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Eel Oil Attenuates Acetaminophen-Induced Acute Liver Injury Through Inhibition of Oxidative Stress in Rats

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ABSTRACT

Health practitioners all over the world have studied liver injury caused by drug side effects. Excessive production of free radicals causes cell damage, which has implications for pathological conditions in both humans and animals. Omega-3 fatty acids are a component of fish that can work as hepatoprotective agents. Eel (*Anguilla bicolor*) is known to contain omega-3 including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). This study aimed to evaluate the hepatoprotective activity of eel oil on rats via inhibiting oxidative stress. Methods: Acetaminophen-induced male Wistar rats were used as liver injury experimental models. Rats were divided into 5 groups, namely normal control, negative control, positive control (silymarin, 100 mg/kg), and two groups of eel oil dose (2000 mg/kg and 4000 mg/kg). The study was conducted for 14 days. The levels of serum glutamic pyruvic transaminase (SGPT), serum glutamic pyruvic transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin, albumin, Malondialdehyde (MDA), and glutathione (GSH) levels of the liver organ were measured. The data were analyzed using statistics and analysis of variance. Results: The study showed that eel fish oil can reduce SGPT and total bilirubin levels of male Wistar rats induced by acetaminophen. Eel oil at a dose of 4000 mg/kg could significantly reduce SGPT and liver bilirubin levels in male Wistar rats ($p<0.05$). Eel oil is effective in reducing malondialdehyde (MDA) levels and increasing glutathione (GSH) levels at a dose of 2000 mg/kg. Conclusion: Eel oil has hepatoprotective activity by inhibiting SGPT, total bilirubin, MDA, and increasing GSH levels in rats.

Keywords: Omega-3; Liver injury; Malondialdehyde; Glutathione

INTRODUCTION

The liver has an important role in the body, including detoxification, secretion, storage of food reserves, hematology, protection, and also plays a role in the metabolic process of biomolecules (Sasongko et al., 2019). Liver injury as a result of side effects of drug use has been studied by scientists and health practitioners around the world (Wu et al., 2019). Toxins and free radicals are detoxified by the liver through conjugation processes, with numerous chemicals generated in the liver, including glutathione, glucuronic acid, glycine, and acetate (Blondet et al., 2018). Excessive free radical generation produces cell damage known as "oxidative damage," which has consequences for pathological disorders such as damage to cells, tissues, or organs in both humans and animals. Liver damage can be caused by several things, including viruses, drugs, metabolic disorders, cardiovascular disease, and a fatty liver. Acetaminophen is one of the drugs that can cause liver damage, particularly in overdose or long-term use (Sasongko and Sugiyarto, 2018).

Indications of liver damage are increased blood biochemical levels such as serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transferase (SGOT), serum alkaline phosphatase (ALP), bilirubin, and gamma glutamine-transferase (γGT) (Farid et al., 2019); (He et al., 2017). In addition, there was a decrease in endogenous antioxidant compounds such as superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) (Tzankova et al., 2017). Eel (*Anguilla bicolor*) is an aquacultured fish that is grown in large numbers in Asia, especially Indonesia. Eel oil is known to contain omega-3 which can work as an antioxidant for hepatoprotection (Kusharto et al., 2014); (Sasongko et al., 2017). Omega-3 can improve the health liver through anti-inflammation and suppress oxidative stress (Di Minno et al., 2012); (Qiu et al., 2012); (Pineda-Peña et al., 2018).

Studies on the hepatoprotective activity of eel fish oil are still very limited, so this research can be a reference for the use of fish oil for health.

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The purpose of this study is to determine whether eel oil can have a hepatoprotective effect in rats induced by acetaminophen through the inhibition of oxidative stress. Analysis of MDA and GSH levels in the liver was carried out to see the role of eels in oxidative stress.

