The effect of red algae extract (Eucheuma spinosum) on dexamethasone induced MDA levels of wistar male white rats (Rattus norvegicus)

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INTRODUCTION

Type 2 diabetes mellitus (DMT2) is a metabolic disorder characterized by high blood sugar levels and insulin resistance. It is commonly associated with non-alcoholic fatty liver disease (NAFLD) or metabolic-associated fatty liver disease (MAFLD), which can cause liver damage ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) and cirrhosis of the liver (SH). Individuals with both type 2 diabetes and NAFLD have a higher risk of developing cardiovascular disease than those with type 2 diabetes alone.1

Insulin resistance in muscle and adipose tissue, hyperinsulinemia, impaired pancreatic islet cell response, and hepatic insulin resistance are the pathophysiological basis of diabetes in liver disease. The first trigger (first hit) is steatosis caused by increased free fatty acids absorbed by the liver due to insulin resistance. This is followed by various complex interactions (multiple-second hits) involving liver cells, stellate cells, adipose cells, Kupffer cells, inflammatory mediators, and reactive oxygen species (SOR) which can trigger inflammation (NASH) or develop into SH. Increased levels of malondialdehyde (MDA) can be used as a benchmark for suspicion of NAFLD, although it is not very specific and sensitive according to most epidemiological studies.2,4

The red algae Eucheuma spinosum contains a flavonoid that lowers blood fat levels by stimulating the PPAR pathway resulting in beta-oxidation of fatty acids. This reduces fatty liver and insulin sensitization while increasing the expression of adiponectin. Additionally, red algae may protect the liver and reduce fatty liver.5-7 Long-term use of corticosteroid agents can cause insulin resistance. Corticosteroids are steroid hormones secreted by the adrenal cortex.
Dexamethasone is a commonly used corticosteroid with anti-inflammatory, strong immunosuppressant, anti-allergic, and anti-nausea properties. However, long-term use of this drug can result in various adverse effects, including fat accumulation in the liver and NAFLD.8-11

The study aimed to analyze the effect of dexamethasone on MDA levels in alloxan-induced male Wistar rats. It also examined the differences in administering *Eucheuma spinosum* extract on serum MDA levels in rats induced with dexamethasone. The research intends to determine the effect of diabetes induced by dexamethasone on MDA levels and the ability of *Eucheuma spinosum* extract to repair liver damage caused by dexamethasone. Two students conducted the study, which focuses on exploring marine biota's role in disease prevention in humans.

**MATERIAL AND METHODS**

The experiment protocols conducted in this study received approval from the Ethics Committee Faculty of Medicine-Universitas Hang Tuah with reference number 1/1031/UHT.KEPK.03/IV/2023, located in Surabaya, Indonesia. These procedures were carried out following ethical standards, according strictly to the guidelines and objectives of animal research. This study used a post-test control group design on three groups of male white rats. Group K1 was the negative control group given standard feed, K2 was the control group, and K3 was the treatment group given red algae extract prophylactically. All three groups were induced by dexamethasone 8 mg/kg BW and measured serum MDA levels at the end of the study.12

The study used male Wistar strain rats (*Rattus norvegicus*) aged 9-12 weeks, weighing 150-200 grams, and in good health. Inclusion criteria required male rats, while exclusion criteria were anatomical abnormalities, unhealthy appearance, and refusal to eat. Animals who died or contracted unrelated diseases were dropped from the study. The experiment used a total of 30 rats divided into 3 groups. The sample size was calculated using the Federer formula13 with a minimum of 9 rats per group. Ten rats were used per group, to account for dropouts. The research sample was taken through simple random sampling.14

Before extracting red algae, taxonomic identification is performed to determine the type of algae. Suitable *Eucheuma spinosum* seaweed is washed, dried, cut into smaller sizes, and weighed at approximately 1 kg. The pieces are then macerated 3 times in 24 hours at room temperature with ethanol solvent in a jar. The macerated results are filtered using Whatman paper no. 42 to produce residue and filtrate. The filtrate is evaporated with a rotary vacuum evaporator at 40°C until a thick extract is obtained. 1 ml of 1% CMC- Na is added as a solvent to the extract.15

The preparation used was dexamethasone sodium phosphate injection 5mg/ml. According to Hamdy's research in 2019, the dose was 8 mg/kg BW.16 The experimental animal weighed 0.15 kg, so the dose was 0.24 mg/day. The research operational framework in Figure 1 illustrates the stages of the study. The rats were anesthetized with intramuscular ketamine at a dose of 50 mg/kg BW, and blood samples were taken from the heart or intracardiac using a 3 cc syringe. The blood was slowly poured into a test tube and shaken before being centrifuged at 12,000 rpm for 10 minutes to calculate the serum MDA level.18 Animals must be anesthetized before euthanasia by cervical dislocation. MDA levels were measured using a modified TBA acid test.

