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

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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*

Original Research Article

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 metabolites 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 8^{th} 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 1^{st} until 10^{th} 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

Group	Mean (nmol/g)
G-	2207,50
G+	3321,63
Gt	2757,50

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

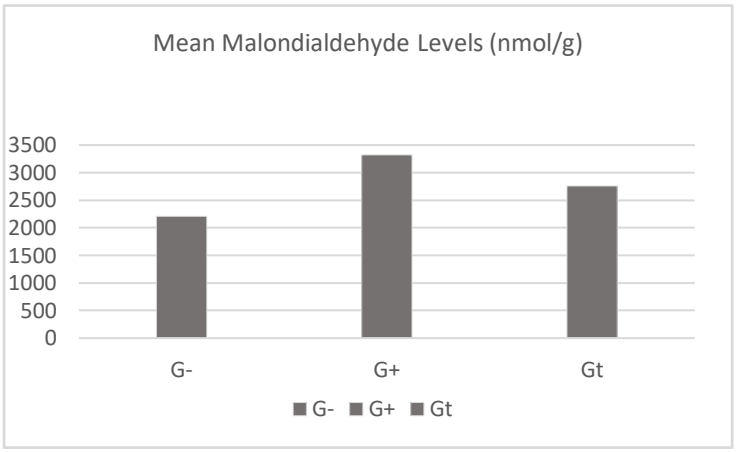

Figure 1. Mean MDA Levels

MDA Levels Normality Test Results

Table 2. Normality Test Results

Group	Shapiro-Wilk	
	Estrogen Statistics	Sig.
G-	0,879	0,184
G+	0,934	0,553
Gt	0,905	0,323

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.

Table 3. Variance Homogenity test results

Data	*Levene Statistics*	Sig.
MDA	3.812	0.039

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 4. Kruskal-Wallis test results

	MDA Levels
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

	Negative control	Positive control	Treatment
Negative control	-	0.005*	0.144
Positive control	-	-	0.016*
Treatment	-	-	-

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 (Fe^{2+}) 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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REFERENCES

Ahmad, A., Manjrekar, P., Yadav, C., Agarwal, A., Srikantiah, R. M., & Hegde, A. (2016). Evaluation of ischemia-modified albumin, malondialdehyde, and advanced oxidative protein products as markers of vascular injury in diabetic nephropathy. *Biomarker Insights*, *11*, 63–68. https://doi.org/10.4137/BMI.S39053

Ahmed, K. A., Korany, R. M. S., El Halawany, H. A., & Ahmed, K. S. (2019). *Spirulina platensis* alleviates arsenic-induced toxicity in male rats: Biochemical, histopathological and immunohistochemical studies. *Advances in Animal and Veterinary Sciences*, *7*(8), 701–710. https://doi.org/10.17582/journal.aavs/2019/7.8.701.710

Alrahmany, R. (2012). *Antioxidant and antimicrobial activities of phenolic acids rich extracts released from oat bran with the aid of carbohydrases*. *Thesis*. Food Science and Nutrition. Carleton University, Ontario.

Anggraini, W., Rousdy, D. W., Wardoyo, E. R. P. (2021). Nilai Malondialdehid Hepar Mencit yang diinduksi Parasetamol pada Pemberian Ekstrak Metanol Kulit Kayu *Vitex pubescens Vahl,. Protobiont,10*(1), 6-11

Baylan, M., Devrim Özcan, B., Işik, O., & Akar, M. (2012). A Mini Review on Spirulina. *Türk Bilimsel Derlemeler Dergisi*, *5*(1), 31–34. www.nobel.gen.tr

Belay, A., Berestov, V., Bertolin, T. E., Pilatti, D., Cristina, A., Varrone, V., Bavaresco, C. S., Colla, L. M., Alberto, J., Costa, V., Chu, W. L., Lim, Y. W., Radhakrishnan, A. K., Lim, P. E., Falquet, J., Herrero, M., Ibanez, E., Senorans, F. J., Cifuentes, A., … Ibrahim, Z. K. (2008). Factors Affecting Oocyte Quality: Who is Driving the Follicle? *Reproduction in Domestic Animals*, *5*(3), 809–822.

