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In Vitro and *In Vivo* Malondialdehyde Inhibition Activities of *Stichopus hermanii* and *Spirulina platensis*

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ABSTRACT

Previous research showed that *Stichopus hermannii* and *Spirulina platensis* had an antioxidant activity. It is indicated by the reduced malondialdehyde (MDA) level in the liver of the diabetic rats. However, the STZ administration did not significantly increase MDA concentration of diabetic rats' blood serum for 14 days. This research aimed to determine *in vitro* and *in vivo* MDA inhibition of *S. hermanii* and *S. platensis*. The *in vitro* antioxidant activity test was conducted using the MDA-TBA method, and a positive control used α-tocopherol. For *in vivo* experiment, diabetic rats (DM) were induced by streptozotocin for 21 days. Twenty-five rats which were divided into five groups: normal rat group (NA), diabetic rat group (DA), diabetic rat group + glibenclamide (DG), diabetic rats + *Stichopus hermanii* (SH), and diabetic rats + *Spirulina platensis* (SP). The *in vitro* results showed that the antioxidant activity of 25 ppm *Spirulina platensis* had the same MDA inhibitory activity as 200 ppm α-tocopherol, but 200 ppm *Stichopus hermanii* had lower inhibition than 200 ppm α-tocopherol. The *in vivo* result showed that *Stichopus hermanii* treatment was more effective in suppressing blood serum MDA concentration, but *Spirulina platensis* was more effective in suppressing liver MDA concentration.

1. Introduction

The condition of hyperglycemia in diabetes mellitus triggers lipid abnormalities (Mohamed *et al.* 2016), and this leads to excessive reactive oxygen species (ROS), which stimulates lipid peroxidation and oxidative stress. This oxidative stress plays a pivotal role in damaging blood vessels, nerves, and organ structures and produces malondialdehyde in the body (Ayala *et al.* 2014). High malondialdehyde (MDA) levels in the blood cause damage, both micro and macrovascular (Marjani 2010). Malondialdehyde (MDA) is a product of lipid peroxidation which is toxic to cells in the body. MDA is used as a biomarker of oxidative stress in various health problems, one of which is diabetes (Khoubnasabjafari *et al.* 2015). Malondialdehyde is an end-product generated by the decomposition of arachidonic acid and larger PUFAs. Once formed, it can be enzymatical or react on cellular proteins or DNA to form adducts resulting in biomolecular damages. Early studies showed that a probable biochemical route for MDA metabolism involves its oxidation by mitochondrial aldehyde dehydrogenase followed by decarboxylation to produce acetaldehyde, which is oxidized by aldehyde dehydrogenase to acetate and further to CO_2 and H_2O (Ayala *et al.* 2014).

Golden sea cucumbers (*Stichopus hermanii*) (SH) and Spirulina (*Spirulina platensis*) (SP) are two marine resources that may scavenge free radicals and to have hepatoprotective effects on the liver (Windari *et al.* 2019). The antioxidant activity of *Stichopus hermanii* methanol extract using the DPPH method has an IC_{50} value of 65.08 ppm, which indicates that the extract has a vigorous antioxidant activity because the IC_{50} value is lower than 200 ppm (Rasyid 2012). The crude extracts of methanol, acetone, and ether pigments in microalgae spirulina have a stable value of inhibition against DPPH 51.0 ppm and 34.85 ppm, which indicates that the extract has a very strong antioxidant activity because the IC_{50} value is lower than 200 ppm (Yudiati *et al.* 2011). Previous research showed that a diabetic condition (induction of 50

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mg/kg bw STZ in rats) in the study period of 14 days can increased MDA level in the liver significantly ($p<0.05$), but MDA levels in rats blood serum were not significantly different ($p<0.05$) between the normal group and diabetics group (Windari *et al.* 2019). The induction of 50 mg/kg bw STZ in rats was able to make a diabetic condition in the study period of 14-21 days (Lenzen 2008). This study aimed to determine the effects of the antioxidant activities of *Stichopus hermanii* from Labuan Bajo, Flores, NTT Indonesia, and *Spirulina platensis* from Jepara, Central Java, Indonesia, in inhibiting malondialdehyde *in vitro* and *in vivo* on blood serum and livers of diabetic and wounded rats for 21 days.

2. Materials and Methods

2.1. Preparation of Golden Sea Cucumber (*Stichopus hermanii*) and *Spirulina platensis*

The *Stichopus hermanii* obtained from Flores East Nusa Tenggara Indonesia are then culled the unnecessary body parts and washed it. Next, cut the cleaned *Stichopus hermanii* into small pieces, and put into a bowl to be heated at medium heat temperature for 13 minutes with a wet weight of approximately 1 kg. *Stichopus hermanii* was set aside for a few moments, poured into a bottle, and stored in a freezer at a temperature of -20°C (Mu'alimah 2017). The powder of pure Spirulina platensis was obtained from Jepara, Central Java, Indonesia.

2.2. *In Vitro* Analysis

2.2.1. *Stichopus hermanii* Extraction

The fresh Stichopus hermanii were dried using a (BUCHI-B190) freeze dryer at a temperature of -50°C for 24 hours. The simplicia yield was extracted with methanol (Merck) using the maceration method. The ratio between the simplicia yield and the solvent was 1:5 (b/v). Then, the mixture was macerated using a shaking incubator with a speed of 125 rpm at room temperature. In this study, the maceration time was shortened to 24 hours. The mixture was filtered, and the obtained pulp was dissolved in methanol. This maceration method was replicated four times. The obtained filtrate was subsequently concentrated using a (the 1110S-wd eyela) rotary evaporator at a temperature of 40°C until it yielded an extract paste (Rasyid 2012).

