Effect of *Spirulina platensis* on Liver Malondialdehyde Levels of Male White Rat Induced by High Dose Paracetamol

Anggilia Vanny1, Sihning E. J. T*, Indri Ngesti Rahayu, Roostantia Indrawati

**Abstract**

Paracetamol is widely used in therapy, yet it becomes a hepatotoxic agent in a high dose. The CYP450 enzyme metabolizes a small portion of paracetamol to a reactive substance, NAPQI. A high dose of paracetamol causes an extensive increase in NAPQI levels. NAPQI then binds to hepatocytes in conditions of oxidative stress. *Spirulina platensis* contains C-phycocyanin, flavonoids, and phenolics that inhibit lipid peroxidation and reduce oxidative stress. This study aims to determine the effect of *Spirulina platensis* on MDA levels in the liver tissue of male Wistar rats induced by high dose of paracetamol. This research was experimental with a Randomized Post-test Only Control Group design using 24 white male rats divided into three groups; negative control group (G−), positive control group (G+) was given paracetamol at a dose of 1,250 mg/kg on the 8th day, and treatment group (Gt) was given an amount of *Spirulina platensis* extract 360 mg/kg BW/day for ten days from 1st until 10th day and paracetamol at a single dose of 1,250 mg/kg BW on the 8th. At the 10th day of the treatment, all groups were terminated for liver tissue collection. TBA test was used to calculate MDA levels. Statistical tests analyzed the results of the data. This statistical tests by SPSS software: MDA levels in the treatment group did not significantly differ (p = 0.144) compared to the negative control group. There was a significant difference of liver tissue MDA levels in the treatment group (p = 0.016) compared to the positive control group. This study concludes that administering *Spirulina platensis* at a dose of 360 mg/kg/day for ten days can help prevent on increase in tissue MDA levels and maintain liver conditions close to their original state.

**Keywords:** antioxidant, malondialdehyde (MDA), *Rattus norvegicus*, *Spirulina platensis*

**INTRODUCTION**

Paracetamol is widely used in therapy and is a potent hepatotoxic agent at higher doses (Chellappan et al., 2016). The liver metabolizes Paracetamol at average doses via glucuronide and sulfate conjugation to a more air-soluble conjugated product that facilitates excretion to the kidneys (Esperanza et al., 2021). CYP450 enzymes metabolize a small portion of paracetamol to produce a highly reactive substance, N-acetyl-p-benzoquinone imine (NAPQI) (Rahmawati et al., 2018). Following a paracetamol overdose, NAPQI levels extensively reduce GSH. NAPQI binds to hepatocytes because GSH cannot convert and form NAPQI-adduct protein which causes the liver to experience oxidative stress and hepatocellular necrosis (Verma et al., 2013).
Malondialdehyde is the primary metabolite of arachidonic acid which can be used as a biomarker under oxidative stress conditions (Singh et al., 2014). MDA is a mutagenic and highly reactive three-carbon dialdehyde produced during the peroxidation of polyunsaturated fatty acids and arachidonic acid metabolism (Ahmad et al., 2016). The higher the antioxidant status, the lower the MDA content. The MDA test can be used as a biomarker of oxidative stress because MDA formation increases following oxidative stress (Rahmawati et al., 2018).

*Spirulina platensis* is a single-celled, spiral-shaped, filamentous blue-green microalgae (cyanobacterium) with many health benefits from its antioxidant activity (Ahmed et al., 2019; Baylan et al., 2012). Spirulina contains about 20% dry weight of the blue pigment phycocyanin extraction (Güroy et al., 2017). The phycocyanin contained in Spirulina, sp. depends on nitrogen supply. Phycocyanin produces a bright blue color. Phycocyanin is a protein complex that can boost immunity and has anticancer and antioxidant properties (Ridlo et al., 2016). Phycocyanin contained in Spirulina can significantly inhibit lipid peroxidation up to 65% (Belay, 2002). Spirulina, sp. also produces secondary organic metabolites, which result from metabolism through secondary reactions of primary organic substances (carbohydrates, fats, and proteins) (Enzing et al., 2014; Yuniati et al., 2020). There are three secondary metabolite groups: phenolics, alkaloids, and terpenoids. The secondary metabolites found in this plant are flavonoids, steroids, alkaloids, terpenoids, and tannins (Sukmaningbayu et al., 2016). This study was conducted to see the effect of blue-green microalgae extract as a hepatoprotection on the metabolic function of male white rats Wistar strain that induced by high dose of paracetamol.