Ben-Shachar, R., Chen, Y., Luo, S., Hartman, C., Reed, M., & Nijhout, H. F. (2012). The biochemistry of acetaminophen hepatotoxicity and rescue: A mathematical model. *Theoretical Biology and Medical Modelling*, *9*(1). https://doi.org/10.1186/1742-4682-9-55

Burhan, H. W., Mewo, Y. M., & Assa, Y. A. (2021). *Efek Antioksidan dari C-Fikosianin pada Spirulina*. *9*(1). https://doi.org/10.35790/ebm.9.1.2021.31908

Chellappan, D. K., Ganasen, S., Batumalai, S., Candasamy, M., Krishnappa, P., Dua, K., Chellian, J., & Gupta, G. (2016). The Protective Action of the Aqueous Extract of Auricularia polytricha in Paracetamol Induced Hepatotoxicity in Rats. In *Recent Patents on Drug Delivery & Formulation*, *10*(1), 72-6. doi: 10.2174/1872211309666151030110015. PMID: 26517821.

de Andrade, K. Q., Moura, F. A., dos Santos, J. M., de Araújo, O. R. P., Santos, J. C. de F., & Goulart, M. O. F. (2015). Oxidative stress and inflammation in hepatic diseases: Therapeutic possibilities of N-acetylcysteine. *International Journal of Molecular Sciences*, *16*(12), 30269–30308. https://doi.org/10.3390/ijms161226225

Engli, K. A. (2012). Defisiensi Glukosa-6-fosfat dehidrogenase (G6PD). *Tugas Biokimia Kedokteran.* Universitas Brawijaya

Enzing C, Ploeg M, Barbosa M, Sijtsma L, authors Vigani M, Parisi C, Rodriguez Cerezo E, editors. (2014). Microalgae-based products for the food and feed sector: an outlook for Europe. EUR 26255. Luxembourg (Luxembourg): Publications Office of the European Union. https://doi.org/10.2791/3339

Esperanza, Y., Prabowo, S., & Handajani, F. (2021). Pengaruh Pemberian Curcumin Terhadap Perbaikan Fungsi Hepar Tikus Putih (Rattus Novergicus) yang diinduksi Parasetamol Dosis Tinggi. *Jurnal Ilmiah Kedokteran Wijaya Kusuma*, *10*(2), 208. https://doi.org/10.30742/jikw.v10i2.1250

Fernandes, R., Campos, J., Serra, M., Fidalgo, J., Almeida, H., Casas, A., Toubarro, D., & Barros, A. I. R. N. A. (2023). Exploring the Benefits of Phycocyanin: From Spirulina Cultivation to Its Widespread Applications. *Pharmaceuticals*, *16*(4). https://doi.org/10.3390/ph16040592

Frank, J. E. (2005). Diagnosis and management of G6P deficiency. *American Family Physician, 72*(7), 1277–82. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16225031D

Ghneim, H. K., & Alshebly, M. M. (2016). Biochemical markers of oxidative stress in Saudi women with recurrent miscarriage. *Journal of Korean Medical Science*, *31*(1), 98–105. https://doi.org/10.3346/jkms.2016.31.1.98

Güroy, B., Karadal, O., Mantoğlu, S., & Irmak Cebeci, O. (2017). Effects of different drying methods on C-phycocyanin content of *Spirulina platensis* powder. *Ege Journal of Fisheries and Aquatic Sciences*, *34*(2), 129–132. https://doi.org/10.12714/egejfas.2017.34.2.02

Hall, J. E. (John E. (2016). *Guyton and Hall textbook of medical physiology* (13th ed.).