The yields of freeze-drying and extraction were calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{Final extract weight (g)}}{\textit{Stichopus hermanii} \text{ dried weight (g)}} \text{x } 100\%$$

2.2.2. *Spirulina platensis* Extraction

The *Spirulina platensis* simplicia was extracted with methanol (Merck). The ratio between the simplicia and the solvent was 1:10 (b/v). Then, the mixture was macerated using a shaking incubator with a speed of 125 rpm at room temperature. In this study, the maceration time was shortened to 24 hours. This maceration method was replicated two times. The obtained filtrate was then concentrated using a (the 1110s- wd eyela) rotary evaporator at a temperature of 40°C (Rasyid 2012).

The yield of *Spirulina platensis* extraction was calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{Final extract weight (g)}}{\textit{Spirulina platesis} \text{ dried weight (g)}} \text{x } 100\%$$

2.2.3. Antioxidant Activity Analysis using the Thiobarbituric Acid-malondialdehyde Method

The antioxidant activity analysis used the standard curve of 1, 1, 3, 3-tetra methoxy propane (TMP) for the determination of malondialdehyde. The standard curve of 1, 1, 3, 3-tetra methoxy propane solution (Sigma Aldrich) was created at various concentrations of 0, 5, 8, 10, 15, 18, 20 µM. Each solution was pipetted as much as 1 ml and added with 2 ml of 20% TCA (Merck) and 2 ml of 1% TBA (Merck) solution in 50% acetic acid (Merck). The reaction mixture was heated in a 100°C water bath for 10 minutes, cooled, and centrifuged at 3,000 rpm for 15 minutes. Its absorbance was measured at a wavelength of 532 nm. 1 ml of distilled water, which had been treated like the other TMP concentration solution (0 TMP concentration), was used as a blank solution. Determination of linoleic acid incubation time was conducted using the TBA method. A mixture consisting of 6 ml of phosphate buffer (0.1 M pH 7) (Merck) was added with linoleic acid 100 mM (Sigma Aldrich) in 99.8% ethanol (Merck) and 3 ml of deionized water. 1 ml of the mixture was transferred into a dark bottle and incubated at 40°C. Absorption intensity was measured by mixing 1 ml of linoleic acid that had been incubated with 2 ml of 20% TCA solution (Merck) and 2 ml of 1% TBA solution (Merck) in 50% acetic acid (Merck). The reaction mixture was heated in a 100°C water bath for 10 minutes, cooled, and centrifuged at 3,000 rpm for 15 minutes. Next, its wavelength was read at 532 nm using a HITACHI UV/VIS U2800 BRUKER Spectrophotometer. The measurements were then taken every day until a maximum absorption was obtained. The blank solution contained 1 ml of a mixture, 3 ml of 99.8% ethanol, and 2 ml of 0.1 M phosphate buffer pH. It was then added by 2 ml of

20% TCA and 2 ml of 1% TBA in 50% acetic acid. The blank solution was heated in a 100°C water bath for 10 minutes, cooled, and centrifuged at 3,000 rpm for 15 minutes (Kikuzaki and Nakatani 1993).

The % inhibition of the extract was calculated as follows:

$$\text{Inhibition (\%)} = \frac{([\text{MDA}]\ \text{negative control} - [\text{MDA}]\ \text{extract})}{[\text{MDA}]\ \text{negative control}} \times 100\%$$

2.3. *In Vivo* Malondialdehyde Analysis

2.3.1. Experimental Design

Twenty-five Sprague Dawley rats of the same age of 12 weeks and weighed <200 g were obtained from the Indonesian National Agency of Drug and Food Control (BPOM). They were adapted for one month until the body weight was 200 g. This study was conducted under the supervision of the Institutional Animal Ethics Committees (R.05-17-IR) from PT. Bimana Indomedical Indonesia. The 25 Sprague Dawley rats were divided into five groups. Five rats were randomly chosen to be the control group, and 20 rats became streptozotocin-induced rats (50 mg/kg bb). STZ was dissolved in citrate buffer (4.5 pH) at a concentration of 1 mg/ml. 1 ml syringe was used for intraperitoneal STZ induction and after being induced, they were left alone for 48 hours (2 days). During the treatment, the rats were still given feeds and water.

The five rats that were not induced by STZ were then injected with 0.9% NaCl and 2 ml of distilled water for 21 days, and they were categorized as the control group. The twenty streptozotocin-induced rats were divided into four groups. (SA) group was fed with 2 ml of distilled water, (SG) group was fed with glibenclamide 3 mg/kg bb, (ST) group was fed with golden sea cucumber at a dose of 2,840 mg/kg bb, and (SS) group was fed with spirulina at a dose of 81 mg/kg bw. The blood rat was taken for malondialdehyde levels test on days 0 and 21. Meanwhile, the liver malondialdehyde measurement was evaluated on day 21. Bodyweight measurement and food intake were conducted every seven days (the adaptation and experimental periods).

The process of making wounds was carried out using a surgical approach on the back of rats. The rats were given ketoprofen 5 mg/kg BW as an analgesic and anesthetized using ketamine 75 mg/kg BW and xylazine 5 mg/kg BW. Aseptically, the wound was made based on a mold in the shape of a rectangle measuring a depth of 1 x 0.5 cm until the muscle tissue was identified (skin area to the subcutis) using a sterile scalpel.

2.3.2. Blood Serum Preparation

The rats were deprived of food for ±16 hours before their blood was drawn from their tails. The blood was collected in an Eppendorf tube and centrifuged at 3,000 rpm with a 12 cm rotor radius for 10 minutes. The obtained serum was stored at -4°C until the malondialdehyde analysis session day came (Safithri *et al.* 2012).

