EFFECTS OF GOLDEN SEA CUCUMBER EXTRACT (*Stichopus hermanii*) ON FASTING BLOOD GLUCOSE, PLASMA INSULIN, AND MDA LEVEL OF MALE RATS (*Rattus norvegicus*) INDUCED WITH STREPTOZOTOCIN

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ABSTRACT

Diabetes mellitus (DM) is one of disease that its incidence increases every year worldwide. The condition of DM can cause various complications caused by oxidative stress. Stichopus hermanii (SH) or golden sea cucumber extract contains antioxidant compounds that have been proven to reduce oxidative stress conditions. The purpose of this study was to investigate the effect of Stichopus hermanii extract on condition of diabetes mellitus by looking at changes in fasting blood glucose, plasma insulin, and malondialdehyde levels in animal models of Wistar rats. This study was a laboratory experimental study using Randomized Control Trial Design with Post-test only control group design. Thirty-five male Wistar rats divided into five groups, i.e normal control group, positive control, negative control and two treatment groups with SH extract dose 8.5 and 17 mg / kgBW for 14 days once daily after induction of Streptozotocin at the Biochemistry Laboratory of the Faculty of Medicine, Airlangga University. Fasting blood glucose level was measured by a glucometer, plasma insulin measured by ELISA and MDA level was measured by a spectrophotometer. Data were analyzed statistically by using One Way ANOVA test and Kruskal Wallis. There were significant results of SH extract can reduce fasting blood glucose (Kruskal Wallis, p=0.030) and MDA (Kruskal Wallis, p=0.042) but not in plasma insulin (ANOVA, p=0.130). The lowest MDA level occurs in the K4 group that given SH extract dose 17 mg/kg BW than another experimental group. As the conclusion, this study showed SH extract can decrease fasting blood glucose and oxidative stress in diabetic-induced rats.

***Keywords**: Stichopus hermanii; diabetes mellitus; FBG; insulin; MDA*

ABSTRAK

Diabetes melitus merupakan salah satu penyakit yang insidensinya meningkat setiap tahun di seluruh dunia. Kondisi diabetes melitus dapat menyebabkan berbagai komplikasi yang disebabkan adanya stress oksidatif. Ekstrak Stichopus hermanii mengandung senyawa antioksidan yang telah terbukti dapat meredam kondisi stress oksidatif. Tujuan dari penelitian ini adalah untuk membuktikan bahwa ekstrak teripang emas (Stichopus hermanii) dosis 8,5 dan 17 mg/kgBB dapat menurunkan kadar glukosa darah, meningkatkan kadar insulin plasma, dan menurunkan kadar malondialdehid (MDA) pada hewan model tikus wistar yang diinduksi Streptozotocin. Penelitian ini merupakan penelitian ekperimental laboratoris menggunakan rancang bangun acak lengkap dengan post test only control group design pada tiga puluh lima tikus putih jantan galur wistar yang dibagi menjadi lima kelompok dan diamati selama 14 hari setelah induksi Streptozotocin di Laboratorium Biokimia Fakultas Kedokteran Universitas Airlangga. Pengukuran kadar glukosa darah puasa dilakukan dengan glukometer, kadar insulin plasma diukur dengan ELISA sedangkan kadar MDA diukur dengan spektrofotometer. Data dianalisis secara statistik dengan uji One Way ANOVA dan Kruskal Wallis. Hasil penelitian menunjukkan bahwa pemberian ekstrak SH dapat signifikan menurunkan glukosa darah puasa (Kruskal Wallis, p=0.030) dan kadar MDA (Kruskal Wallis, p=0.042) tetapi tidak pada kadar insulin plasma (ANOVA, p=0.130). Kadar MDA terendah dari kelompok uji adalah pada kelompok K4 yang diberi ekstrak SH dosis 17 mg/kg BB. Kesimpulan penelitian ini adalah pemberian ekstrak teripang emas dapat menurunkan kadar glukosa darah puasa dan stress oksidatif pada tikus model diabetes melitus.