**MATERIALS AND METHODS**

**Research design**

This study uses true experimental with a randomized post-test-only control group in the biochemistry laboratory at the Hang Tuah Faculty of Medicine, Surabaya. The random sample used in this study was 24 white males Wistar strain *Rattus norvegicus* divided into three groups. This experimental animal will receive different treatments for ten days during the research period. Paracetamol will be given to positive control (G+) and treatment group (Gt) on 8th day with a single dose of 1,250 mg/kg BW. On the other hand, Spirulina suspension will be given to treatment group every day from the 1st until 10th day with a dose of 360 mg/kgBW/day.

**Material**

The material used in this study was Paracetamol suspension, *Spirulina platensis* suspension, aquadest, Alcohol 95%, Ketamine, and CMC-Na 1%.

**Experimental Animal Treatment Stage**

At the initial phase of the study, the 24 rats were divided into three groups. Adaptation or acclimatization was carried out for seven days in the new environment. During this adjustment period, the experimental animals continued to receive standard feed and filtered local water product. Furthermore, the experimental animals will receive different treatments for ten days during the research period.

The negative control group in this study will consist of eight experimental animals without treatment. The positive control group will consist of eight experimental animals, which on the 8th day of the study, were given paracetamol at a dose of 1,250 mg/kg BW. The treatment group of 8 experimental animals would be given *Spirulina platensis* suspension at a dose of 360 mg/kg BW/day and paracetamol at a 1,250 mg/kg BW given on the 8th day of the study. After the research period ended, surgery was performed to take experimental animal tissue. Calculation of Malondialdehyde levels in liver tissue will be carried out by the Thiobarbituric acid (TBA) test.
Data Analyst
The results of the data obtained will then be analyzed using statistical tests by SPSS software. First, a normality test will be carried out using the Shapiro-Wilks test. The second step that needs to be done is to do an ANOVA test to see if the data is normally distributed. Later, the data to be analyzed comes from the results of trials in the negative control, positive control, and treatment group. Each will be compared, resulting in three data analysis groups.

RESULTS
Malondialdehyde (MDA) Levels Examination Results

Table 1. Mean MDA Levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-</td>
<td>2207.50</td>
</tr>
<tr>
<td>G+</td>
<td>3321.63</td>
</tr>
<tr>
<td>Gt</td>
<td>2757.50</td>
</tr>
</tbody>
</table>

G-: Negative Control Group; G+: Positive Control Group; Gt: Treatment Group

![Figure 1. Mean MDA Levels](image)

MDA Levels Normality Test Results

Table 2. Normality Test Results

<table>
<thead>
<tr>
<th>Group</th>
<th>Shapiro-Wilk Estrogen Statistics</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-</td>
<td>0.879</td>
<td>0.184</td>
</tr>
<tr>
<td>G+</td>
<td>0.934</td>
<td>0.553</td>
</tr>
<tr>
<td>Gt</td>
<td>0.905</td>
<td>0.323</td>
</tr>
</tbody>
</table>

The results of the normality test in table 2 obtain a significance value of p > 0.05, thus it indicates that the data distribution from the five groups is normally distributed.

Results of Homogeneity Tests of Experimental Animal Group Variants
After conducting a normality test and normally distributed data, proceed to perform a variant homogeneity test. The homogeneity test used is Levene’s test.
Levene test results of MDA levels in table 3 showed a significance value of 0.039 meaning that MDA levels are not homogeneous (p < 0.05).

**Kruskal-Wallis Test Results**

*Kruskal-Wallis* non-parametric statistical tests were conducted to prove the hypothesis in this study.

<table>
<thead>
<tr>
<th>Table 3. Variance Homogenity test results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>MDA</td>
</tr>
</tbody>
</table>

The result of the data in Table 3 showed a significance value of 0.039 meaning that MDA levels are not homogeneous (p < 0.05).

**Table 4. Kruskal-Wallis test results**

| Asymp. Sig. | 0.005 |

The result of the data in Table 4 has a significance value of 0.005. This shows a p < of 0.05, thus there is significant difference of MDA levels in liver tissue in all groups of experimental animals.

**Mann-Whitney U post-hoc test results**

**Table 5. Mann-Whitney U post-hoc test results**

<table>
<thead>
<tr>
<th>Negative control</th>
<th>Positive control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>-</td>
<td>0.005*</td>
</tr>
<tr>
<td>Positive control</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Treatment</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5 shows a difference in average of MDA levels between negative control and positive control group with the significance value between these two are 0.005 (p<a). Furthermore, a significance between negative control and treatment groups are 0.144 (p>a), indicating that the mean total MDA levels are not significantly different. Meanwhile, the positive control and treatment groups are significantly different at the value of 0.016 (p<a), indicating that the total average of MDA levels between these two groups are significantly different as well.

