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

Dita Sukmaya Prawitasari1, Indri Safitri2, Harianto Notopuro2

1Master Candidate, 2Department of Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

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 meningkatkan 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 eksperimental laboratorium menggunakan rancangan post-test only control group design pada tiga puluh lima tikus wistar jantan galur wistar, dibagi menjadi lima kelompok yang terdiri dari normal control group, positive control, negative control dan dua kelompok eksperimental dengan dosis ekstrak SH 8,5 dan 17 mg/kgBB untuk 14 hari secara daily. Fasting blood glucose level diukur dengan glucometer, kadar plasma insulin diukur dengan ELISA dan kadar MDA diukur dengan spektrofotometer. Data analisis dilakukan dengan One Way ANOVA test dan Kruskal Wallis. Hasil penelitian menunjukkan bahwa pemberian ekstrak SH dosis 8,5 dan 17 mg/kgBB dapat menurunkan kadar glukosa darah (Kruskal Wallis, p=0.030) dan MDA (Kruskal Wallis, p=0.042) tetapi tidak pada kadar plasma insulin (ANOVA, p=0.130). Kadar MDA terendah ditemukan 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 diadakan pada tikus model diabetes melitus.

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

Correspondence: Dita Sukmaya Prawitasari, Master Candidate, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

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 (Veerabhadradi, 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.

108
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).

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

| Variables               | Mean ± SD          | P   |
|-------------------------|--------------------|-----|
| FBG level (mmol/L)      | K0 4.93±0.59       |     |
|                         | K1 13.99±10.6      |     |
|                         | K2 7.02±3.55       |     |
|                         | K3 5.26±0.86       |     |
|                         | K4 8.41±3.8        |     |
| Plasma insulin level (mIU/L) | K0 0.41±0.93 |     |
|                         | K1 0.36±0.33       |     |
|                         | K2 0.52±0.15       |     |
|                         | K3 0.46±0.11       |     |
|                         | K4 0.49±0.07       |     |
| MDA level (nmol/ml)     | K0 1.91±1.69       |     |
|                         | K1 4.45±1.51       |     |
|                         | K2 4.61±1.94       |     |
|                         | K3 3.11±1.88       |     |
|                         | K4 2.54±1.79       |     |

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.

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.
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 increased 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 equivalent 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.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to all Department of Biochemistry's staffs at Medical Faculty, Airlangga University for the continuous support of my postgraduate program study and related research in Medical Faculty, Airlangga University.

REFERENCES

Bajaj S, Khan A (2012). Antioxidant and diabetes. Indian Journal of Endocrinology and Metabolism 16, 267-271a
Barky ARE, Ali EMM, Mohamed TM (2017). Marine sea cucumber saponins and diabetes. Austin Pancreatic Disorders 1, 1002
Ceriello A, Testa R (2009). Antioxidant anti-inflammatory treatment in type 2 diabetes. Diabetes Journals care 32, S232-236
Elekofehinti OO (2015). Saponins: Anti-diabetic principles from medicinal plants. A review. Elsevier, Pathophysiology 22, p 95-103
Firdaus, Rimbawan, Marliyati SA, Roosita K (2016). Model tikus diabetes yang diinduksi streptozotocin-sukrosa untuk pendekatan penelitian diabetes melitus gestasional. Jurnal MKMI 12, 29-34
Mu’allimah I (2017). Aktivitas antihiperglikemik sediaan teripang (Stichopus hermanii) dan spirulina (Spirulina planteis) pada tikus putih sprague dawley yang diinduksi streptozotocin. Bogor, Institut Pertanian Bogor
Olokoba AB, Obasteru OA, and Olokoba LB (2012). Type 2 diabetes mellitus: A review of current trends. Oman Medical Journal 27, 269-273
Pangestuti R, Arifin Z (2017). Medicinal and health benefit effects of functional sea cucumbers. Journal of Traditional and Complementary Medicine xxx, 1-11
Purwanto B, Liben P (2014). Model hewan coba untuk penelitian diabetes. Kelompok Kajian animal Model Departemen Faal FKUA. Surabaya, Revka Petra Media
Reviandi S, Soetjipto, Rahayu RP (2016). Protective role of Stichopus hermanii ethanol extract supplementation to oxidative stress and oral hyperkeratosis in smoking exposed rats. International Journal of ChemTech Research 9, 408-417
Suryadinata RV, Wirjatmadi B, Adriani M (2017). Efektivitas penurunan malondialdehyde dengan kombinasi suplemen antioksidan superoxide dismutase melon dan gliadin akibat paparan rokok. Global Medical and Health Communicaiton 5, 79-83
Veerabhadra GGK, Rahman MA, Patil S (2016). A study on antioxidants status in type 2 diabetes mellitus patients. International Journal of Biomedical and Advance Research 7, 278-280