



## Antioxidant Activity of *Spirulina platensis* and Sea Cucumber *Stichopus hermanii* in Streptozotocin-Induced Diabetic Rats

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DOI: <https://doi.org/10.21315/tlsr2019.30.2.9>

### Highlights

- Diabetes can cause by the high blood glucose level which have an influence on the imbalance of MDA and antioxidant enzyme (SOD and Catalase).
- Sample of *Spirulina platensis* could reduce MDA of liver in Streptozotocin-induced diabetic rats.
- Sea cucumber *Stichopus hermanii* has antioxidant activity which is reduction of free radicals like MDA in Streptozotocin-induced diabetic rats.

## Antioxidant Activity of *Spirulina platensis* and Sea cucumber *Stichopus hermanii* in Streptozotocin-Induced Diabetic Rats

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**Published date:** 18 July 2019

**To cite this article:** Hema Aprilia Setyo Windari, Kustiariyah Tarman, Mega Safithri and Iriani Setyaningsih. (2019). Antioxidant activity of *Spirulina platensis* and sea cucumber *Stichopus hermanii* in streptozotocin-induced diabetic rats. *Tropical Life Sciences Research* 30(2): 119–129. <https://doi.org/10.21315/tlsr2019.30.2.9>

**To link to this article:** <https://doi.org/10.21315/tlsr2019.30.2.9>

**Abstract:** Uncontrolled blood glucose level of diabetic people can lead to the production of excessive Reactive Oxygen Species (ROS) as the trigger of lipid peroxidation. Antioxidant can defend the human body from overreacting to lipid peroxidation. The study aimed to determine the antioxidant activity of *Spirulina platensis* and sea cucumber *Stichopus hermanii* based on the number of blood serum and liver malondialdehyde (MDA) levels as well as superoxide dismutase (SOD) and catalase (CAT) enzymes' activity of red blood cells in streptozotocin-induced diabetic rats. A group of Sprague Dawley rats (n=3) were the normal group (aquadest 2 mL/day, p.o.) and group of DM, Sp (*S. platensis* 81 mg/kg body weight; p.o.), Tr (*S. hermanii* 284 mg/kg body weight; p.o.), (Combination *S. platensis* 81 mg/kg + *S. hermanii* 284 mg/kg body weight; p.o.) prior to a single dose of streptozotocin (STZ, 50 mg/kg body weight; i.p.). The results showed that *S. platensis*, sea cucumber (*S. hermanii*), and the mixture could suppress rats' body weight by 2–7%. The MDA concentration of liver organ of rats prior to STZ administration increased. However, the STZ administration did not significantly increase MDA concentration of blood serum and decreased activity SOD and CAT of red blood cells in the normal group for 14 days. This study concluded that *Spirulina platensis* demonstrated antioxidant activity indicated by the reduction of MDA level in the liver of diabetic rats induced by STZ (50 mg/kg body weight; i.p.).

**Keywords:** Antioxidant, Diabetes, Sea Cucumber, *Spirulina platensis*, Rats

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## INTRODUCTION

The prevalence of diabetic people in Indonesia has reached 9.8%, which puts the country as the seventh highest in the world, after China, India, the United States, Brazil, Russia, and Mexico. The International Diabetes Federation (IDF) in 2015 reported that the number of diabetic people had reached 381 million in the world, and this was predicted to significantly increase 2–3 times in 2040 (Shaukat 2015). In Indonesia the prevalence may increase to 16% in 2030 (Guariguata *et al.* 2014). Therefore, significant efforts in dealing with diabetes mellitus (DM) are necessary (Mathers & Loncar 2006).