METHODOLOGY

Material

Silymarin (Sigma-Aldrich), acetaminophen (pharmaceutics), reagent serum SGPT, SGOT, ALP, total bilirubin, and albumin from *Diasys.* Reagents, 0.15 M Tris-HCl solution (pH 7.4), 2-thiobarbituric acid (TBA) (Merck), sodium decocyl sulfate (Sigma-Aldrich), trichloroacetic acid (TCA) (Sigma-Aldrich), Ellman reagents (5, 5-dithiobis-2-nitrobenzoic acid) (Sigma-Aldrich), acetic acid, NaCl 0.9% (WIDA), sodium citrate (Merck Millipore), aquabidest (Ikapharmindo).

Eel Oil Extraction

One kilogram of eel fillets is boiled for 5 hours at 70–80 C. The results of boiling formed two phases, namely oil, and water, and then the upper phase (oil) was taken. The oil was then centrifuged at 4000 rpm for 10 minutes to separate the remaining water (Sasongko et al., 2017).

Animal experiments

Male Wistar rats (weighing 150–200 g) were used in the experiment. Animals were divided and placed in polycyclic cages with no more than three experiment animals per cage, and then placed in standard laboratory conditions (20–26^{0}C) with 12-hour light and 12-hour dark cycle. Experiment animals were fed 15 g of pellets per day and given standard water. The experiment animal was first acclimated to laboratory conditions for a week before the experiment. All processes carried out in this study have received approval from the health research ethics committee of Dr. Moewardi Hospital (No.230/II/HREC/2018).

Study of Hepatoprotective

Twenty-five rats were separated into five groups, with five animals in each group: Normal control: aqua dest treatment; Negative control: 0.25% CMC-Na per-oral; Positive control: 100 mg/kg silymarin per-oral; Dose 1: eel oil at a dose of 2000 mg/kg per-oral; Dose 2: eel oil at a dose of 4000 mg/kg per-oral.

The experiment was carried out for 14 days, and 30 minutes after the completion of the experiment, a dose of 2000 mg/kg of acetaminophen oral solution was administered. Blood samples were collected after 48 hours of acetaminophen administration, and the animals were euthanized. Blood samples were analyzed for SGPT, SGOT, ALP, albumin, and bilirubin.

Glutathione Measurement

Homogenate of liver organs was created using rat liver tissue that had been washed in cold saline and ground up with a mortar and pestle. It would be used to measure MDA and GSH. Five hundred milligrams of liver were mixed with 5 mL of a 0.15 M tris-HCL solution (pH 7.4), which was then compounded to form a 10% w/v homogenate (Novianto et al., 2015).

Glutathione levels are measured by the Ellman (1959) method, in which 0.75 mL of homogenate supernatant has been made with 0.75 mL of 10% TCA that contains 1 mM of EDTA and then centrifuged with a velocity of 200 rpm for 10 minutes. The supernatant was then combined with 1.8 mL of Ellman reagent (5,5'-dithio bis-2-nitrobenzoic acid) at 0.01 mM, which was prepared in a 0.3 M phosphate buffer containing 1% sodium citrate. The solution is measured in wavelength of 412 nm which is compared with blanks. Glutathione levels were determined using the extinction coefficient of 14.15 nm^{-1} cm^{-1} (Novianto et al., 2015).

$$\text{GSH level} = \frac{\text{absorbance} \times \text{dilution factor}}{\text{cuvette dense} \times 14{,}15\ \text{mM}^{-1}\ \text{cm}^{-1}} \times \frac{1}{\text{liver weight}}$$

