealed that cell death or necrosis increased with increasing treatment doses. The percentage value of the damaged parts was categorized into minor damage which was characterized by the number of necrotic cells <25 %. A study conducted highlighted that when total cholesterol value is close to normal, the kidney tissues will experience improvement (27). This observation suggests that the increased levels of lipids in the blood makes the organism susceptible to a high risk of kidney disfunction.

The pancreas histopathological examination of hyperlipidemic rats (Table 7) after eel fish oil extract administration indicated that there were histological changes in each treatment group. There was a damage to the pancreas in the form of hydropic degeneration, fat degeneration, and bleeding. The value of the average necrosis of the cells in the pancreas of the white rats (Figure 3) pointed out that there was an increase in cell necrosis following the increase in the test dose administered. From this percentage, the pancreatic cell damage was included in the scoring of Group 1; the percentage of damage ranged from 1-35 %. According to a reasearch, scoring for the destruction of pancreatic cells involved a score of 0 when there was no cell inflammation/ normal (0 %); a score of 1 when cell inflammatory section, normal cell lines (1-35 %); a score of 2 when there was inflammation in part of the cells, some of the cell formed were necrotic (36-50 %); a score of 3 when part of the cells was inflammatory, many cells had necrosis (51-70 %); and a score of 4 when necrosis of all pancreatic cells (> 71 %) was observed(28).

The anti-inflammatory activity of eel fish oil extract can be observed from its ability to inhibit the formation of oedema on the soles of rats' feet (29). Plethysmometer was used to measure the volume of inflammation in the soles of the rats’ feet. The operational principle of the instrument is based on Archimedes' law, which proposes that an object that is partially or completely immersed in a liquid will experience an upward force equal to the weight of the liquid displaced by the object. The measurement of the volume of inflammation in the soles of the rats' feet was carried out before treatment, and every 60 minutes after administration of the last test material for 6 hours of observation.

It was expected that an increase in the dose of the test extract would lead to a corresponding increase in the anti-inflammatory activity by a reduction of the inflammation volume, but it was observed (Table 8) that a dose of 44 mg/ 200 g of the eel fish oil extract showed the highest anti-inflammatory activity comparable to the positive control. The decrease in inflammation volume in the positive control group was due to a drug activity that inhibited the formation of prostaglandins. There was a decrease in the volume of inflammation in the soles of the rats. Meanwhile, the ability to reduce inflammation as a result of eel fish oil extract is thought to be due to the cell contents such as EPA, DHA, palmitic acid, stearic acid, and oleic acid which have anti-inflammatory effects. EPA acts to partially replace arachidonic acid in the phospholipid membrane in the cyclooxygenase process that inhibits the formation of Prostaglandin E2 (PGE2) and Leukotriene-4 (LT4), resulting in decreased inflammatory processes (30). The mechanism of DHA as an anti-inflammatory agent are inhibition of the activity of desaturase and elongase enzymes, leading to a corresponding inhibition of the formation of arachidonic acid (8). Palmitic acid induces the production of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and cyclooxygenase-2 (COX-2). The anti-inflammatory effect is thought to be the action of saturated and unsaturated fatty acids such as palmitic acid, EPA, DHA, stearic acid and oleic acid contained in eel fish oil extract.

The largest volume of inflammation was observed in a decreasing order: 1.5 % PEG negative control group (71.31 %) > the dose of 22 mg/200 g fish oil extract treatment group (48.49 %) > the dose of 44 mg/200 g eel fish

oil extract treatment group (44.03 %) > the diclofenac sodium positive control (43.60 %) > the 88 mg/200 g eel fish oil extract treatment group (32.21 %). This observation is consistent with the percentage inhibition of inflammation among the four groups compared to the negative control; the dose of 88 mg/200 g eel fish oil extract treatment group (54.48 %) > the diclofenac sodium positive control group (38.59 %) > the dose of 44 mg/200 g eel fish oil extract treatment group (37.94 %) > the dose of 22 mg/200 g eel fish oil extract treatment group (31.73 %). Although the percentage inhibition of inflammation obtained was different after the statistical one-way ANOVA, where the values of all the test groups were not significantly different (significance = 0.208 [p> 0.05]). This suggested that the eel fish oil extract with three dose variations had the same anti-inflammatory activity as the positive control (diclofenac sodium treatment). Therefore, the use of the lowest dose of 22 mg/200 g eel fish oil extract is sufficient in inflammation therapy.

The most effective dose for the anti-inflammatory activity was the 22 mg/200 g eel fish oil extract, which had an effect comparable to the positive control and offered the highest potential to reduce oedema. On the 39th day of testing, the diclofenac sodium positive control group showed a significant decrease in inflammation volume, while a decrease in inflammation volume was observed in the 1.5 % PEG negative control group, but was more likely to increase the inflammation volume over a period of 6 hours.

CONCLUSION

The findings from this study indicated that eel fish oil extract has anti-hyperlipidemic activity on white rats and is effective at a dose of 72 mg/kg. Histopathological testing of the heart, liver, kidneys and pancreas at doses of 18, 36 and 72 mg/kg classified as mild damage (<25%) which was in the safe category for treatment. Also, eel fish oil extract has anti-inflammatory activity in the egg white-induced inflammation in rats and was effective at a dose of 22 mg/ 200 g.

SUGGESTION

It is necessary to purify using natural or synthetic adsorbents from extracted fish oil and characterization to obtain pure oil that complies with IFOS/IFOMA and SNI standards.

It is necessary to test the security level so that it can become basic data in further testing.

It is necessary to carry out further pharmacological testing to determine the effect of fish oil before and after it is purified.

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