Data management, including coding, was carried out from the start of the research in the laboratory. The data was then cleaned, processed, and analyzed using descriptive statistics to calculate the mean and standard deviation of the variable serum MDA levels. If the normality test results are not normally distributed, a non-parametric statistical test is performed using the Kruskal-Wallis One-Way Test. All tables and graphics (figures, reaction schemes, and chemical structures) are inserted within the manuscript text where they are first discussed.

**RESULT**

**Research Data**

Data was collected from male white rats (*Rattus norvegicus* Wistar strain) induced by dexamethasone to measure malondialdehyde (MDA) levels. The rats were divided into three groups: a negative control group with no treatment, a positive control group with dexamethasone-induced diabetes, and a treatment group prophylactically given red algae extract (*Eucheuma spinosum*) 200 mg/kg BW, then induced with dexamethasone 8 mg/kg BW. The samples were randomized.

**Research Results**

The results of the descriptive analysis of the dependent variable can be seen in Table 1 below. The data obtained from the results of the study are then described in Figure 1 below. According to Table 1 and Figure 1, the mean serum Malondialdehyde (MDA) level was highest in K3 (872.17 + 103.33 nmol/g) and lowest in K1 (672.50 + 286.49 nmol/g). The K3 group, which was given prophylactic red algae extract (*Eucheuma spinosum*) 200 mg/kg BW and induced by dexamethasone 8 mg/kg BW, showed an increase in serum MDA level compared to the K2 group, the positive control group. The study results were described and tested with a significance level of 95% (p<0.05).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA serum</td>
<td>K1</td>
<td>672.50</td>
<td>286.49</td>
</tr>
<tr>
<td>(nmol/g)</td>
<td>K2</td>
<td>848.50</td>
<td>157.75</td>
</tr>
<tr>
<td></td>
<td>K3</td>
<td>872.17</td>
<td>103.33</td>
</tr>
</tbody>
</table>

Note:
K1: The negative control group, namely the white male rats of the Wistar strain (*Rattus norvegicus*), did not receive any treatment (10 rats).
K2: Positive control group, white male rats with Wistar strain (*Rattus norvegicus*) with diabetes model induced by dexamethasone at 8 mg/kg BW (10 individuals).
K3: The treatment group, namely a group of white male rats with Wistar strain (*Rattus norvegicus*) diabetes model induced by dexamethasone at 8mg/kg BW and given red algae extract (*Eucheuma spinosum* extract) at a dose of 200 mg/kg BW (10 individuals).
THE MEAN OF MALONDIALDEHYDE (MDA) SERUM LEVELS OF WHITE RATS

Figure 1. Bar chart descriptive analysis of serum Malondialdehyde (MDA) levels of white rats.

Table 2. Non-parametric test results (Kruskal-Wallis) for serum Malondialdehyde (MDA) levels of white rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Kruskal Wallis H</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA serum</td>
<td>2,933</td>
<td>0.231</td>
</tr>
</tbody>
</table>

Non-parametric test results (Kruskal-Wallis)

The study used serum MDA levels of white rats as non-homogeneous data and continued with a Kruskal-Wallis test. The results are presented in Table 2.

A non-parametric Kruskal-Wallis test was conducted on MDA levels in white rat serum. The test result was not significant (p>0.05), indicating no difference in the average serum MDA levels of white rats in each group of samples. The groups were negative control, positive control, administration of red algae extract (Eucheuma spinosum) 200 mg/kg BW and induced by dexamethasone 8 mg/kg BW (10 mice).

DISCUSSION

According to Hamdy’s 2019 research, 0.24 mg/day of dexamethasone sodium phosphate injection at 8 mg/kg BW was given intraperitoneally to rats weighing 0.15 kg. The increase in serum MDA levels of white rats was not significant using the non-parametric Kruskal-Wallis test. Dexamethasone administration leads to insulin resistance by suppressing Adiponectin and decreasing transcription of Insulin receptor substrate-1 (IRS-1), resulting in increased insulin levels, lipid breakdown, and release of free fatty acids into the blood. When dexamethasone increases glucocorticoids in the blood, it also raises glucagon levels, stimulating lipolysis. Insulin resistance boosts hormone-sensitive lipase activity and triggers increased lipid breakdown. This leads to a surge in free fatty acid levels in the blood, which the liver absorbs and stores as lipid droplets. The amount of absorption is directly proportional to the high levels of glucocorticoids in the blood and leads to the emergence of fatty liver.