***Kata kunci**: Stichopus hermanii; diabetes mellitus; FBG; insulin; MDA*

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic related-disease that its prevalence increasing every year worldwide (Olokoba et.al, 2012). It happens due to the high level of morbidity, disability, mortality and expensive health cost due to complications and long-term treatment. Many factors commonly associated with risk factors of

DM such as lifestyle, genetics and medical conditions, but oxidative stress also contributes to the pathogenesis complication of DM (Bajaj 2012). Metabolic process disorders in DM can cause permanent and irreversible functional changes of cells in the body, especially those involved in the vascular system. Overproduction of ROS (reactive oxygen species) work in making tissue damage is accelerated by a variety of important molecular mechanisms that are activated as a result of chronic hyperglycemia conditions. So, longer exposure of hyperglycemia conditions will lead to high oxidative stress then so many tissue damage will be born and makes various complication both micro and macroangiopathy (Veerabhadra 2016, Ceriello & Testa 2009).

Indonesia is an archipelagic country with an abundant marine product. One of the marine products that are very popular is sea cucumbers that believed to have medicinal properties as well as food ingredients especially by people in Asia and Middle East continent since long time ago. From 1716 species of sea cucumber in Asia Pacific region, there are about 350 species can be found in Indonesia's marine, include a type calls *Stichopus hermanii* (SH) (Pangestuti and Arifin, 2017). The high protein and low fat content, as well as other ingredients such as glycosaminoglycan sulphate, vitamins, minerals, triterpene glycosides (saponins), carotenoids, collagen, chondroitin sulfate and so on, making sea cucumbers have high medical value as wound healers, neuroprotective, antitumor, anticoagulant, antimicrobial and antioxidant (Pangestuti and Arifin, 2017; Elekofehinti, 2015). Other benefits such as saponins, for example, it is said can also lower blood glucose levels by modulating insulin signals, increase insulin release by the pancreas, restore insulin response, inhibit disaccharide activity etc. so it is potential to be used as an antidiabetic compound (Barky et al 2017).

Various study related to sea cucumber that effect of antioxidants has also been tried. *Stichopus hermanii* ethanol extract was proven to decrease oxidative stress and hyperkeratosis rats exposed cigarette smoke (Revianti et al, 2016). Mu'allimah (2017) is her research showed that sea cucumbers have an anti-glycemic effect. Saponins can regulate blood glucose levels and prevent diabetic complication from antioxidant activity and may also reduce the risk of atherosclerosis and cardiovascular disease in diabetic patients (Barky, 2017).

That made a study about DM is still continued until today. The most common biological marker that used to study oxidative stress as one of the causes of DM complication with the easiest way is malondialdehyde (MDA), a lipid peroxidation product. Examination of blood glucose and fasting plasma insulin level are used for predicting the condition of pancreatic beta cells, insulin resistance and monitoring therapy for DM patient.

MATERIALS AND METHODS

This study was a pure experimental study using randomized control trial with post test only control group design. Thirty five male Wistar strain white rats were divided into five groups randomly: K0 (standard group), K1 (diabetes-induced STZ), K2 (diabetes-induced STZ and metformin), K3 (diabetes-induced STZ and SH with dose 8,5 mg/kgBW) and K4 (diabetes-induced STZ and SH with dose 17 mg/kgBW). Supplementation of SH sea cucumber in this study was taken from the tip of Raas Island, Sumenep, Madura, East Java which is extracted with ethanol solvent and the dose refers to previous research by Revianti et.al (2016).

After seven days of acclimation, all group except K0 induced STZ with dose 50 mg/kgBW in 0,05 M citric acid buffer with pH of 4.3 - 4.5 intraperitoneal (Purwato dan Liben, 2014). Three days after that, the fasting blood glucose was measured. Diabetes mellitus is said if blood glucose shows value >126 mg/dl after fasting about 10 hours (Firdaus dkk, 2016). The K0 dan K1 group was given standard feed and administration of distilled water ad libitum until the 15th days. Meanwhile, K2, K3, and K4 groups were given each metformin, SH with dose 8,5 mg/kgBW, and SH with dose 17 mg/kgBW of rat once per day by sonde orally.