2.3.3. Analysis of Malondialdehyde Levels in Rat Blood and Liver

Measurement of malondialdehyde level in blood serum used the standard curves were created using 6 M TMP stock (sigma) that had been diluted by distilled water at the following levels: 0.9; 1.8; 2.7; 3.6; 4.5; and 5.0 µM. Each solution was pipetted into a test tube as much as 2 ml. Each tube was added with 0.5 ml of 1% TBA (Merck) in 50% acetic solvent. The mixture was heated at 95°C for 60 minutes and cooled at room temperature. Furthermore, 0.5 ml of distilled water and 2.5 ml of *n*-butanol: pyridine (15:1 v/v) (Merck) were transferred into the test tube and centrifuged at 3,000 rpm for 15 minutes. Two phases appeared, and the phase taken was the pink phase, which was on the top of the test tube. Its absorbance was spectrophotometrically measured at 532 nm.

The modification of this method is the volume of solvent used in the test and the blood serum was centrifuged at 3,000 rpm for 15 minutes before the test was carried out. The obtained blood serum was then stored at -4°C. As much as 0.2 ml of blood serum was added with 1.2 ml of H_2SO_4 (0.083 N) (Merck), set aside for 10 minutes, added with another 0.15 ml phosphotungstic acid 10% (Merck) and set aside for another 5 minutes at room temperature. This stage was carried out two times. Next, the obtained pellets were added with 0.5 ml of distilled water and 0.5 ml of 1% TBA reagent (Merck) in 50% acetic acid (Merck). The mixture was then heated at 95°C for 60 minutes and cooled at room temperature. Then, as much as 0.5 ml of distilled water and 2.5 ml of *n*-butanol: pyridine (15:1 v/v) (Merck) were transferred into a test tube and centrifuged at 3,000 rpm for 15 minutes. Two phases appeared, and the phase taken was the pink phase, which was on the top of the test tube. Its absorbance was spectrophotometrically measured at 532 nm.

Measurement of malondialdehyde levels of the liver organ was conducted by the Okhawa (1979) method with modification. Rinsing off the 0.9% NaCl (Merck) on 1 g rat liver and drying it with a filter paper. Liver homogenates were made at a concentration of 10% (b/v) in 0.9% NaCl. 1 ml of homogenate, which

had been centrifuged at 4,000 rpm for 20 minutes, was taken out from the centrifuge and transferred into the test tubes. Each test tube was added with 0.2 ml of 8.1% SDS (Merck) and 1.5 ml of acetic acid, and the pH was then adjusted to 3.5 with 1 M NaOH (Merck). As much as 1 ml of distilled water and 1.5 ml of 1% TBA (Merck) were added into the test tubes. The mixture was heated at 95°C for 60 minutes and cooled at room temperature. Furthermore, as much as 1 ml of distilled water and 5 ml of *n*-butanol: pyridine (15:1 v/v) (Merck) were transferred into the test tube and centrifuged at 4,000 rpm for 10 minutes. Two phases appeared, and the phase taken was the pink phase, which was on the top of the test tube. Its absorbance was spectrophotometrically measured at 532 nm.

3. Results

3.1. *In Vitro* Malondialdehyde Assay

The yield of *Stichopus hermanii* was 26.87%, and Spirulina platensis was 8.66% (both were based the on dry base). The *in vitro* result showed that malondialdehyde levels increased from day 0 to 7. The maximum malondialdehyde levels obtained from linoleic acid oxidation occurred on the 7th day (Figure 1) and then it decreased afterwards. *Spirulina platensis* extract can inhibit the formation of malondialdehyde more than *Stichopus hermanii* extract (Figure 2). The extract of *Spirulina platensis* at a concentration of 200 ppm had a higher inhibition (66.69%) compared to a-tocopherol at a concentration of 200 ppm (57.67%), yet it was not significantly different (P<0.05). The 200 ppm of *Stichopus hermanii* extract had a lower inhibition standing at (41.26%) and was significantly different (P<0.05) with a-tocopherol at a concentration of 200 ppm (57.67%).

3.2. *In Vivo* Experiment

3.2.1. Body Weight and Food Intake of Rats

The beginning of adaptation that is seven days before fed, the average body weight of rats was 188.95 g, and the rat body weight during the adaptation period increased reaching 212.5 g (Figure 3). The bocy weight of diabetic rats treated with aquadest, glibenclamide, *Stichopus hermanii* and *Spirulina platensis* for 21 days decreased except in the normal group. For the DA, DG, SH, and SP groups, the rats body weight on the 21st day was significantly different (p<0.05) with the NA group. The weight sequence was 34.84% (DA), 31.20% (SH), 30.95% (DG) and 30.23% (SP). The administration of *Stichopus hermanii* and *Spirulina platensis* could suppress 1-4% body weight of STZ-induced rats.

The amount of feed intake in rats at the beginning of adaptation conditions was not significantly different (p>0.05) from the average intake of 16.5 grams (Figure 4). The rats experienced an increase in feed intake during the adaptation period, which was significantly different (p>0.05) on day 0, and feed intake decreased after 21 days of STZ induction. The 21 days of treatment showed that rats had significantly different food intake (p<0.05), respectively, by 27.99% (SG), 23.81% (DA), 22.24% (SH), and 20.34% (SP). The administration