The study found that dexamethasone induction increased serum MDA levels in white rats, indicating liver damage with necrosis releasing MDA into the blood. The red algae extract Eucheuma spinosum 200 mg/kg couldn’t improve MDA levels or inhibit dexamethasone-induced liver damage. Non-alcoholic fatty liver disease (NAFLD) is linked to high-carbohydrate, high-fat, and low-nutrient diets and is prevalent in overweight children and adults. NAFLD is associated with metabolic syndrome, including obesity, high blood pressure, diabetes, peripheral insulin resistance, hypertriglyceridemia, and hyperinsulinemia. The pathogenesis of NAFLD is largely due to insulin resistance, oxidative stress, and inflammation, which occur in obese individuals and those with dyslipidemia.

This study hopes that the induction of white rats with dexamethasone will result in hyperglycemia and a DM-like state due to damage to the β-pancreatic cells of the rats due to excessively forced insulin production which in turn will trigger non-alcoholic fatty liver disease (NAFLD). If NAFLD has occurred, necrosis will occur in the liver cells of white rats.

MDA is a marker of increased free radicals in the body that are formed due to oxidative damage. Lipid peroxidation in cell membranes includes reactions between free radicals (hydroxy radicals) and PUFAs to produce the final product, MDA. Aldehyde products that are toxic to cells are decomposed by hydrogen peroxide and will then produce the main aldehyde, MDA. The positive control group showed an increase in serum MDA levels which indicated that there was an increase in the formation of free radicals in the body which were formed due to oxidative damage. However, after administration of red algae extracts Eucheuma spinosum 200 mg/kg BW in the treatment group, the mean increased, although not significantly from the Kruskal Wallis non-parametric test. The statistical results indicated that the mean difference in serum MDA levels was insignificant between groups because p > 0.05.

Increased lipid absorption will trigger an increase in fatty acid oxidation and result in mitochondrial dysfunction due to disturbances in the mitochondrial respiratory chain. The impaired function will be continued by increased production of SOR and cause an increase in inflammatory cell infiltration in the liver. This will trigger an increase in oxidative stress which in turn is a stimulant for increasing the production of pro-inflammatory cytokines through the induction pathway by Nuclear Factor-κB (NF-κB). Administration of red algae extract Eucheuma spinosum 200 mg/kg BW in this study did not successfully reduce serum MDA levels of white rats.

Theoretically, Flavonoids can reduce inflammation through PPARα stimulation, inhibiting NF-κB. Flavonoids are also found to have a radical scavenging effect that simultaneously reduces inflammatory cell infiltration. However, in this study, it was found that the administration of...
red algae extracts *Eucheuma spinosum* 200 mg/kg BW was not able to stop the inflammation that had already occurred in the liver, so serum MDA levels were still increasing in the treatment group. This indicates that there is still inflammation in the liver due to dexamethasone induction although it is not as strong as when the red algae extract *Eucheuma spinosum* 200 mg/kg BW is not given. This event shows that the red algae extract *Eucheuma spinosum* 200 mg/kg BW cannot completely stop the inflammatory process but can inhibit the inflammatory process that occurs. The dose of *Eucheuma spinosum* (red algae extract) 200 mg/kg BW given may still be insufficient to stop the entire inflammatory process that occurs due to dexamethasone induction.

This study’s limitation is that it did not examine the antioxidant levels of *Euchema spinosum*, so it cannot compare the oxidative stress produced by administering dexamethasone and the antioxidants produced by *Eucheuma spinosum*. Therefore the suggestions for forthcoming research were: 1) more in-depth research on the appropriate *Eucheuma spinosum* dosage that prevents the increase of MDA serum; 2) further research is needed regarding the impact of *Eucheuma spinosum* on endogenous antioxidants so that it can provide evidence that *Eucheuma spinosum* can prevent oxidative stress; 3) future studies should incorporate human test subjects to demonstrate the effectiveness of *Eucheuma spinosum* in preventing the increase of oxidative stress.

**CONCLUSION**

The induction of dexamethasone in this study increased liver cell necrosis, characterized by an increase in the mean serum MDA level in the positive control group. Giving red algae extract (Eucheuma spinosum) 200 mg/kg BW to white rats has not reduced serum MDA levels of white rats induced by dexamethasone 8 mg/kg BW.

**FUNDING**

Indri Ngesti Rahayu, Asami Rietta Kumala, Anang Mufti, I Putu Bagus Dirgantara

Dusak, Larasati Kirana Damayanti, and Lestari Dewi, are supported by the Internal Research and Community Service (LPPM), Hang Tuah University.

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