DM is a disease caused by a metabolic disorder, which is characterised by high blood glucose level (hyperglycemia). The high blood glucose level, which is uncontrolled, can cause the production of Reactive Oxygen Species (ROS). It can be the first cause of oxidative stress (Nelson & Cox 2008). Oxidative stress is a condition caused by the imbalance between oxidant and antioxidant in the human body (Khattab *et al.* 2015). The change of oxidative status is marked by changes in endogenous antioxidant activity and increase of oxidative damage to form biomolecules (Kamlesh *et al.* 2012). Therefore, exogenous antioxidant has a beneficial role to inhibit oxidative damage in diabetic patients. An exogenous antioxidant can be given to diabetic patients by mouth (Dauqan *et al.* 2012).

Consuming supplements or health foods containing antioxidants from natural ingredients may be a preventive action for diabetic patients (Mamelona *et al.* 2007). Indonesia has abundant natural resources in the coastal and marine region. *Spirulina platensis* and sea cucumber (*Stichopus hermannii*) are two marine resources that have a high potential as antioxidants (Celik & Akkaya 2009). NADFC explained that consuming *Spirulina platensis* for human is 3 g/day and sea cucumber (*Stichopus hermannii*) is 10,5 g/day by mouth (Sparringa 2014). Marine resources also provide beneficial properties for human health, such as free radical scavenging, as well as inhibition of hydrolytic and oxidative enzymes (Eman *et al.* 2015).

Marine resources of Indonesia, such as *Spirulina platensis* contain vitamins, minerals, and carotenoids that serve as antioxidants encompass a carotene type pigment, chlorophyll A and phycocyanin pigments (Setyaningsih *et al.* 2015). Sea cucumber (*S. hermannii*) contains chondroitin, triterpene glycoside (saponins), phenol (flavonoid) (Sara *et al.* 2011), peptides and essential amino acids that are not toxic based on the results of acute toxicity tests in diabetic rats (Althunibat *et al.* 2009), and has several bioactivities, such as natural sex aphrodisiac and antioxidant (Kustiariyah 2006). Therefore, this research focused on the potential of *S. platensis* and sea cucumber (*S. hermannii*) as antioxidants in vivo using diabetic rats. This study aimed to determine the antioxidant activity of *Spirulina platensis* and sea cucumber, based on the number of blood serum and liver malondialdehyde (MDA) levels as well as superoxide dismutase (SOD) and catalase (CAT) enzymes' activity of red blood cells on streptozotocin-induced diabetic rats.

## MATERIALS AND METHODS

### Chemical and Material

*S. platensis* was obtained from Jepara, Central Java while sea cucumber (*S. hermanii*) was obtained from Labuan Bajo, Flores, East Nusa Tenggara. *Sprague Dawley* albino rats (age of 12 weeks, body weight 210–250 g) were obtained from BPPOM Republic of Indonesia by proposal Animal Care and Use Committee number R.05-16-IR.

Chemicals used for MDA analysis were 1,1,3,3-tetramethoxypropane 6 M (Sigma-Aldrich Ltd.), 2-thiobarbituric acid (Merck Millipore, Germany), glacial acetic acid (Merck Millipore), sulfuric acid 95–97% (Merck Millipore), phosphotungstic acid hydrate (Sigma-Aldrich Ltd., USA), GlucoDR and strips of GlucoDr (All Medicus, Korea), SOD kit (BioVision, USA), catalase kit (BioVision, USA), microplate reader (BioRad 3550, USA) and ELISA reader (BioRad 3550, USA).

### Experimental Design

*Sprague Dawley* rats were divided into five groups: normal group (N), diabetic rats group (DM), *Spirulina platensis* group (Sp), sea cucumber group (Tr) and combined group of *Spirulina platensis* and sea cucumber group (Sp–Tr). Normal group (N) was maintained under standard laboratory conditions and fed with appropriate diet till the completion of the experiment and added distilled water. Meanwhile, the diabetic rats' group (DM) was induced by streptozotocin (50 mg/kg body weight; i.p.) and distilled water (2 mL/day; p.o.). *Spirulina platensis* group (Sp) was induced by streptozotocin (50 mg/kg body weight; i.p.) and treated *Spirulina platensis* (81 mg/kg body weight; p.o.). Sea cucumber (*S. hermanii*) group (Tr) was induced by streptozotocin (50 mg/kg body weight; i.p.) and treated Sea cucumber (284 mg/kg body weight; p.o.). Furthermore, the combined group (Sp–Tr) was induced by streptozotocin (50 mg/kg body weight; i.p.) and treated *Spirulina platensis* (81 mg/kg body weight; p.o.) and then Sea cucumber 284 mg/kg body weight; p.o.). Under these conditions, the rats were fed with their appropriate diet till the completion of the experiment. All the groups were treated orally for 14 days.