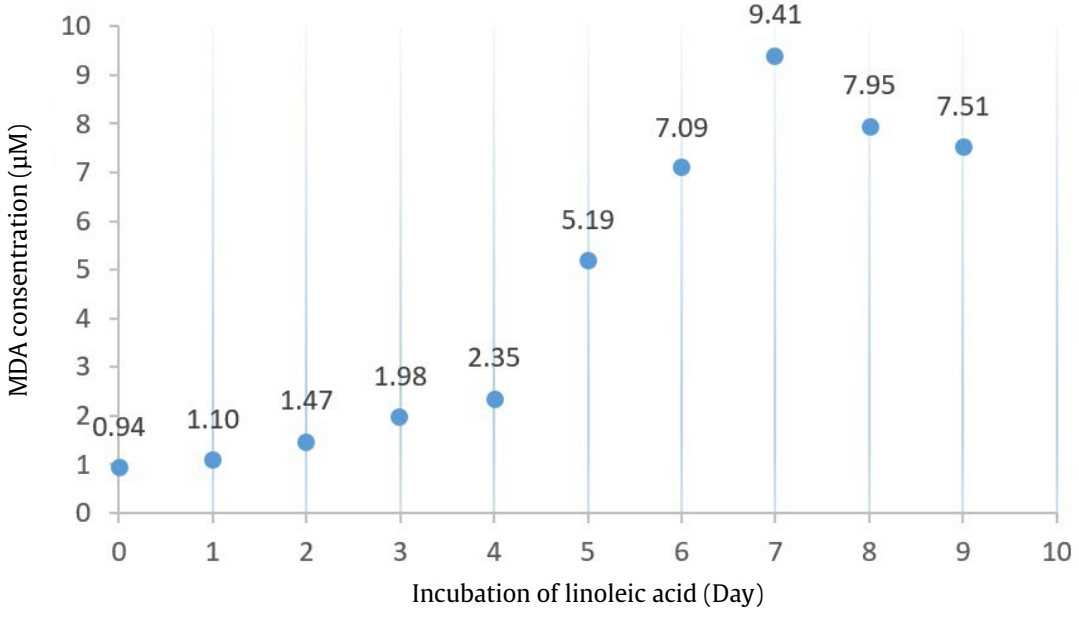

Figure 1. Malondialdehyde concentration of linoleic acid incubation for nine days

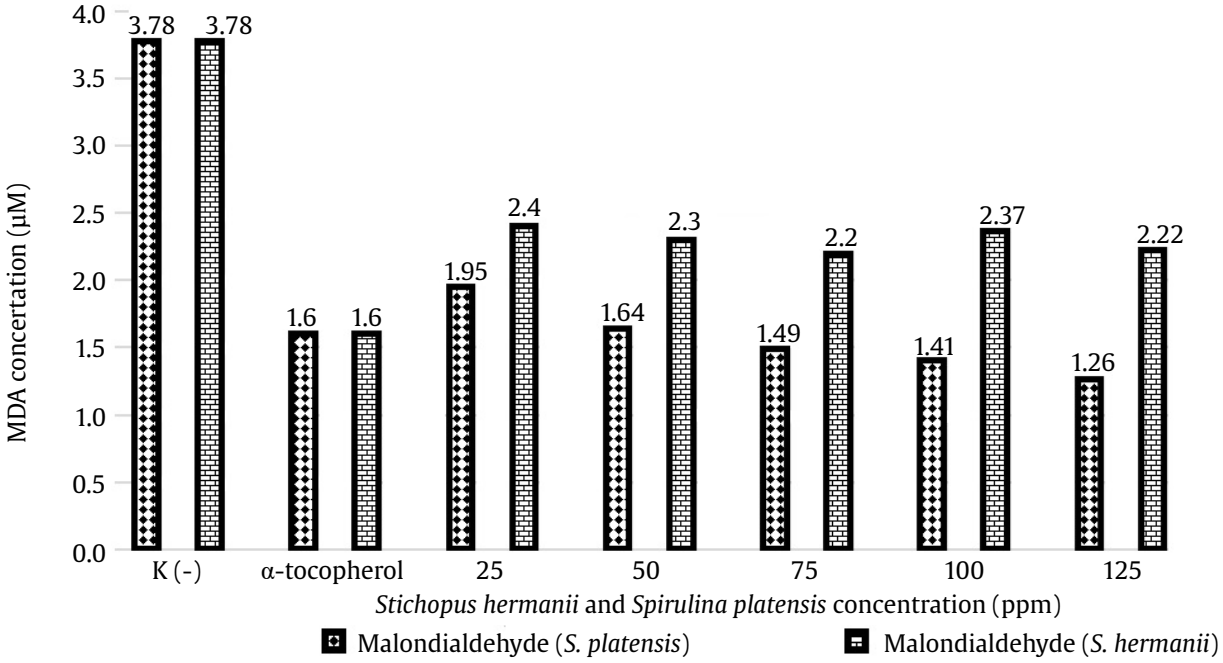

Figure 2. The effect of *Stichopus hermanii* and *Spirulina platensis* extracts on the formation of malondialdehyde as a result of linoleic acid peroxidation