Hidayati, H., & Kustriyani, A. (2020). Paracetamol, Migraine, and Medication Overuse Headache (Moh). *JPHV (Journal of Pain, Vertigo and Headache)*, *1*(2), 42–47. https://doi.org/10.21776/ub.jphv.2020.001.02.5

Novirianthy, R., & Sekarutami, S. M. (2015). Pengaruh Kadar Malondialdehid dan Aktivitas Antioksidan Katalase terhadap Toksisitas Akut Radiasi pada Kanker Serviks Stadium Lanjut Lokal. *Radioterapi & Onkologi Indonesia, 6*(2): 81-92

Rahmawati, N., Sugiyanta, & Sakinah, E. N. (2018). Pengaruh Pemberian Cuka Apel "A" terhadap Kadar MDA Hepar Tikus Wistar Jantan yang Diinduksi Parasetamol Dosis Toksik. *Pustaka Kesehatan*, *6*(2), 272-277. https://doi.org/10.19184/pk.v6i2.7667

Ridlo, A., Sedjati, S., Supriyantini, E. (2016). Aktivitas Anti Oksidan Fikosianin Dari Spirulina Sp. Menggunakan Metode Transfer Elektron Dengan DPPH (1,1-difenil-2-pikrilhidrazil). *Jurnal Kelautan Tropis, 18*(2), 58–63. DOI: https://doi.org/10.14710/jkt.v18i2.515

Riza, M. M., & Devi, N. A. (2015). Kandungan Total Fenol Dan Aktivitas Antioksidan Ekstrak Air Daun Kersen (Muntingia calabura L.) Total Content of Fenol and Antioxidant Activity of The Aqueous Extract of Cherry Leaf (Muntingia calabura L.). *Jurnal Kedokteran Yarsi*, *23*(3).

Sayed, A. A., Soliman, A. M., Taha, M. A., & Sadek, S. A. (2022). Spirulina and C-phycocyanin mitigate titanium dioxide nanoparticle-induced hematobiochemical and hepatorenal disorders through antioxidative pathway. *Food Chemistry Advances*, *1*(January), 100035. https://doi.org/10.1016/j.focha.2022.100035

Singh, Z., Karthigesu, I. P., Singh, P., & Kaur, R. (2014). Use of Malondialdehyde as a Biomarker for Assessing Oxidative Stress in Different Disease Pathologies: a Review. In *Iranian J Publ Health, 43*(3). http://ijph.tums.ac.ir

Sukmaningbayu, A. J., Sudjarwo, S. A., & Setiabudi, R. S. (2016). Efek Terapeutik Ekstrak *Spirulina platensis* pada Gambaran Histopatologi Kerusakan Hati Tikus (Rattus norvegicus) yang diinduksi Ethanol. *Veterina Medika, 9*(3): 23-30.

Verma, P. K., Raina, R., Sultana, M., Prawez, S., & Jamwal, N. (2013). Hepatoprotective mechanisms of Ageratum conyzoides L. on oxidative damage induced by acetaminophen in Wistar rats. *Free Radicals and* Antioxidants*, 3*(2), 73–76. https://doi.org/10.1016/j.fra.2013.05.009

Warshaw, J. B., Wilson, C. W., Saito, K., & Prough, R. A. (1985). The responses of glutathione and antioxidant enzymes to hyperoxia in developing lung. *Pediatric Research*, *19*(8), 819–823. https://doi.org/10.1203/00006450-198508000-00008

Yuniati, R., Zainuri, M., & Kusumaningrum, H. (2020). Qualitative Tests of Secondary Metabolite Compounds in Ethanol Extract of *Spirulina platensis* from Karimun Jawa Sea. *Indonesia. Biosaintifika: Journal of Biology & Biology Education*, *12*(3), 343–349. http://dx.doi.org/10.15294/biosaintifika.v12i3.23153

Yuniastuti, A. (2016). *Dasar Molekul Glutation dan Perannya sebagai Antioksidan , Buku Monograft.* Semarang: FMIPA Press.

Zulaikhah, S. T. (2017). The Role of Antioxidant to Prevent Free Radicals in The Body. *Sains Medika : Jurnal Kedokteran Dan Kesehatan*, *8*(1), 39. https://doi.org/10.30659/sainsmed.v8i1.1012