Indonesian Journal of Environmental Management and Sustainability
e-ISSN:2598-6279 p-ISSN:2598-6260

IJEMS

Research Paper

Effects of *Spirulina platensis* extract on serum *Malondialdehyde* (MDA) levels and differential leukocyte count on motor vehicle exhaust emissions exposure (in vivo study on Wistar rats)

Dea May Fitry [1]*, Nani Maharani [2], Renni Yuniati [3]

[1] *Graduate School of Biomedicine, Faculty of Medicine, University of Diponegoro, Semarang, Indonesia*
[2] *Department of Pharmacology and Therapy, Faculty of Medicine, Diponegoro University, Semarang, Indonesia*
[3] *Department of Dermatovenerology, Faculty of Medicine, Diponegoro University , Semarang, Indonesia*
***Corresponding author e-mail**: deamfitry@gmail.com

Abstract
The particulate matter in vehicle emission has been shown to cause an increase in free radical levels which can increase serum MDA levels and leukocyte count. *Spirulina platensis* is an algae that has antioxidant effects that can scavenge free radicals. In this research, twenty-four of wistar mice are divided into 4 groups. Groups X1 and X2 received vehicle emission smoke exposure treatment and were given oral *Spirulina platensis* extract at a dose of 500 and 750 mg / kgBW / day for 14 days, the negative control group (C2) was given 0.9% NaCl orally and the normal control group did not get vehicle emission smoke exposure and *Spirulina platensis* extract. Serum MDA levels were checked by ELISA method and leukocyte count by giemsa staining. Data analysis was performed by using the one-way ANOVA test followed by Post Hoc Bonferroni. The results showed the mean serum MDA levels there was a significant difference between group C2 and group X2. In the mean absolute value of neutrophils and lymphocytes, there were significant differences between the C2 group with the administration of X1 and X2. In the mean absolute value of eosinophils and monocytes, there were significant differences between groups C1 and C2 and group X2 and between groups X1 and C1. Furthermore, the given doses of *Spirulina platensis* extract at a dose of 750mg / kgBW had an effect on serum MDA levels as well as the number of neutrophils and lymphocytes and the administration of doses of 500 and 750mg / kgBW affected the number of eosinophils and monocytes.

Keywords
Spirulina platensis, PM2.5, MDA, Leukosit

Received: 8 September 2020, Accepted: 28 September 2020
https://doi.org/10.26554/ijems.2020.4.3.85-89

1. INTRODUCTION

Ambient air pollution (outdoor air) is the biggest world problem of environmental health. According to WHO, there were an estimated 4.2 million deaths in 2016 due to ambient air pollution. Adverse health effects can occur as a result of short or long-term exposure. The main effect of ambient air pollutants is particulate matter (PM), ozone (O_3), nitrogen dioxide (NO_2) and sulfur dioxide (SO_2). One of the main sources of PM is vehicle emission from diesel and gasoline (WHO, 2016). The presents of free radicals and the inflammatory response of ambient air pollution can cause several health problems (Lu et al., 2018; Manzetti and Andersen, 2016; Gong et al., 2013; Lee et al., 2018). Free radicals will cause cell damage, lipid peroxidation and protein oxidation. The result of lipid peroxidation is malondialdehyde (MDA)(Birben et al., 2012). The inflammation that occurs in the short term can increase the risk of exacerbation of several diseases such as COPD, asthma and autoimmune diseases and in the long term can cause various cardiorespiratory diseases. One marker of inflammation is leukocytes (Lee et al., 2018).

As similarly reported by Gong et al. (2013) in china, showed the increasing MDA urine percentage and exhaled breath condensate (EBC) during the Beijing Olympics in which air pollution was increased dramatically. According to Yan et al. (2017) showed an increase in MDA d levels in mice with were given exposure to PM 2.5 (particles $<2.52\mu$m in size) compared to control rats. Lee et al. (2018) also reported that in 2010 to 2016 showed an increase in the number of leukocytes due to long-term exposure to ambient air pollution in South Korea. However, Indonesian marine biota has a huge opportunity to be explored as a source of natural antioxidant compounds. One of the microorganisms

that have the potential as a source of natural antioxidants is *Spirulina platensis* (Yudiati et al., 2011).