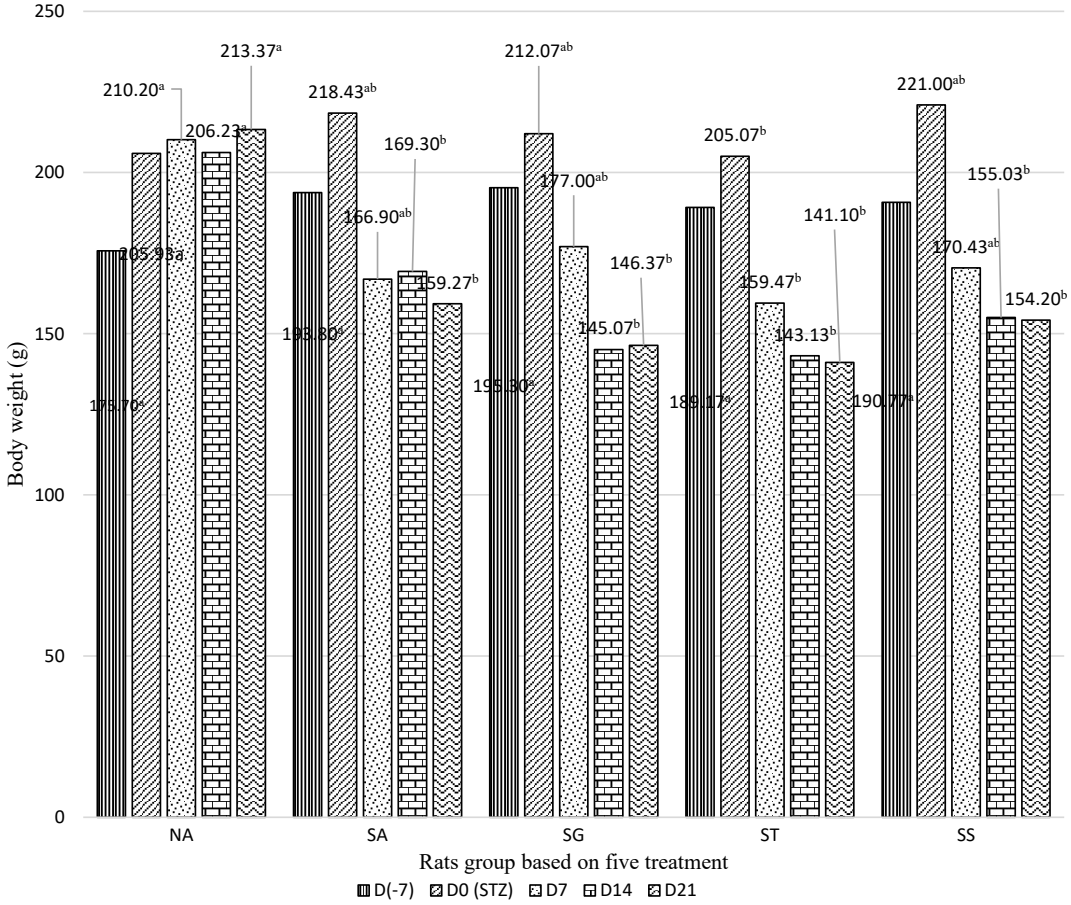

Figure 3. Changes in body weight of rats during the adaption and experimental periods based on the five groups, NA:0.9% NaCl injection and distilled water induction, SA:STZ injection and distilled water induction, SG:STZ injection and glibenclamide induction, ST:STZ injection and Stichopus hermanii induction, SS:STZ injection and *Spirulina platensis* induction. Numbers following by the same letters indicated that they were not significantly different at $p<0.05$, n = 3

of *Stichopus hermanii* and *Spirulina platensis* could suppress 1-4% food intake of STZ-induced rats.

3.2.2. Malondialdehyde Concentration in Rat Blood Serum

Measurement of blood serum malondialdehyde concentration was undertaken on day 0 and at the end of the study (day 21) i.e. on the 21st day of treatment (Figure 5). All rat groups on day 0 had no significant differences ($p>0.05$) in serum malondialdehyde concentration from the normal group. Blood serum malondialdehyde concentration increased on the 21st day of treatment, and the lowest percentage of malondialdehyde levels was found in the Stichopus hermanii group (39.46%). The administration of Stichopus hermanii and Spirulina platensis could decrease the percentage of serum malondialdehyde concentration levels of diabetic rats by 86.49% and 59.35%.