**DISCUSSION**

Paracetamol is widely used in therapy and is a potent hepatotoxic agent at higher dose. Paracetamol, given at average doses, will go through glucuronide and sulphate conjugation in the liver and become conjugated products so that they can be excreted through the kidneys (Verma et al., 2013). A small portion of paracetamol will be metabolized by cytochrome p450 into a reactive product, namely NAPQI, which, if in average amounts, will be quickly conjugated by GSH (Ben-Shachar et al., 2012). Giving paracetamol in high dose causes an excessive increase in NAPQI levels, so GSH cannot convert and form NAPQI-protein adducts (Hidayati & Kustriyani, 2020). Excess levels of NAPQI in the liver lead to lipid peroxidation, characterized by increased liver malondialdehyde (MDA) levels, decreased GSH levels, and hepatic cell necrosis (de Andrade et al., 2015). MDA results from lipid peroxidation, which will increase in tissues as a biomarker under oxidative stress conditions (Singh et al., 2014).

The decrease in the average level of hepatic tissue MDA could be due to the administration of blue-green microalgae extract (*Spirulina platensis*) at a dose of 360 mg/kg BW, which contains C-phycocyanin, flavonoids, vitamins, and phenolic compounds which can be antioxidants and reduce MDA levels in liver tissue through controlling oxidative stress in tissues.
C-phycocyanin is one of the phycobiliprotein pigments found in blue-green microalgae, which helps reduce free radical damage by reacting with hydroxyl radicals, thereby preventing lipid peroxidation, increasing antioxidant defences in plasma lipoproteins and restoring the body's antioxidant function (Fernandes et al., 2023). The mechanism of C-phycocyanin in fighting oxidative stress can help protect and increase GSH levels. The C-phycocyanin contained in Spirulina can significantly inhibit lipid peroxidation by up to 65% (Belay, 2002). The C-phycocyanin content in Spirulina can significantly reduce MDA levels and be a defense mechanism against lipid peroxidation induced by carbon tetrachloride (Burhan et al., 2021; Sayed et al., 2022).

The content of C-phycocyanin found in Spirulina platensis is also helpful in increasing G6PD. Glucose-6-phosphate dehydrogenase (G6PD) deficiency can occur in cells under oxidative stress. G6PD plays a role in protecting cells from oxidative stress in the form of NADPH (Frank, 2005). Hepatic glutathione (GSH) is converted to its oxidized form, namely glutathione disulphide (GSSG), in a reaction catalyzed by glutathione reductase (Warshaw et al., 1985). After oxidizing, GSH will be hydrolyzed again by glutathione reductase, using NADPH as the electron donor (Ghneim & Alshebly, 2016). Under conditions of oxidative stress, GSSG buildup will occur so that the role of NADPH can help convert it back into GSH, which will then play a role in NAPQI conjugation (Engli, 2012; Yuniastuti, 2016).

Catalase is an enzymatic antioxidant that catalyzes hydrogen peroxide into water and oxygen. Catalase helps reduce high amounts of hydrogen peroxide (Zulaikhah, 2017). Administration of Spirulina platensis containing C-phycocyanin helps increase catalase activity to prevent the accumulation of free radicals (Novirianthy & Sekarutami, 2015). Flavonoids have three ways to reduce free radicals: donating hydrogen to break the proxy chain, reducing singlet oxygen, and releasing hydrogen peroxide through metal iron (Fe²⁺) bonds in the Fenton reaction, so that it can inhibit the formation of hydroxyl radicals (Alrahmany, 2012). The Fenton reaction is a chemical bond-breaking reaction of a compound under the influence of a metal ion catalyst which produces two free radical molecules. Flavonoids play an essential role in reducing NAPQI radicals because the hydroxyl groups of flavonoids are highly reactive hydrogen donors and can form stable flavonoid-NAPQI complexes (Anggraini et al., 2021). Flavonoids are phenolic components abundant in Spirulina sp. They can improve hepatic MDA levels due to their complex antioxidant role in reducing free radicals (Riza & Devi, 2015).

CONCLUSION
Spirulina platensis with a dose of 360 mg/kg/day have shown the ability to decrease liver malondialdehyde (MDA) levels in experimental animal groups induced by high dose of paracetamol. Therefore, Spirulina platensis is considered to be a preventive agent against liver tissue damage due to administration of high dose of paracetamol.

CONFLICT OF INTEREST
All authors declare that there is no conflict of interest in this study

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