Streptozotocin (50 mg/kg body weight) was injected intraperitoneal (i.p.). Forty-eight hours after injection, administration of *Spirulina platensis*, sea cucumber (*S. hermanii*) and their combination was carried out. Body weight and blood glucose of the rats were observed on days 0, 7, and 14 after induction of streptozotocin. Blood serum and liver organ were taken at 12 to 16 hours after fasting on the 14th day for analysis of MDA, SOD, and CAT enzyme activity. All the experimental procedures and protocols used in this study were reviewed by the institutional animal ethics committee (R.05-16-IR/2016) from PT. Bimana Indomedical and were in accordance with the guidelines of the Animal Rodent.

## **Preparation of *Spirulina platensis* and Sea cucumber (*Stichopus hermanii*)**

Dried *Spirulina platensis* was used in this research. For the preparation *Spirulina* was mixed with distilled water. Fresh sea cucumber (*Stichopus hermanii*) was cleaned from unwanted materials, washed and cut into small pieces. The cut were then heated for 13 min and then stored at  $-20^{\circ}\text{C}$ . Each of these rats was given doses according body weight rats in each day for 14 days.

## **Determination of Blood Serum and Liver MDA Levels**

Measurement of malondialdehyde (MDA) was based on the reaction of thiobarbituric-acid (TBA) and MDA to yield a red pigment. The TBA reaction is advantageous in its high sensitivity. Since malondialdehyde is unstable, tetramethoxypropane was used as standard. To determine MDA in the serum and organ, tetramethoxypropane were precipitated along with the serum and organ solvent to remove water-soluble TBA-reactive substances. Standard MDA 1,1,3,3-tetramethoxypropane  $60\ \mu\text{g}/\text{mL}$  was made at various concentrations of the stock sample. For the liver samples, the analysis was performed according to Ohkawa *et al.* (1979). The liver was crushed in a mortar to make supernatant after that was reacted with TBA. Supernatant was heated at  $95\ ^{\circ}\text{C}$  for 60 minutes. For the blood serum samples, the procedure was performed according to Yagi (1987). It was purified and after that was reacted with TBA. The solution of serum samples and organ samples were analysed using UV-Vis Spectrophotometer at  $\lambda = 532\ \text{nm}$ .

## **Determination of Superoxide Dismutase (SOD) Activity**

The SOD activity was measured according to BioVision (2010). SOD enzyme was measured based on the inhibition rate of ferri-sitochrom c reduction by superoxide anions produced by xanthine/xanthine oxidase. Xanthine was oxidised to uric acid, while superoxide anions formed subsequently reducing ferri-sitochrom c. In this method 4 solutions were used such as blank 1, blank 2, blank 3 and blank 4. After they reacted with superoxide anions all the blank wells were incubated at  $37^{\circ}\text{C}$  for 20 minutes, then the solution was measured using microplate and ELISA reader (BioRad 3550, USA) at  $\lambda = 450\ \text{nm}$ .