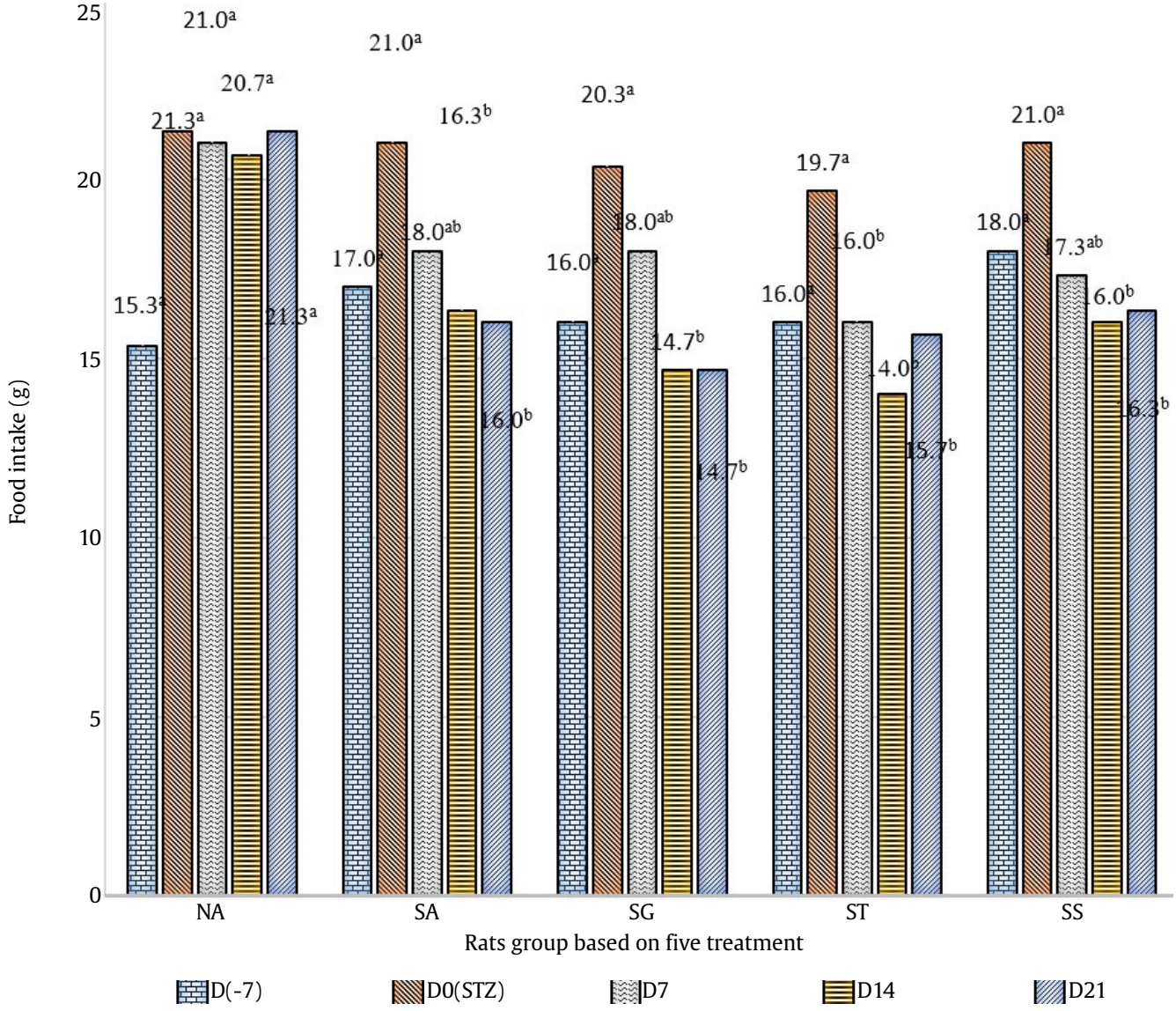

Figure 4. Food intake during the adaptation and experimental periods of rats treated by 0.9% NaCl injection and distilled water induction for NA group, STZ injection and distilled water induction for SA group, STZ injection and glibenclamide induction for SG group, STZ injection and *Stichopus hermanii* induction for ST group, STZ injection and *Spirulina platensis* induction for SS group. Numbers following by the same letters indicated that they were not significantly different at $p<0.05$, n = 3

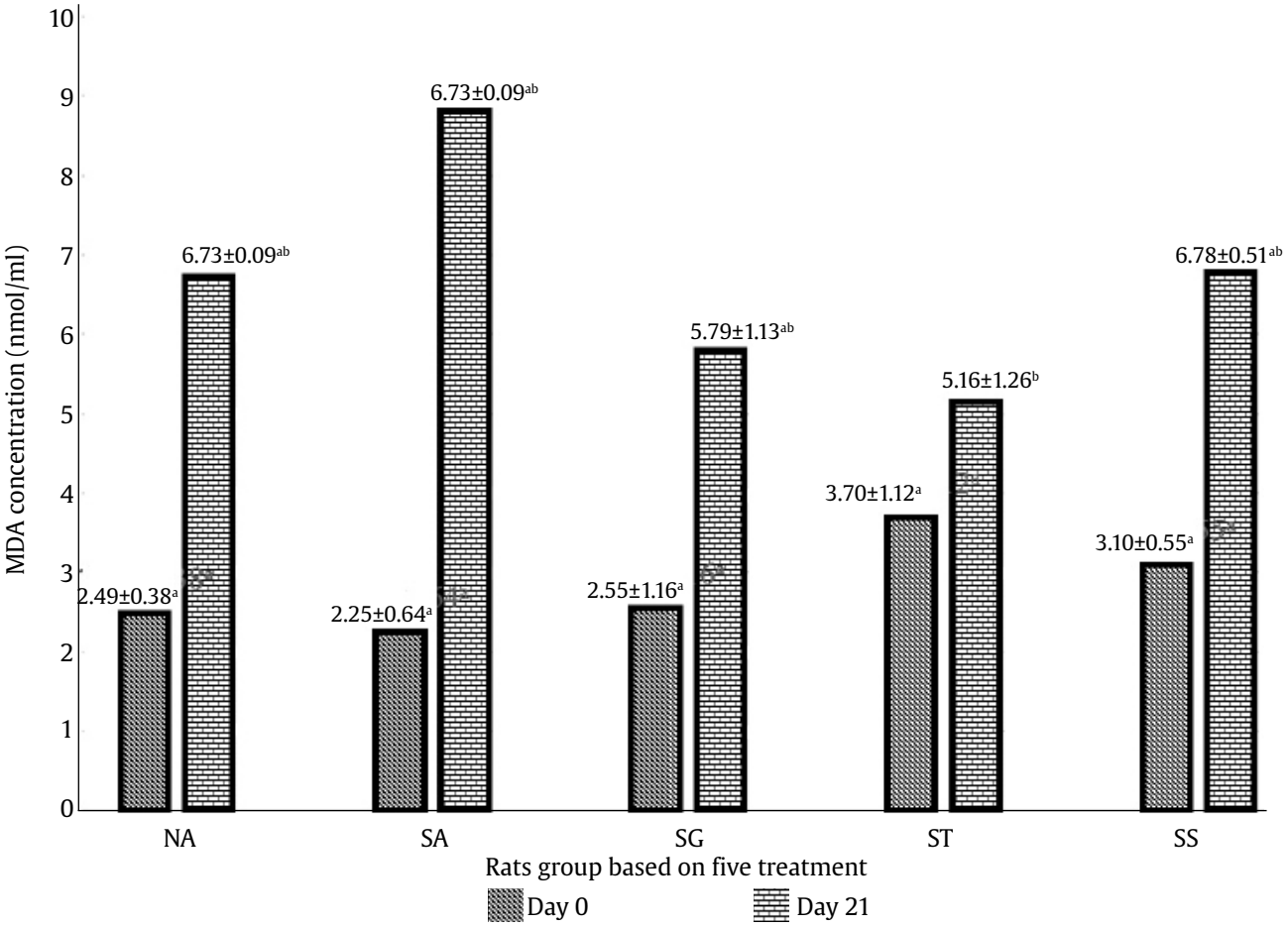

Figure 5. Comparison of malondialdehyde concentration between day 0 and day 21 in blood serum of rats treated by 0.9% NaCl injection and distilled water induction for NA group, STZ injection and distilled water induction for SA group, STZ injection and glibenclamide induction for SG group, STZ injection and *Stichopus hermanii* induction for ST group, STZ injection and *Spirulina platensis* induction for SS group. Numbers following by the same letters indicated that they were not significantly different at $p<0.05$, n = 3

3.2.3. Malondialdehyde Concentration in Rat Livers

Malondialdehyde concentration in rat livers on day 21 of all groups were significantly different (p0.05). Therefore, SH and *Spirulina platensis* administration was able to suppress the malondialdehyde concentration in rat livers by 18.5% and 38.67% while the value of rats induced by distilled water stood at 49%.

