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In-vivo Antipyretic Effect of Eel (*Anguilla bicolor bicolor*) Oil on Yeast-induced Fever on Mice

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ABSTRACT

Fish oil has been studied for medicinal purposes, including its antipyretic properties. Eel (*Anguilla bicolor bicolor*) oil, which contains vitamins and fatty acids, including Omega-3 (EPA and DHA), is also expected to have the antipyretic effect. This research aimed to examine the antipyretic activity of eel oil on white mice (*Mus musculus* L.). An in-vivo study was done on thirty Swiss-Webster strain males mice that previously got 20% yeast-induced fever. Six treatments were applied including normal group (untreated), a negative control group (yeast-treated), a positive control group treated with acetaminophen (1.764 mg/20 g body weight), and three groups treated with eel oil (0.048, 0.096 and 0.192 g/20 g body weight, respectively). The data was analyzed statistically using one way ANOVA then was continued with LSD post hoc test. The results showed that eel oil has significantly reduced yeast-induced hyperthermia on mice five hours after application at doses 0.096 and 0.192 g/20 g body weight. Our finding suggests that eel oil possess antipyretic properties when was applied in certain doses, and this effect is presumably attributed to its high content of fatty acid, including EPA and DHA.

Keywords: Antipyretic, Eel oil, Fever, Omega-3, DHA, EPA

INTRODUCTION

Fever is defined as an elevation of core body temperature above normal (> 37.7 ºC) centered in body thermoregulatory set-point and triggered by the presence of pyrogens (Zaino et al., 2014). As one of non-steroidal anti-inflammatory drug (NSAID), acetaminophen is commonly used for fever treatment as it possesses an antipyretic effect due to its ability to inhibit prostaglandin biosynthesis (Tesema and Makonnen, 2015). It is one of drug-induced liver injuries when it is applied in toxic dose, but it is quite safe and well-tolerated in therapeutic doses (Brune et al., 2015; James et al., 2003).

Alternative sources of antipyretic drugs might come from marine products, especially fish oil. One of the potential alternatives is eel (*Anguilla bicolor bicolor*) oil. In previous studies, eel oil analysis using the heating method indicated a good quality of eel oil with appropriate standards. The oil parameters that have been studied are specific gravity value, acid value, peroxide value, saponification value, iodine value (Sasongko et al., 2017[a]), and metal content (Sasongko et al., 2017[b]). Eel is a type of fish that contains high protein, vitamins, and fatty acid like omega-3 (EPA and DHA) (Nafsiyah et al., 2018). Omega-3 content in eel oil is expected to be higher than omega-3 content in other fish oils (Widyasari et al., 2014; Ahn et al., 2015; Farah et al., 2018). For instance, a study reported by Widyasari et al. (2014) shows that eel oil has 5.16% omega-3. This number is considerably higher than omega-3 in milkfish oil (*Chanos chanos forsskal*), which only 2.4% (Aziza, 2015).

Omega-3 content in fish oil is thought to have a significant role in reducing inflammation, pain (Sasongko et al., 2019), and fever because it can reduce prostaglandins (PGs) production in the body. It competes with arachidonic acid and also suppresses monocyte capacity to synthesize interleukin-1 (IL-1) and tumor necrosis factor (TNF) (Calder, 2013). IL-1 and TNF are the primary mediators of inflammation that can induce fever (Simopoulos, 2002). Blockage of the cyclooxygenase (COX) enzyme would inhibit the conversion of arachidonic acid to the pro-inflammatory PGs that mediates the classic inflammatory response, temperature increase (Maroon and Bost, 2006). Therefore, this research aimed to investigate the antipyretic effect of eel oil on induced fever in mice.