## **Determination of Catalase Activity**

The CAT activity was measured according to BioVision (2011). CAT enzyme activity was measured based on the removal of hydrogen peroxide. The CAT enzyme was oxidised to a high-valent iron intermediate which hydroperoxidases is reduced back to the resting state by further reacting it with hydrogen peroxides. Hydrogen peroxide is harmful, therefore must be removed as soon as it is produced in the cell. Measurement of this activity takes place at a temperature of  $25^{\circ}\text{C}$ . Catalase reaction was made by adding  $\text{H}_2\text{O}_2$  into each well of samples. Moreover, each well

was prepared a solution to be added including solvent 1, solvent 2 and solvent 3. Afterwards, solution in each well was mixed and incubated at 25°C for 10 minutes and then measured using microplate and ELISA reader (BioRad 3550, USA) at  $\lambda = 570$  nm.

## Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) and significant differences between means were compared by The Tukey T Test at  $p < 0.05$ . All data were expressed as mean  $\pm$  SD.

## RESULTS

The results demonstrated that the body weight of treated rats decreased. Table 1 shows the reduction of rat body weight observed at the 7th and 14th days of the experimental period. For the normal group, the body weight of rats did not significantly decrease, but it increased in the 7th day (0.80%) and 14th day (1.15%). For the diabetic rat group (DM), the reduction of body weight was significantly different from the normal group, and showed a remarkable decline in the 7th day (11.64%) and 14th day (24.39%).

**Table 1:** Condition of Body Weight Rats in different treatment.

Treatment (mg/kg)	Average of body weight (g)			
	Before STZ	D0 (After STZ)	D7	D14
N	207.27 $\pm$ 17.8 <sup>a</sup>	207.93 $\pm$ 15.8 <sup>a</sup>	208.93 $\pm$ 16.2 <sup>a</sup>	210.40 $\pm$ 11.1 <sup>a</sup>
DM	211.67 $\pm$ 4.3 <sup>a</sup>	210.40 $\pm$ 6.8 <sup>a</sup>	182.97 $\pm$ 10.2 <sup>b</sup>	156.57 $\pm$ 5.1 <sup>c</sup>
Sp (81)	217.93 $\pm$ 7.3 <sup>a</sup>	207.80 $\pm$ 6.5 <sup>a</sup>	184.27 $\pm$ 4.7 <sup>c</sup>	157.83 $\pm$ 4.1 <sup>d</sup>
Tr (284)	210.97 $\pm$ 2.5 <sup>a</sup>	211.23 $\pm$ 10.8 <sup>a</sup>	178.00 $\pm$ 19.9 <sup>bc</sup>	167.70 $\pm$ 11.4 <sup>c</sup>
Sp-Tr (81 + 284)	227.23 $\pm$ 11.2 <sup>a</sup>	208.87 $\pm$ 9.4 <sup>abc</sup>	195.37 $\pm$ 17.1 <sup>abc</sup>	176.93 $\pm$ 18.7 <sup>c</sup>

All the values were expressed as means $\pm$ SD. The different superscript letters within the same raw were significantly different at ( $p < 0.05$ )

The three treatment groups were *S. platensis* group (Sp), Sea cucumber group (Tr) and the combination of *S. platensis* and sea cucumber. The body weight percentage of the three treatments were not significantly different from the normal group (N) on the day 7. The body weight percentage of the *S. platensis* group was not significantly different from the Diabetic rats' group (DM) but significantly different from the normal group on the day 14. In contrast, the body weight percentage of sea cucumber group and combination group were not significantly different from the normal group (N) on the day 14.

Table 2 shows a comparison of MDA in blood serum and antioxidant enzymes (SOD and CAT) on the day 14. Levels of MDA were determined in blood serum and liver of rats. The result showed that MDA levels in rats blood serum were not significantly different ( $p < 0.05$ ) between the normal group, DM group, Tr group, and Sp-Tr group. Only *Spirulina platensis* group (Sp) was significantly different from the normal group. This indicated that the level of MDA in blood serum was unaltered in 14 days after induction of streptozotocin (50 mg/kg body weight; i.p). The result also revealed that MDA levels in rat liver after 14th days showed a significant difference ( $p < 0.05$ ) between normal group and treatment groups (Sp, Tr, Sp-Tr). This indicated that the MDA level in rats liver was altered in 14 days after induction of streptozotocin (50 mg/kg body weight; i.p).