Spirulina platensis is a filamentous and multicellular blue-green alga capable of reducing inflammation and also manifesting antioxidant effects. *Spirulina platesis* is a natural source of protein, vitamins, macronutrients and micronutrients such as amino acids, gamma linoleic acid, chlorophyll and phycocyanin (Abd El-Hakim et al., 2018) Previous studies have shown the antioxidant potential of *Spirulina platensis*. El-Baz et al. (2013)conducted a study which showed that *Spirulina platensis* extract had a beneficial effect in controlling hyperglycemia and reducing MDA levels in streptozoticin-induced DM type 2 rats (STZ). In addition, research conducted by Al-Qahtani and Binobead (2019) *Spirulina platensis* is produced in different areas. In this research, the antioxidant potential possessed by Spirulina platensis is expected to inhibit lipid peroxidation and inflammation which can be measured by seeing a decrease in MDA levels.

2. EXPERIMENTAL SECTION

2.1 *Spirulina Platensis* extract

This study used *Spirulina platensis* powder with the registration number FDA 15594742028 and CERES number 50OGA1200043 (9241). *Spirulina platensis* powder was extracted by maceration (immersion), with a ratio of 1:10, namely 1 part of the *Spirulina platensis* microalgae sample powder soaked in 10 parts of 95% ethanol solution. Maceration is carried out for 5 days in a glass container. Shake the glass container every day for a few minutes. After 5 days the solution was filtered with Whatman filter paper. The next step is evaporation using a rotary evaporator at the boiling temperature of ethanol until a concentrated extract is obtained.

2.2 Vehicle emission and exposure setting system

The vehicle emission carried out by comes from a 125 cc automatic motorcycle was product in 2017 and fueled by pertalite. The exposure was carried out by setting the system i.e., the exhaust is connected to the sucking pump, where only particles less than 2.5μm in diameter can pass (using Whatman filter paper no 42). The sucking pump will flow the smoke into the exposure chamber. Then the motorcycle engine is operated for 80 seconds. The PM2.5 concentration in the exposure chamber was measured using a kanomax (Figure 1). The inhalation time of PM2.5 per day per 80 seconds of fumigation is 100 seconds where the PM2.5 dose exposed to mice is 0.625 + 0.069mg/m^3.

2.3 Procedure of wistar mice exposure

Twenty-four male Wistar mice aged 2-3 months with a weight of 100-200gr were acclimatized for 7 days with room temperatures ranging from 25-28°C and room humidity levels ranging from 70-75%. A light reception is set to 12 hours bright and 12 hours dark. Rats were given feed in the form of AD II pellets and reverse osmosis drinking water which was given ad libitum. On the 8th day, Twenty-four male Wistar rats that had met the inclusion criteria were then divided into 4 groups randomly (randomization) where each group consisted of 6 Wistar rats. Groups X1 and X2 received vehicle emission exposure treatment and were given oral *Spirulina platensis* extract at a dose of 500 and 750 mg/kgBW/day, the negative control group (C2) was given exposure to smoke and NaCl 0.9% orally and the normal control group did not get vehicle emission exposure and *Spirulina platensis* extract.

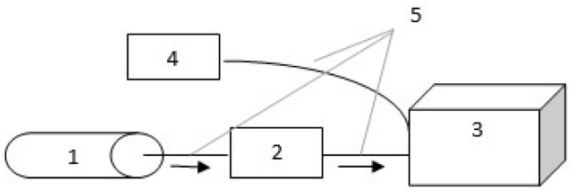

Figure 1. Procedure for exposure to vehicle emission

(Note: 1 = motorcycle exhaust; 2 = sucking pump + filter paper; 3 = exposure chamber; 4 = kanomax; 5 = connecting pipe)

After fourteen-day treatment, the wistar rats were anesthetized by giving ketamine injection at a dose of 60 mg/kgBW (i.m). Then two separate blood samples were taken from the retroorbital veins of each mouse. The first sample was used for examination of serum MDA levels by the ELISA (Enzyme linked immunosorbent assay) method according to the protocol specified in the commercial kit used (Catalog no: E-EL-0060: Elab Science Biotechnology, Texas, USA). The second sample was used for manual examination of leucocyte count with Giemsa staining according to the procedure. This research has been approved by the Medical / Health Research Bioethics Commission, Faculty of Medicine, Sultan Agung Islamic University, Semarang with the Ethical Clearance number 521 / VIII / 2019 / Bioethics Commission.