Next, all experimental rats euthanized by ether inhalation to obtained blood samples 14 days after supplementation of metformin and SH extract. The measurement of fasting blood glucose level done by glucometer, fasting plasma insulin by ELISA and MDA by a spectrophotometer with the maximum wavelength of 535 nm.

Data were analyzed statistically for parametric by One Way ANOVA and for non-parametric by Kruskal-Wallis test. All statistical comparisons were made using SPSS 22, and a p-value of < 0.05 was considered significant.

RESULTS

The result of this study was done by comparing fasting blood glucose, plasma insulin and MDA level of Wistar rat induced STZ among all group showed in Table 1.

This table shows mean and standard deviation of fasting blood glucose (FBG), plasma insulin and MDA between K0 group (standard or normal group), K1 (negative control rats group with induced STZ), K2 (positive control rats group with metformin), K3 (experimental rats group with induced STZ and given supplementation of SH 8,5 mg/kg BW orally once daily), and K4 (experimental rats group with induced STZ and given supplementation of SH 17 mg/kg BW orally once daily).

Figure 1 shows that the highest mean of fasting blood glucose (FBG) level is obtained in K1 group and the lowest in K0 (standard group). Figure 2 shows that the highest mean of plasma insulin level is in the K2 group and the lowest in K1 group. Figure 3 shows that the highest level of MDA is in K2 group and the lowest is in K0 level. This figure also shows that MDA level has a lower level in all supplementation of SH extract group.

Table 1. Mean and standard deviation of fasting blood glucose, plasma insulin, and MDA level each group

Variables	Mean ± SD					P
	K0	K1	K2	K3	K4	
FBG level (mmol/L)	4.932±0.59	13.995± 10.6	7.026±3.55	5.261±0.86	8.413±3.8	0.030
Plasma insulin level (mIU/L)	0.419±0.93	0.367±0.33	0.521±0.15	0.463±0.11	0.493±0.07	0.130
MDA level (nmol/ml)	1.91±1.69	4.445±1.51	4.619±1.94	3.113±1.88	2.543±0.79	0.042

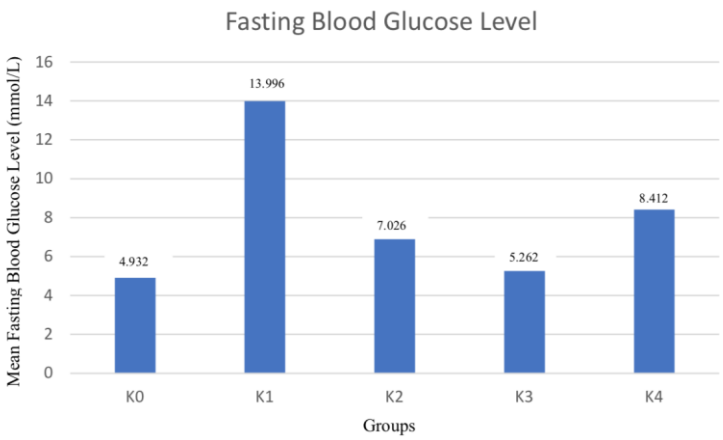

Fig. 1. Mean fasting blood glucose level (mmol/L) on the role of SH extract in diabetic-induced rats.