4. Discussion

The yield of *Stichopus hermanii* methanol extract obtained in this study was 26.87% lower than the yield of *Stichopus hermanii* methanol extract from the same place (Labuan Bajo, East NusaTenggara, Indonesia) (Susanto *et al.* 2017), 37.58%. The yield of *Spirulina platensis* methanol extract was 8.66% obtained was lower than *Spirulina platensis* methanol extract from the same place, 10.91% (Jepara, Central Java, Indonesia) (Zhafira 2016). The difference in yield value could be influenced by the extraction times, solvent types, ratios between the number of samples and solvents, extraction temperatures, and particle sizes of the sample (Bustan *et al.* 2008).

The extraction time can affect the yield of African leaves extraction. The extraction which has been carried out for 48 hours resulted in a smaller yield value of 4.003% (Sukmawati *et al.* 2017) than the extraction for 72 hours that resulted a yield value of 9.07% (Prastika 2020). Increased the extraction time is also can lead to the higher yields by increasing solvent exposure to the samples, this is because the extraction time is an important parameter that influences the extraction yield of bioactive compounds (Pichai and Krit 2015). The type of solvent used for extracting

can affect the yield of the extraction result. *Celosia cristata* L. resulted different yield values in 3 types of solvents, that are 6.366% methanol solvent, 4.328% water solvent, and 1.655% ethanol solvent (Ajima 2021). The ratio of sample and solvent is also can affect the yield of the extraction results. The solid-to-solvent ratio (1:30, 1:50, and 1:100 were tested) to extract the carotenoid (Lutein) from *C. vulgaris*. The highest carotenoid yield was achieved a solid-to-solvent ratio of 1:100 (g/ml). (Dianursanti *et al.* 2020). The extraction with temperature at 60°C in *Sargassum* sp.extraction resulted a higher yield value (2.59%) and significantly different from extraction with temperature at 90°C (1.82%) (Sukma *et al.* 2017). The last factor that can affect the yield of extraction result is particle size. The yield of natural color extract from *Pandanus tectorius* fruit with a particle size of 40 mesh (3.58%) is smaller than a result from sample with particle size of 60 mesh (4.57%) (Antari *et al.* 2015).

In vitro, antioxidant activities showed that *Spirulina platensis* extract had an equivalent inhibition value of malondialdehyde to a positive control (α-tocopherol). Antioxidants can prevent or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. The phenolic compounds, such as phenolic acids, phenolic diterpenes, flavonoids, have antioxidative effects. The redox properties of phenolic compounds play a primary role in scavenging free radicals (Kasote *et al.* 2015). *Spirulina platensis* contains active compounds such as flavonoids, phycocyanin, chlorophyll, β-carotene, vitamin A and α-tocopherol, which inhibit and reduce free radical formations (Wang *et al.* 2007). *In vitro, Stichopus hermanii* extract antioxidant activities can inhibit the formation of malondialdehyde because of saponin presence (Zhafira 2016). Saponin compounds included in the phenolic group could inhibit free radicals (Firdiyani *et al.* 2015).

The body weights of all rats increased during the adaptation period (before STZ induction), which indicated that the body weight in each rat group was not significantly different ($p>0.05$) (Figure 3). Sprague Dawley experienced increased body weight during the adaptation period of 7 to 15 days (Falah *et al.* 2010). It indicated that all rats were healthy and normal during the adaptation period (Sihombing and Raflizar 2010). The normal rats experienced an increase in body weight by 9.4% during the 16 days of the study (Hasibuan *et al.* 2016).

The rats that were induced by STZ a significantly decreased body weight and food intake in this research. Rats were induced by STZ for 21 days without any treatment experienced a decrease in body weight by 31.91% (Dogan *et al.* 2015). A significant decrease in body weight that occurred continuously after the induction of diabetogenic substances STZ was a diabetes symptom. STZ induction influenced uncontrolled weight loss and caused glucose intolerance. Rats could not control the use of glucose in their body to be converted into energy in cells. Due to the occurrence of insulin resistance, the body responds to produce energy in other ways such as glycogenolysis, gluconeogenesis, lipolysis, and this causes loss of protein tissues (Skovso 2014). The fat contained in various tissues was catabolized through the β-oxidation process to produce energy, and loss of fat resulted in a decrease in body weight (Hikmah *et al.* 2015). The administration of *Stichopus hermanii* and *Spirulina platensis* could suppress the amount of food intake of diabetic rats better than glibenclamide positive control. *Spirulina platensis* contains flavonoids such as quercetin, genistein, and kaempferol (Papalia *et al.* 2019). *Stichopus hermanii* contains saponins such as triterpene glycoside (Bordbar *et al.* 2011). Flavonoids have been proven as an antioxidant, as a substance improving hyperlipidemia and hyperglycemia condition in diabetic rats by regulating fatty acid metabolism and cholesterol (Anulianni'aim *et al.* 2012).