METHODS

Material Preparation

Acetaminophen (Kimia Farma®) was purchased from the local pharmacy, Apotek Sebelas Maret Surakarta. Yeast was purchased from Pasar Gede Traditional Market. Other chemicals such as NaOH (Merck®), Na_2SO_4 (Merck®), methanol (Merck®) pro analytic grade, distilled water, and NaCl 0.9% were obtained from Pharmaceutical and Pharmacology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret.

Experimental animal

The experiment used 30 males of Swiss Webster mice **(**20 – 30 g) that were locally purchased from animal house breeding in Surakarta, Central Java. Animal handling procedures have been approved by the ethics committee of the Faculty of Medicine, Universitas Sebelas Maret.

Eel Oil

Eel oil was extracted from an average of 7 month-old Eel (*Anguilla bicolor bicolor*) weighing about 100 – 200 grams each. The eels were purchased from eel growing center, business development unit of Universitas Sebelas Maret, Surakarta.

Eel Oil Extractions

Reflux extraction technique was performed to extract oil from fresh Eels. Eels were cut into small pieces and then refluxed using distilled water under controlled temperature (70 – 80 ºC) for about 6 hours. The oil phase then was separated using filter paper (Sasongko et al., 2017[a]).

Eel Oil Analysis

The yielded eel oil then was analyzed for its total protein using the Kjeldahl method, a total of fatty acid using gravimetric analysis, and vitamin A and vitamin E using High-Performance Liquid Chromatography (HPLC). HPLC method was conducted in LiChrosper RP-18 column (5 μm), 4 mm x 250 mm, at a flow rate of 1 mL/min. Methanol was used as the mobile phase, and the volume injection was 10 μL. Spectral profiles were sampled at a rate of 10 points/sec using 277-nm PDA detector at a column temperature of 40 ± 15 ºC. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were analyzed using gas chromatography (GC), 30 m TG-WAXMS column with FID detector at a temperature of 250 ºC.

Antipyretic Test

The mice were previously adapted to the new cage and given free access to food and water for one week. Food access then was restricted for 11 hours before treatments, but access to water was still given. This fasting procedure was conducted to reduce bias due to food intake.

The antipyretic test was conducted by evaluating six groups of antipyretic treatment on fever-induced mice. The treatment including normal group (untreated), a negative control group (yeast treated), a positive control group treated with acetaminophen (1.764 mg/20 g body weight), and three groups treated with a different dose of eel oil (0.048,

0.096 and 0.192 g/20 g body weight, respectively). Each treatment was given to a group of 5 mice.

Fever induction was carried out according to a modified method by Sengar et al. (2015). The mice were given with sub-cutan injection of 20 mL/kg body weight (BW) of 20% yeast suspension below the nape of the neck. Body temperatures were measured in the rectal organ of mice (Souza et al., 2002) at the time before yeast induction as t_0 and 3 hours after yeast induction. Antipyretic treatment was orally given to mice that showed hyperthermia symptom (increasing body temperature). The post-induction measurement of body temperature was carried out at 1-hour interval for the first 5 hours. The serial measurements would give information about onset duration of the drug. Onset is the time needed for the drug to cause an effect.

Statistical Analysis

The effect of the six antipyretic treatments on yeast-induced fever on mice was analyzed using one-way analysis of variance (ANOVA). Priory, Shapiro-Wilks tests and homogeneity test were performed to confirm that the data were normally distributed and had homogeneity of the variances. Least significant difference (LSD) Post Hoc test was performed to determine the differences between each group of treatment.

RESULTS AND DISCUSSION

Eel Oil Analysis

The results showed that eel oil contains a high percentage of total fatty acid compounds, up to 88.43% w/w (Table 1). This value more than different marine fish species like forkbeard (40.5 %), Atlantic cod (44.3%), European pilchard (18 %), Atlantic mackerel (12.4 %), great weever (68.9 %), *etc.* (Guil-Guerrero et al., 2011). Eel oil has been known for its high content of saturated fatty acids (SAFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) (Nafsiyah et al., 2018). As much as 0.8 % w/w EPA and 4.17 % w/w DHA was also measured in the Eel oil. The EPA and DHA are thought to provide an antipyretic effect, providing anti-inflammation activity through inhibition of prostaglandin biosynthesis (Camuesco et al., 2005; Mullen et al., 2010).