The measurement of SOD and CAT enzymes activity was performed on rats' red blood cells for 14th days. The results showed that the SOD activity was not significantly different between normal, DM and treatment group (Table 2). Similarly, catalase (CAT) activity was not significantly different among normal, DM and treatment groups. This finding indicated that SOD and CAT activity was unchanged in 14 days after induction of streptozotocin (50 mg/kg body weight; i.p).

**Table 2:** The concentration of serum MDA, liver organ MDA, the red blood SOD and catalase activity in different treatment.

Treatment (mg/kg)	[MDA] serum (nmol/mL)	[MDA] liver organ (nmol/mL)	SOD activity (U/L)	Catalase activity (U/L)
N	2.02 ± 0.92 <sup>c</sup>	15.19 ± 0.06 <sup>b</sup>	3.23 ± 0.10 <sup>a</sup>	15.65 ± 0.66 <sup>ab</sup>
DM	2.70 ± 0.90 <sup>c</sup>	53.95 ± 18.97 <sup>a</sup>	3.14 ± 0.21 <sup>a</sup>	16.55 ± 1.26 <sup>ab</sup>
Sp (81)	6.10 ± 2.23 <sup>a</sup>	15.92 ± 3.06 <sup>b</sup>	3.01 ± 0.01 <sup>a</sup>	14.50 ± 0.66 <sup>a</sup>
Tr (284)	2.39 ± 0.38 <sup>c</sup>	16.50 ± 6.84 <sup>b</sup>	2.77 ± 0.01 <sup>a</sup>	17.20 ± 0.80 <sup>b</sup>
Sp-Tr (81 + 284)	2.97 ± 0.38 <sup>c</sup>	18.81 ± 2.25 <sup>b</sup>	2.49 ± 0.55 <sup>a</sup>	16.20 ± 1.06 <sup>ab</sup>

All the values were expressed as means±SD. The different superscript letters within the same column were significantly different at ( $p < 0.05$ ).

## DISCUSSIONS

This study showed that there was an antioxidant effect on streptozotocin-induced diabetic rats after treatment with *S. platensis*, sea cucumber (*S. hermannii*) and the combination of *Spirulina platensis* and sea cucumber. These three treatment groups were not associated with a reduction of rat body weight. The determination of rat body weight was to investigate the effects of given treatment against physiological changes and responses in diabetic rats (Musalmah et al. 2002). Meanwhile, the administration of bio-active plant resources compounds for 28th days could suppress the body weight of rats induced by 50 mg/kg body weight of streptozotocin (Hasibuan et al. 2016).

In this present work, administration of *Spirulina platensis*, sea cucumber (*S. hermanii*), and the combination for 14 days led to the body weight reduction of 2–7% (presented in Table 1). Imbalance of ROS levels and antioxidant enzyme concentrations was caused by various diseases and body weight reductions (Aher *et al.* 2011). Free radicals can interfere with the metabolic process. Superoxide anions is one of ROS, which can activate several damaging pathways in diabetes, which have been proven to be involved in micro- and macrovascular complication (Rodwell *et al.* 2015). American Diabetes Association (ADA) reported that the decreasing body weight was one of the symptoms for diabetic patients (Matsunami *et al.* 2010). Body weight changes in diabetic rats can be caused by several factors including feed treatment. Thus, excessive hunger condition (*Polyphagia*) on rats was imbalance with intake of feed given (Nathan 2014).