Measurement MDA

Measurement of MDA levels was carried out using the *thiobarbituric acid reactive substances* (TBARS) spectrophotometric method. Added 0.2 mol of the liver homogenate to the mixture, 0.2 mL *sodium decocyl sulfate* (SDS) 8.1%; 1.5 mL acetate acid 20%, and 1.5 mL TBA 0.8%. The volume of the mixture was made into 4 mL by adding aquadest and heated at 95^{o}C in the *water bath* for 60 minutes. After incubation is complete, leave it at room temperature. Then the solution was added with 4 mL of 10% TCA and vortexed for 2 minutes, then centrifuged at 3000 rpm for 10 minutes. After that, read the absorbantion at the wavelength of 523 nm. MDA level can be expressed by *Thiobarbituric Acid Reactive Substance* (TBARS)/mg protein using extinction coefficient 1.56 x 10^5 $M^{-1}cm^{-1}$ (Dhage et al., 2021); Simeonova et al., 2020)

$$\text{MDA level} = \frac{\text{Absobantion x Diluent Factor}}{(\text{Thick Cuvette x } 1{,}56 \text{ x } 10^5\ \text{M}^{-1}\text{cm}^{-1})} \text{ x } \frac{1}{\text{Hepar Weight}}$$

Statistical Analysis

Data are presented as mean ± SD and statistically analyzed. The data obtained were tested for normality and homogeneity using the

Shapiro-Wilk and Levene's test methods. If the data is normally distributed and homogeneous, then it is continued with *one-way* ANOVA and *post hoc* tests which are the Least Significant Difference (LSD) ($p \leq 0.05$). If the data is not normally distributed and homogeneous, then the *kruskal wallis* test is carried out followed by a *post hoc* test in the form of *the mann whitney* test ($p \leq 0.05$).

RESULT AND DISCUSSION

Table I shows the effects of eel oil on SGPT, SGOT, ALP, total bilirubin, and albumin levels. The results showed that the administration of a toxic dose of acetaminophen to rats could significantly increase the levels of SGPT, SGOT, and total bilirubin. Only a dose of 4000 mg/kg of eel oil can significantly inhibit the increase in SGPT and total bilirubin after 14 days of administration ($P \leq 0.05$).

Table II shows the effect of 14-day eel oil administration on liver tissue antioxidant enzyme activity markers in acetaminophen-induced hepatotoxicity in rats. There are different levels of MDA and GSH in the livers of rats receiving eel oil at doses 2000 – 4000 mg/kg and silymarin compared to negative controls ($p<0.05$).

The consumption of acetaminophen in excessive amounts is one of the toxicants that can cause damage to the liver. This is partly since the metabolism of acetaminophen in general through glucuronate and sulfate is saturated, which results in a significant number of reactive NAPQI (N-acetyl-p-benzo-quinone-imine) metabolites. Excessive concentrations of NAPQI can lead to a decrease in glutathione levels, malfunctions in the mitochondria, and oxidative stress, all of which can cause damage to the liver (Du et al., 2016; Wu et al., 2019). An increase in blood biochemical markers such as SGPT, SGOT, ALP, bilirubin, and protein serum are sign that the liver has been damaged (Setyawati, 2018; Singal et al., 2015).

The findings of this study show that eel oil can lower blood biochemical levels that rise when acetaminophen is taken in high quantities. Eel oil is efficient in shielding liver cell membranes from damage induced by NAPQI when administered at a dose of 4000 mg/kg. It is characterized by a decrease in SGPT levels and total bilirubin. Eel oil does not have a substantial impact on ALP and albumin concentrations.

Malondialdehyde is a metabolite produced when free radicals peroxide lipids (Aflanie et al., 2015). While glutathione is the principal antioxidant, it acts by inhibiting the generation of new free radicals by converting existing free radicals into molecules that have a less active impact (Balasaheb Nimse and Pal, 2015). This process prevents new free radicals from being formed. These two molecules serve as parameters for determining the condition of oxidative stress, which is characterized by a shift in the equilibrium between the formation of free radicals and antioxidants and occurs when the levels of free radicals are greater than the levels of antioxidants (Asao and Asaduzzaman, 2018).