Malondialdehyde is a biomarker of oxidative stress in various health problems, one of which is diabetes mellitus (Khoubnasabjafari *et al.* 2015). In this study, the normal rats that were not induced by STZ experienced increased blood serum malondialdehyde (Figure 5). It was influenced by the wounds made on the rats' back. The value of blood serum malondialdehyde of the normal rats given wounds on their backs and smeared with distilled water for ten days stood at 5.189±0.94 nmol/ml (Andini 2016). It was revealed that the wound could trigger an increase in oxidative stress. The giving wound was carried out to determine its effect on increasing stress conditions in normal rats. Oxidative stress was an imbalance condition between antioxidants and prooxidants influenced by several factors such as age, chemical drugs, toxicities, and inflammations (Ullah *et al.* 2016). Stress conditions trigger the formation of ROS compounds in the body, one of which is Hydroxyl

radicals (HO*). This compound is a chemically reactive species that can form hydrogen peroxide (H_2O_2) (Ayala *et al.* 2014). The MDA concentration of blood serum in STZ-induced rats at a dose of 55 mg/kg bb for 21 days was 7.957 nmol/ml (Yuniasih 2016). Induction of streptozotocin can reduce superoxide dismutase (SOD) and catalase (CAT) levels in the rat body (Safithri 2012).

Measurement of malondialdehyde concentration in the liver was evaluated after 21 days of treatment. The value of malondialdehyde concentration in a tissue indicates the level of free radical against lipids (Gwarzo *et al.* 2014). Streptozotocin induction at a dose of 50 mga/kg for 21 days had proven to increase liver malondialdehyde concentration significantly ($p<0.05$) higher than the malondialdehyde of normal rats (Figure 5). The malondialdehyde concentration of normal rat liver was 21.70±5.66 (Noerman *et al.* 2011). Rats induced by streptozotocin (50 mg/kg bb) for 21 days had a liver MDA level of 45.01±5.78 nmol/gram (Dogan *et al.* 2015).

Spirulina platensis antioxidant activity had proven to suppress the increase in blood serum malondialdehyde in diabetic rats (Figure 5). This result was consistent with *in vitro* malondialdehyde inhibition. *Spirulina platensis* extract at a concentration of 50 ppm had an MDA inhibition value of 56.53%. It indicated that this was statistically not different from α tocopherol at a 200 ppm (Figure 2). The antioxidant activity of *Spirulina platensis* was generated by the presence of phycocyanin pigment (81.33 mg/gr) (Farihah *et al.* 2014). Phycocyanin was a powerful antioxidant that quickly dissolves in water and can break down free radicals to inhibit fat oxidation by peroxide. These phycocyanins could effectively reduce peroxynitrite (ONOO-) toxins generated from STZ induction (Pirenantyo and Limantara 2008). *Spirulina platensis* and *Stichopus hermanii* contain amino acid glycine and glutamate (Maryanti 2017) which amino acids produce glutathione. Glutathione is a primary antioxidant found in the body containing amino acid glycine, cysteine, and glutamate (Diez *et al.* 2015). Glutathione works by donating electrons to superoxide (O2-*) and hydrogen peroxide (H_2O_2) to form H_2O molecules with the help of the glutathione peroxidase enzyme (GPx) (Nelson and Cox 2013). This reaction may stop the formation of free radicals in the body so that the lipid peroxidation process can also be reduced; therefore, the Stichopus hermanii and Spirulina platensis antioxidant activities reduce the serum malondialdehyde concentration in diabetic rat blood by 86.49% and 59.35%.

Stichopus hermanii and *Spirulina platensis* have antioxidant activities *in vitro* and *in vivo* by inhibiting malondialdehyde formation in the blood serum and liver of the rats. *Stichopus hermanii* has been detected more active in inhibiting the formation of MDA in rat blood serum because *Stichopus hermanii* contains eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which can reduce blood serum triglyceride concentrations. Decreases in triglyceride concentration can reduce the occurrence of lipid peroxidation (Nugraha 2018). Diabetic rats induced by STZ 50 mg/kg bw for 21 days for the diabetes group had higher triglyceride concentrations at the end of the study than the treatment group (Dogan *et al.* 2015). The methanolic extract of *Stichopus hermanii* contains bioactive compounds of alkaloids, saponins and triterpenoids (Zhafira 2016). Triterpenoids contained in an extract can significantly reduce cholesterol, low density lipoprotein (LDL) and triacylglycerol (TAG) concentrations. These compounds can also act as antioxidants in plasma and blood serum to reduce DNA damage, inflammation and levels of oxidative stress (Han and Bakovic 2015). *Spirulina platensis* is proven to be able to inhibit the formation of MDA in rat liver. The antioxidant activity of Spirulina platensis is obtained from the presence of the phycocyanin pigment content of 81.33 mg/gr (Farihah *et al.* 2014). Phycocyanin is a strong antioxidant that is easily soluble in water and has the potential to break down free radicals so that it can inhibit lipid oxidation by peroxides. These phycocyanins can reduce peroxynitrite (ONOO-) toxins that are harmful to the body resulting from STZ induction (Pirenantyo and Limantara 2008). *Spirulina platensis* is also contains fiber which has an effect on fat absorption by binding to fatty acids. Several mechanisms for fat metabolism include pancreatic lipase enzyme reactions. In addition, fiber is able to withstand the enterohepatic cycle (reabsorption of bile in the intestine to the liver) (Nugraha 2018).This study showed that there was a synergistic effect between *Stichopus hermanii* from Labuan Bajo, East Nusa Tenggara, Indonesia, and Spirulina platensis from Jepara, Central Java, Indonesia, in reducing MDA in liver and blood serum. In future research, it is necessary to carry out more diverse analyzes such as analysis of superoxide dismutase (SOD) and catalase activity, SGOT/AST

activity, SGPT/ALT activity in blood serum and liver histopathology. This needs to be done in order to support the results of research in knowing how effective the administration of Stichopus hermanii and Spirulina platensis combination.

Conflict of Interests

The authors declare no conflict of interest on the publication of this article.

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