The MDA profile in blood serum can describe the condition of the liver organ due to raising lipid peroxidation by free radicals streamed into the venous vessel (Pham-Huy *et al.* 2008). In addition to the formation of superoxide anions, free radicals can also encourage lipid peroxidation which can further form MDA. Therefore MDA is chosen as a parameter because it is the marker of damage or the product of oxidative stress (Rodwell *et al.* 2015). The serum MDA profile serves as a marker of cellular damage due to the decline of free radicals (Ming-Lu *et al.* 2010). In this work, determination of level the serum MDA aimed to discover the effects of streptozotocin induction (50 mg/kg body weight; i.p) in diabetic rats on diabetes complication diseases (Khattab *et al.* 2015).

The results showed that serum MDA in the Sp group (*Spirulina platensis* 81 mg/kg body weight; p.o) was significantly different ( $p < 0.05$ ) from the other groups (Table 2). The level of serum MDA from lipid peroxidation could be determined on 4–8 weeks (Ming-Lu *et al.* 2010). Gwarzo *et al.* (2014) suggested that the level of serum MDA was found at  $8.5 \pm 0.76$  nmol/mL measured on the 21st day in normal rats. Although the level of serum MDA was higher in the Sp group, Setyaningsih *et al.* (2015) found that *Spirulina platensis* contained antioxidant compounds such as phycocyanin and saponin. Even though the MDA level at normal group is higher than DM group, which is still below the normal limit of the MDA level in rats. Sea cucumber and combined groups showed that the level of serum MDA was not significantly different from the normal group, Rasyid (2012) reported that sea cucumber showed antioxidant activity and possessed bioactive compounds in steroids and saponin form. Marrelli *et al.* (2016) reported that saponin could demonstrate the ability to reduce free radicals like malondialdehyde (MDA).

The level of liver MDA could indicate scavenging and inhibitory activity of free radicals on the process of lipid peroxidation (Gwarzo *et al.* 2014). The level of liver MDA in the DM group showed a significant difference from the normal and treatment groups. The result revealed that induction of streptozotocin (50 mg/kg body weight; i.p) could raise the level of liver MDA during 14 days. Dogan *et al.* (2015) found that administration of streptozotocin (50 mg/kg body weight; i.p) enhanced the liver MDA concentration during 21 days. Furthermore,



Salvayre *et al.* (2010) found that streptozotocin administration to rats promoted the production of free radicals by 5–8% and caused damage to tissue and several organs.

SOD and CAT enzymes activity was determined on rat red blood cells. An increase in high blood glucose levels can cause the formation of free radicals and oxidative stress (Qujeq & Rezvani 2007). Oxidative stress is defined in general as excess formation and insufficient removal of highly reactive molecules such as ROS. ROS include free radicals such as superoxide, hydroxyl, peroxy, hydroperoxy, as well as nonradical species such as hydrogen peroxide (Johansen *et al.* 2005). Superoxide has been dismutated to hydrogen peroxide by SOD. If the amount of superoxide anion is excessive, then the SOD and CAT enzymes will be the free radical scavenger. Hydrogen peroxide can be converted to water (H<sub>2</sub>O) by CAT (Nelson & Cox 2008). The results showed that the SOD and CAT enzymes activity was not significantly different ( $p < 0.05$ ) between normal, DM and treatment groups. Celik and Akkaya (2009) suggest that SOD and CAT activity can decrease for 4 weeks (28 days) by 62.5% and 21.1%, respectively. The SOD and CAT activity can be caused by a low state of hyperglycemia, thus increasing the number of free radicals which makes antioxidant enzyme activity more severe (Safithri *et al.* 2012).

## CONCLUSION

Diabetic rats induced by streptozotocin (50 mg/kg body weight) for 14 days showed an increase in liver MDA level, but it did not increase blood serum MDA level. The results demonstrated that reduction of SOD and CAT enzyme activity was not significantly different ( $p < 0.05$ ) between DM and treatment groups. Administration of *Spirulina platensis* in diabetic rats for 14 days showed antioxidant activity indicated by the reduction of liver MDA level by  $15.92 \pm 3.06$  nmol/mL.

## ACKNOWLEDGEMENTS

This research was financially supported by Mr. Thomas Hendrawan MBA.

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