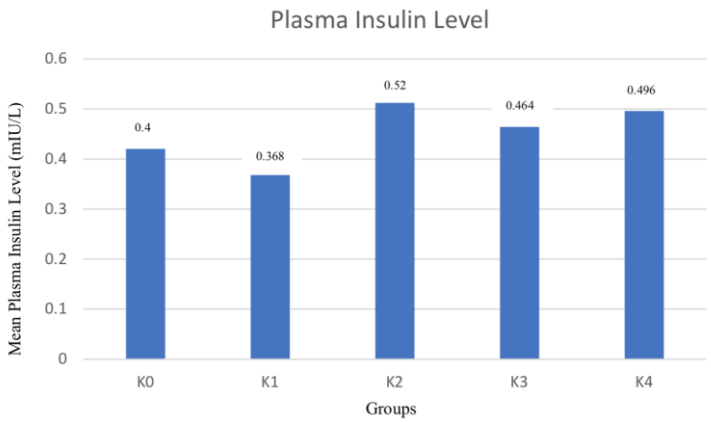

Fig. 2. Mean plasma insulin level (mIU/L) on the role of SH extract in diabetic-induced rats.

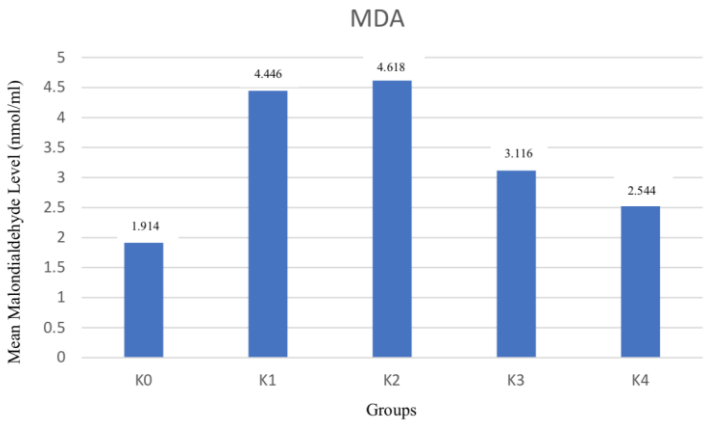

Fig. 3. Mean MDA level (nmol/ml) on the role of SH extract in diabetic-induced rats

There was a significant difference in fasting blood glucose level ($p<0.05$) and MDA ($p<0.05$) among all group, but no significant difference in plasma insulin level ($p>0.05$).

DISCUSSION

Various complication of diabetes mellitus can be caused by increased of oxidative stress (Bajaj, 2012). Oxidative stress induced hyperglycemia is generally associated with a decreased of antioxidant capacity, resulting in damage to some cell components. The fasting blood glucose in this study showed differences among all group but not in plasma insulin level between SH extract group with others. This condition can be produced by STZ that induce diabetic rats affecting glucose oxidation and decrease biosynthesis and insulin secretion by decreasing of GLUT 2 expression. It results in decreased peripheral receptor sensitivity, resulting in increased insulin resistance and elevated blood glucose levels (Firdaus, 2016). Mu'allimah (2016) in his research also said that induction of STZ causes inflammation of the pancreatic beta cells resulting decreases of insulin production. This condition also equivallen with current condition which negative control group (K1) and group with metformin (K2) still survive without insulin that indicate animal model is in nonphase of insulin requirement (NIR) (Purwanto and Liben, 2014).

Hyperglycemic conditions stimulate oxidative stress level that can lead tissue damage through molecular mechanism such as increasingly protein kinase C, hexosamine, polyol and AGEs pathways (Ceriello dan Testa, 2009). This study shows that there was a significant difference of decreasing MDA level in each treatment group given SH supplementation compared with negative and positive control group. Malondialdehyde (MDA) level of control positive group shows 4.619?1.94 nmol/L, but MDA level of SH extract in dose 8,5 mg/kgBW shows 3.113?1.88 nmol/ml which continue to decrease along with increasing dose of SH extract (17 mg/kg BW) showing the value of 2.543?0.79 nmol/ml. This is in accordance with previous research by Suryadinata et al (2017) that antioxidant supplements can reduce MDA levels due to oxidative stress. Revianti et al (2016) also mentioned that SH extract can inhibit oxidative stress by reducing MDA concentration along with increasing dose of SH extract.

CONCLUSION

Supplementation of *Stichopus hermanii* extract can decrease fasting blood glucose and inhibit oxidative stress by decrease free radicals but no significant decrease in plasma insulin level.

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