2.4 Statistical analysis using ANOVA

The statistical analysis of this study used the SPSS 25 computer program. The test performed was One-Way ANOVA and was further analyzed by Post Hoc Bonferroni. The significance value in this study was $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Serum MDA levels

The results after measuring serum MDA levels using the ELISA method, the measurement results are obtained as presented in Table 1.

The results of the one-way ANOVA analysis on serum MDA levels showed significant differences between the study groups and then further analyzed using the Bonferroni Post

Table 1. Results of measurement of serum MDA levels (ng / mL)

Groups	Mean+SD	Min-Max	p*
C1	195.0+22.15	163.56 – 220.22	p<0.001
C2	376.1+55.57	293.56 – 441.33	
X1	326.7+44.51	280.22 – 392.44	
X2	178.6+25.22	155.78 – 209.11	

Hoc test. The results of the Post Hoc test showed that there was a significant difference between the negative control group (C2) and the group giving Spirulina platensis extract 750mg/kgBW (X2) and between the two control groups (C1 and C2).

3.2 Differential Leukocyte counts

The value of absolute neutrophil and lymphocyte count, the results are shown in Table 2 and Table 3. The results

Table 2. The value of absolute neutrophil (x10^3/ μL)

Groups	Mean+SD	Min-Max	p*
C1	4.7+2.07	3-8	p<0.001
C2	12.8+3.49	9-18	
X1	4.0+1.52	2-6	
X2	4.7+2.13	2-8	

Table 3. The value of absolute lymphocyte (x10^3/ μL)

Groups	Mean+SD	Min-Max	p*
C1	4.3+2.07	2-7	p<0.001
C2	11.8+5.27	3-19	
X1	5.3+2.50	2-9	
X2	3.8+1.94	1-6	

of the one-way ANOVA analysis on the value of absolute neutrophil and lymphocyte count showed that there were significant differences between the study groups and then further analyzed using the Post Hoc Bonferroni test. The results of the Post Hoc test showed that there was a significant difference between the negative control group (C2) and the group giving *Spirulina platensis* extract 500mg/kgBB (X1) and 750mg/kgBB (X2) and between the two control groups (C1 and C2).

Thus, the results of the one-way ANOVA analysis on the value of absolute eosinophils (Table 4) and monocytes (Table 5) showed that there were significant differences between the study groups and were further analyzed using the Post Hoc Bonferroni test. The results of the Post Hoc test showed that there was a significant difference between the two control groups (C1 and C2) with the group giving *Spirulina platensis* extract 750mg / kgBB (X2) and between the group giving *Spirulina platensis* extract 500mg / kgBB (X1) and the normal control (C1).

Table 4. The value of absolute eosinophils (x10^3/ μL)

Groups	Mean+SD	Min-Max	p*
C1	1.8+1.17	0-3	p<0.001
C2	8.5+1.87	6-11	
X1	7.4+2.12	5-10	
X2	5.1+0.98	4-6	

Table 5. The value of absolute monocytes (x10^3/ μL)

Groups	Mean+SD	Min-Max	p*
C1	1.7+1.21	0-3	p<0.001
C2	10.3+2.16	8-14	
X1	10.4+2.26	7-13	
X2	5.1+1.91	3-8	

The *Spirulina platens*is has various health benefits such as antioxidants, immunomodulators, anti-inflammatory, anticancer, antiviral and antibacterial and has a positive effect on hyperlipidemia, malnutrition, obesity, metal poisoning and anemia (N'Djamena, 2012). *Spirulina platensis* antioxidants have activity against several types of free radicals such as hydroxyl and peroxyl radicals and preventing the occurrence of DNA damage(Soni et al., 2015). Hydroxyl radicals play an important role in the lipid peroxidation process where the final product is MDA(Ayala et al., 2014). This study provides exposure to vehicle emission in experimental animals for 14 days. PM2.5 contained in vehicle emission caused the presents of free radicals which will cause oxidative stress. Oxidative stress will trigger lipid peroxidation which in turn will increase serum MDA levels (Manzetti and Andersen, 2016; Gong et al., 2013).