Increasing GSH levels and decreasing MDA levels are two ways that eel oil, which contains omega-3 fatty acids, may help reduce oxidative stress. Eel oil acts as an antioxidant by stabilizing carbon-nucleated radicals, preventing them from attacking cells, and interrupting the oxidation chain reaction (Atta et al., 2017). Radical compounds will not develop if the oxidation chain is broken (Balasaheb Nimse and Pal, 2015). The negative control group, which received acetaminophen at a dose of 2000 mg/kg had lower levels of GSH and higher levels of MDA than the other groups. Endogenous antioxidant defense mechanisms fail to protect against excess free radicals when GSH levels fall below 70% of normal levels, which is caused by an increase in NAPQI in the liver (Ntamo et al., 2021).

Positive control groups showed a significant difference ($p<0.05$) in their ability to protect liver cells from oxidative stress when compared to the negative control group. Silymarin can be utilized as a hepatoprotective and this shows that the approach used is adequate (Freitag et al., 2015; Vargas-Mendoza et al., 2014).

When compared to the negative controls, the groups that received eel oil at doses of 2000 mg/kg and 4000 mg/kg saw substantial decreases in MDA levels and increases in GSH. It is thought that eel oil can reduce MDA levels and enhance GSH levels in rats' livers. Essential fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) make up the omega-3 fatty acids, which are a class of unsaturated fats (PUFA) (Sprynskyy et al., 2022). By lowering radicals $NO^{-}{}_{2}$, reducing erythrocyte lipid peroxidation, and increasing vitamin E incorporation into membranes, omega-3 protects cells from oxidative stress and the resultant lipid peroxidation caused by an increase in free radicals (DiNicolantonio and OKeefe, 2019; Iraz et al., 2005). The inflammatory inhibitory pathway is a very important part of the hepatoprotective mechanism. It works together with the oxidative stress effect to stop inflammation. In this study, we have not yet conducted an assessment of inflammatory markers, either through cytokine expressions or histopathological necrosis analysis. In future research, an analysis of the inflammatory profile is highly recommended to evaluate the anti-inflammatory mechanism of eel oil.

Table I. Effect of 14-day eel oil administration on serum levels of liver markers in acetaminophen-induced hepatotoxicity in rats (n=5). *P <0,05 significantly different from the negative control group.

Parameters	SGPT (U/l)	SGOT (U/l)	ALP (U/l)	Total Bilirubin (g/dl)	Albumin (g/dl)
Normal	33.45 ± 2.89*	35.62 ± 1.87*	26.19 ± 3.54	0.43 ± 0.05*	0.46 ± 0,06
Positive Control	23.80 ± 2.11*	50.05 ± 4.96*	16.33 ± 1.96	0.50 ± 0.05*	0.37 ± 0.05
Negative Control	288.35 ± 23.64	176.55 ± 18.90	23.29 ± 2.66	0.95 ± 0.12	0.34 ± 0.04
Eel oil 2000 mg/kg	226,52 ± 6.77	177.50 ± 5.05	25.92 ± 3.37	0.68 ± 0.09	0.25 ± 0.03
Eel oil 4000 mg/kg	68.65 ± 6.90*	224.02 ± 8.94	12.48 ± 1.74	0.35 ± 0.04*	0.45 ± 0.06

Table II. Effect of 14-day eel oil administration on liver tissue antioxidant enzyme activities markers in acetaminophen-induced hepatotoxicity in rats (n=5). *P <0.05 significantly different from the negative control group.

Parameters	MDA (µM/g liver)	GSH (µM/g liver)
Normal	0.040 ± 0.017*	16.431 ± 2.79*
Positive Control	0.048 ± 0.048*	19.403 ± 3.09*
Negative Control	0.145 ± 0.023	10.718 ± 2.45
Eel oil 2000 mg/kg	0.045 ± 0.008*	18.067 ± 3.19*
Eel oil 4000 mg/kg	0.038 ± 0.014*	21.253 ± 6.32*

CONCLUSION

Eel oil has hepatoprotective activity by inhibiting MDA and increasing GSH levels in rats. Biochemical markers SGPT and bilirubin can be inhibited after the administration of eel oil.

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