Antipyretic Effect

In this research, yeast induction had successfully triggered hyperthermia on mice by increasing the average rectal temperature of about 1 ^{o}C, three hours after administration

(except the normal group which not treated with yeast). Yeast contains *S. cerevisiae* and could increase rectal temperature through the synthesis of prostaglandins (Ghauri et al., 2017).

Table 1. Measured total protein, fatty acid, and vitamins compounds of Eel oil.

Parameters	Results
Total protein (TP)	0.19% w/v
Total of fatty acid (TFA)	88.43% w/w
Eicosapentaenoic acid (EPA)	0.8% w/w
Docosahexaenoic acid (DHA)	4.17% w/w
Vitamin A (retinol equivalent)	981.49 mcg/100 g
Vitamin E	not detected

The antipyretic treatments with acetaminophen, eel oil 0.096 g/20 g BW and 0.192 g/20 g BW displayed a decrease of hyperthermia-induced rectal temperature in the first hour of oral treatment. Meanwhile, mice treated with eel oil 0.048 g/20 g BW and yeast treatment (negative control) showed a later temperature decrease in the second and third hour. At last, the rectal temperature of not treated mice considerably showed little changes (Figure 1).

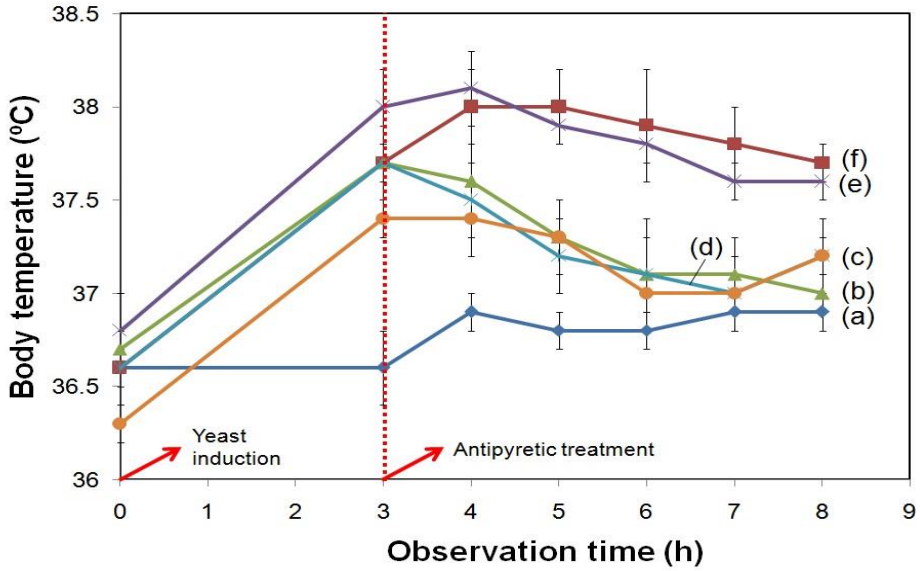

Figure 1. The changes of mice body temperature (°C) after yeast injection of (a) normal group, (b) acetaminophen treatment group, antipyretic treatment group with eel oil (c) 0.192 g / 20 g BW, (d) 0.096 g / 20 g BW, (e) 0.048 g / 20 g BW, and (f) negative control group.

Acetaminophen, as a positive control, showed better body temperature reduction compared to other treatments. Acetaminophen is a non-steroid anti-inflammatory drug

(NSAIDs) that inhibits the activity of the enzyme called cyclooxygenase (COX) which leads to the formation of prostaglandins (PGs) that causes fever (Aronoff and Neilson, 2001; Graham and Scott, 2005). In the positive control treatment, the decrease in body temperature started from 1-hour after drug administration. According to Raffa et al., (2014), this rapid effect is related to the pharmacokinetics of acetaminophen in which can be rapidly absorbed by the digestive tract. The peak serum level is achieved in 30-60 minutes with a half-life of 2 hours (Raffa et al., 2014).