El-Baz et al. (2013) was reported that *Spirulina platensis* extract had a beneficial effect in controlling hyperglycemia and significantly lowering MDA levels of type 2 DM rats compared to a control group. Another study was conducted on mice given furan, a food contaminant and pollutant. Commonly, *Spirulina platensis* supplementation at a dose of 300mg / kgBW can significantly reduce MDA levels compared to the control group (Yudiati et al., 2011). In line with these studies, this study can prove that giving *Spirulina platensis* extract can improve lipid peroxidation conditions that occur in rats with given exposure to vehicle emission. This can be seen from the finding of significant differences in the mean MDA levels between the group given the 750mg / kgBW. *Spirulina platensis* extract and the control group that was given vehicle emission smoke exposure. However, there were no significant differences between the groups given Spirulina platensis extract 500mg / kgBW, this is

probably due to the insufficient dose given to prevent lipid peroxidation. In addition, oxidative stress was increased levels of ROS because of the inflammation (Xu et al., 2013; Cho et al., 2018).

Inflammation is a non-specific immune response to clear pathological agents. The first response to inflammation is the recruitment of neutrophils that kill foreign bodies. Once recruited, neutrophils release cytokines responsible for monocyte recruitment (Xu et al., 2013). In vitro data suggest that the organic and transition metal constituents (e.g., Fe, Cu, Ni, and Zn) contained in PM are capable of producing ROS directly or as a result of its ability to activate neutrophils or other leucocytes (Delfino et al., 2011). According to Steenhof et al. (2014), a positive association between PM and neutrophil count after 2 hours of exposure and monocytes after 18 hours of exposure in populations exposed to PM in underground areas. Another hands by (Al-Homaidan et al., 2016) showed that PM2.5 can significantly increase the number of neutrophils, eosinophils and lymphocytes in bronchoalveolar lavage fluid (BALF) (Yan et al., 2017).

This study shows that giving *Spirulina platensis* extract can affect inflammatory conditions that occur in experimental animals. This can be seen from the finding of significant differences in the mean absolute value of neutrophils and lymphocytes in the group given Spirulina platensis extract at doses of 500 and 750 mg / kgBW with the control group given smoke exposure. Significant differences were also found in the mean absolute values of eosinophils and monocytes between the group given *Spirulina platensis* extract at a dose of 750mg / kgBW and the control group, but there was no significant difference between the group given *Spirulina platensis* extract 500mg/kgBB and the control group.

4. CONCLUSIONS

Based on results of this study, it was found that there was an effect of giving Spirulina platensis extract on serum MDA levels and Wistar rats leukocyte counts exposed to vehicle emission. The administration of *Spirulina platensis* extract at a dose of 750mg/kgBW had an effect on serum MDA levels and the absolute value of eosinophils and monocytes, and doses of 500 and 750mg/kgBW on the absolute values of neutrophils and lymphocytes.

REFERENCES

Abd El-Hakim, Y. M., W. A. Mohamed, and A. E. El-Metwally (2018). Spirulina platensis attenuates furan reprotoxicity by regulating oxidative stress, inflammation, and apoptosis in testis of rats. *Ecotoxicology and environmental safety*, **161**; 25–33

Al-Homaidan, A. A., A. F. Al-Abbad, A. A. Al-Hazzani, A. A. Al-Ghanayem, and J. A. Alabdullatif (2016). Lead removal by Spirulina platensis biomass. *International journal of phytoremediation*, **18**(2); 184–189

Al-Qahtani, W. H. and M. A. Binobead (2019). Anti-inflammatory, antioxidant and antihepatotoxic effects of Spirulina platensis against d-galactosamine induced hepatotoxicity in rats. *Saudi journal of biological sciences*, **26**(4); 647–652

Ayala, A., M. F. Muñoz, and S. Argüelles (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative medicine and cellular longevity*, **2014**

Birben, E., U. M. Sahiner, C. Sackesen, S. Erzurum, and O. Kalayci (2012). Oxidative stress and antioxidant defense. *World Allergy Organization Journal*, **5**(1); 9–19

Cho, C.-C., W.-Y. Hsieh, C.-H. Tsai, C.-Y. Chen, H.-F. Chang, and C.-S. Lin (2018). In vitro and in vivo experimental studies of PM2. 5 on disease progression. *International journal of environmental research and public health*, **15**(7); 1380

Delfino, R. J., N. Staimer, and N. D. Vaziri (2011). Air pollution and circulating biomarkers of oxidative stress. *Air Quality, Atmosphere & Health*, **4**(1); 37–52

El-Baz, F., H. F. Aly, A. El-Sayed, and A. A. Mohamed (2013). Role of Spirulina platensis in the control of glycemia in DM2 rats. *Int J Sci Eng Res*, **4**; 1731–1740

Gong, J., T. Zhu, H. Kipen, G. Wang, M. Hu, P. Ohman-Strickland, S.-E. Lu, L. Zhang, Y. Wang, P. Zhu, et al. (2013). Malondialdehyde in exhaled breath condensate and urine as a biomarker of air pollution induced oxidative stress. *Journal of exposure science & environmental epidemiology*, **23**(3); 322–327

Lee, H., W. Myung, B.-H. Jeong, H. Choi, B. W. Jhun, and H. Kim (2018). Short-and long-term exposure to ambient air pollution and circulating biomarkers of inflammation in non-smokers: a hospital-based cohort study in South Korea. *Environment international*, **119**; 264–273

Lu, X., Z. Ye, S. Zheng, H. Ren, J. Zeng, X. Wang, P. A. Jose, K. Chen, and C. Zeng (2018). Long-Term Exposure of Fine Particulate Matter Causes Hypertension by Impaired Renal D1 Receptor–Mediated Sodium Excretion via Upregulation of G-Protein–Coupled Receptor Kinase Type 4 Expression in Sprague-Dawley Rats. *Journal of the American Heart Association*, **7**(1); e007185

Manzetti, S. and O. Andersen (2016). Biochemical and physiological effects from exhaust emissions. A review of the relevant literature. *Pathophysiology*, **23**(4); 285–293

N'Djamena, C. (2012). Changes in the physico-chemical properties of Spirulina platensis from three production sites in Chad. *Journal of Animal & Plant Sciences*, **13**(3); 1811–1822

Soni, A., M. Dubey, M. Verma, R. Dhankhar, V. Kaushal, R. Atri, and R. Sabharwal (2015). Revisiting the role of phycocyanin in current clinical practice. *International Journal of Pharmaceutical Sciences and Research*, **6**(1); 4950–4600

Steenhof, M., N. A. Janssen, M. Strak, G. Hoek, I. Gosens, I. S. Mudway, F. J. Kelly, R. M. Harrison, R. H. Pieters,

F. R. Cassee, et al. (2014). Air pollution exposure affects circulating white blood cell counts in healthy subjects: the role of particle composition, oxidative potential and gaseous pollutants–the RAPTES project. *Inhalation Toxicology*, **26**(3); 141–165

WHO (2016). Ambient air pollution: A global assessment of exposure and burden of disease

Xu, X., S. Y. Jiang, T.-Y. Wang, Y. Bai, M. Zhong, A. Wang, M. Lippmann, L.-C. Chen, S. Rajagopalan, and Q. Sun (2013). Inflammatory response to fine particulate air pollution exposure: neutrophil versus monocyte. *PLoS One*, **8**(8); e71414

Yan, X.-D., Q.-M. Wang, C. Tie, H.-T. Jin, Y.-X. Han, J.-L. Zhang, X.-M. Yu, Q. Hou, P.-P. Zhang, A.-P. Wang, et al. (2017). Polydatin protects the respiratory system from PM 2.5 exposure. *Scientific reports*, **7**(1); 1–11

Yudiati, E., S. Sejati, S. Sunarsih, and R. Agustian (2011). Aktivitas Antioksidan dan Toksisitas Ekstrak Metanol dan Pigmen Kasar Spirulina sp. *ILMU KELAUTAN: Indonesian Journal of Marine Sciences*, **16**(4); 187–192