Table 2. Mice body temperature changes before and after oral antipyretic treatment as a result of LSD post hoc test.

Groups	Observation Time (Time (hour) ± standart eror of means (SEM))						
	0[#]	3	4	5	6	7	8
Normal Group	36.6±0.2	36.6±0.2	36.9±0.1*	36.8±0.1*	36.8±0.1*	36.9±0.1*	36.9±0.1*
Negative Control Group	36.6±0.1	37.7±0.2	38.0±0.2	38.0±0.2	37.9±0.3	37.8±0.2	37.7±0.1
Acetaminophen 1.764 mg/20g BW	36.7±0.1	37.7±0.1	37.6±0.3*	37.3±0.2*	37.1±0.2*	37.1±0.2*	37.0±0.1*
Eel oil 0.048 g /20 g BW	36.8±0.2	38.0±0.2	38.1±0.2	37.9±0.1	37.8±0.1	37.6±0.1	37.6±0.1
Eel oil 0.096 g /20 g BW	36.6±0.4	37.7±0.3	37.5±0.2*	37.2±0.2*	37.1±0.3*	37.0±0.2*	37.2±0.2*
Eel oil 0.192 g /20 g BW	36.3±0.1	37.4±0.1	37.4±0.2*	37.3±0.1*	37.0±0.1*	37.0±0.2*	37.2±0.1*

Note: [#] The temperatures were just measured before the yeast injection
* The significant differences were obtained by comparing with control group ($p < 0.05$)

The eel oil treatment with a dose of 0.048 g/20 g BW showed little decrease in body temperature but the difference was not significant compared to the negative control group ($p>0.05$), meaning that this dose one has not been able to show a clear antipyretic effect on fever-induced mice (Table 2). Presumably, the dose has not reached the minimum effective level to give a significant result (Benet et al., 1996). While in the doses of 0.096 g/20 g BW and 0.192 g/20 g BW, the antipyretic effect was clearly shown as it was significantly different with the negative control group and the temperature decrease was comparable with the positive control of acetaminophen treatment (Table 2). This antipyretic effect might be attributed to the EPA and DHA contents in eel oil. EPA and DHA are included in the essential fatty acids that are released if there is wound on cell membranes and then competitively inhibit the formation of pro-inflammatory interleukins (IL-1β, IL-6, and IL-12), α factor necrosis factor (TNF α) and prostaglandin (Tesema and Makonnen, 2015). This inhibition of prostaglandins biosynthesis is believed to be the main

key to the antipyretic effect of eel oil as prostaglandin is involved in the regulation of body temperature (Tesema and Makonnen, 2015). Omega 3 especially EPA has a long-chain structure equation with arachidonic acid (AA), thus it can be a competitor to replace arachidonic acid in the metabolic processes of cyclooxygenase (COX) and lipoxygenase (Kobayashi et al., 2006). This theory is strengthened by another study by Laino (2017), suggested that EPA can inhibit the COX mechanism, therefore, prostaglandins are not formed. Blockage of the COX enzyme would inhibit the conversion of AA to the pro-inflammatory PGs that mediate fever (Maroon and Bost, 2006).

CONCLUSION

Eel oil treatment at doses of 0.096 and 0.192 g/20 g BW had shown a clear antipyretic effect and comparable to a common antipyretic drug, acetaminophen. However, the effect was not clear at dose 0.048 g/20 gr BW. This finding indicates that the administration of eel oil on fever-induced mice should meet the minimum doses to give a significant effect. The effect is thought to be the result of prostaglandins biosynthesis inhibition by DHA and EPA.

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