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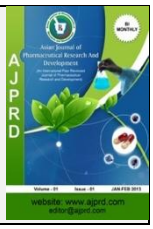

Open Access Research Article

Antihyperglycemic Activity of Sea Cucumber (*Stichopushermanii*) Ethanol Extract Against White Male Rats (*Rattus novergicus*)

Rahmadani[1*], Rosidah[2], Mahdi Jufri[3]

[1]Magister of Pharmacy Study Program, Faculty of Pharmacy, Universitas Sumatera Utara

[2]Department of Pharmaceutical Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara

[3]Department of Pharmaceutical Technology, Faculty of Pharmacy, Universitas Indonesia

ABSTRACT

Objectives: The purpose of this study was to determine antihyperglycemic activity of Sea Cucumber (*Stichopushermanii*) ethanol extract on white male rats, by observing changes in blood glucose levels after being induced by streptozotocin.

Design: The research method used was experimental laboratory, where as test animals were divided into several groups given the test substance. The tests performed on animals were glucose tolerance test and antihyperglycemia test after being induced by streptozotosin.

Interventions: Test animals that have been grouped into each group were given 0.5% Na CMC suspension as a negative control, metformin 65 mg / kg bw as a positive control, extracts of 50, 100, 200, 400 and 800 mg / kg bw as test samples.

Main outcome measure: The results showed good results at a dose of 800 mg / kg bw in the 120th minute on the glucose tolerance test with blood glucose levels of 98.33 ± 7.02, and the antihyperglycemic activity test after the rats were induced by streptozotocin with blood glucose levels of 65.84 ± 2.93 on the 15th day. This value was not significantly different from the positive control (metformin) with a p value≥ 0.05.

Conclusion: Ethanol extract of sea cucumber (*Stichopushermanii*) has antihyperglycemic activity at a dose of 800 mg / kg bw.

Keywords: antihyperglycemic activity, streptozotocin induced, *Stichopushermanii*

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***Address for Correspondence:**

Rahmadani, Magister of Pharmacy Study Program, Faculty of Pharmacy, Universitas Sumatera Utara,Tri Dharma Street No 5, Universitas Sumatera Utara, Medan 20155, Sumatera Utara, Indonesia

INTRODUCTION

The sea cucumbers (*Stichopus hermanii*), including the Stichopodidae family, are one type of sea cucumber that lives in the sea and is one of the edible invertebrates. The distribution of sea cucumbers is not only in the tidal zone but also in the deep sea[1]. The use of sea cucumbers has been empirically used in the community, shows the effectiveness of antidiabetic, has benefits, and one of them is that it has the potential as an alternative to antidiabetic drugs from animal sources. Based on the content contained in *Stichopus hermanii* sea cucumbers, it is hoped that it can be used as a treatment for type 2 diabetes mellitus[2–4].

It is proven in previous studies that sea cucumbers can be used as a supplement to help maintain stable blood glucose levels. Because sea cucumbers, especially the *Stichopus hermanii* species in the wet sample, contain 17.6% α-glucosidase inhibitor at a concentration of 1 µg / mL. Sea cucumbers have the potential to cure diabetes mellitus. Secondary metabolite compounds in sea cucumbers are alkaloid compounds and saponins [5,6]. Alkaloids have the potential to be anti-diabetic by inhibiting the action of the α

glucosidase enzyme. Saponins play a role in increasing tyrosine phosphorylation of the β-subunit insulin receptors, inhibiting tyrosine phosphatase, and stimulating glucose transport activity, such as GLUT 4, as well as saponins which can prevent complications of diabetes [7–9]. Currently, GLUT 4 is often used as a variable in research on the improvement / recovery of type 2 diabetes mellitus[10].

MATERIALS AND METHODS

This research method is an experimental method to see the effect of ethanol extract dose of sea cucumber on blood glucose levels of streptozotosin-induced diabetic rats. The design design used was a completely randomized design then the research data were analyzed using the analysis of variance (ANOVA) method with a 95% confidence level followed by the Tukey HSD test to see the real differences between treatments using the SPSS (Statistical Product and Service Solution) program.

This research consisted of several stages, first collecting sea cucumbers and making ethanol extracts of sea cucumbers, preparing experimental animals, and testing the antidiabetic effect of sea cucumber ethanol extract using the steptozotocin induction method. The research was conducted at the Phytochemical Laboratory, Pharmacology Laboratory of the Faculty of Pharmacy, University of North Sumatra and the Laboratory of Pharmacetics and Pharmacy Formula Development at the University of Indonesia.

Materials and Chemistry

The tools used consist of laboratory glassware, blender, desiccator, drying cabinet, analytical balance, water bath, rotary evaporator, Glucometer and Glucotest strip. The materials used in the study were sea cucumber (*Stichopushermanii*), 96% ethanol, streptozotocin, Na-CMC (sodium carboxy methyl cellulose), and metformin.

Plant Extraction

The sample was collected purposively, that is, without comparing it with other regions. The samples used were fresh sea cucumbers (*Stichopushermanii*)from KepulauanSeribu, Padang beach, Indonesian waters. Sample identification was carried out at the Animal Systematics Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia.

Sea cucumbers are cleaned of dirt and internal organs by washing under running water, then cut to a size of 3 x 3 cm, then drained and weighed. Sea cucumbers are dried in a drying cabinet until they can be broken. This dried sea cucumber is called animal simplicia. The simplicia is pollinated and then weighed and stored in a closed container[11].

The extract was made by maceration using 96% ethanol solvent, carried out by mixing 300 g of simplicia powder with 2.25 liters of 96% ethanol in a vessel and tightly closed. Store in a place protected from light for 5 days, shaking it often. Then filtered, squeezed and the pulp is washed with a liquid filter, kept in a cool place for several days, the liquid is poured out and filtered. Conducted at a temperature of 40–60ºC. Furthermore, the extract was evaporated using a rotary evaporator until a thick extract was obtained, then the extract was dried using a freezedryer[12,13].

Antihyperglycemic Test

The test animals used in this study were white male wistar rats (*Rattus norvegicus* L.) with body weight ranging from the wistar strain aged 2-3 months and weighing 150-300 grams. Before being used for experiments, all animals were acclimatized for 7 days to adjust to their environment. Food and drink during maintenance and experiment were given equally ad libitum. Animals are kept in cages that are well ventilated and clean is always maintained, animal weights are weighed and behavior is observed. The animals that were judged to be healthy, characterized by agile movements of regular weight gain were used in the experiment[14].

Before being given the treatment, the rats' blood glucose levels were measured first, that is, the mice were fasted for 18 hours. Then the body weight was weighed and the fasting blood glucose level was measured by taking blood through the injured tail vein. The blood that comes out is touched on the glucostrip that has been attached to the glucotes. Then the number that appears on the screen is recorded as the initial blood glucose (mg / dl) level.

The glucose tolerance test was carried out using various doses of ethanol extract of sea cucumber 50, 100, 200, 400 and 800 mg / kg bw. Glucose given 30 minutes after giving the test sample orally. Glucose was given to all mice from each group with a glucose concentration of 50% as much as 1% of body weight orally. Then glucose blood level was measured again at 30, 45, 60, 90, and 120 minutes after glucose administration[15,16].

The next test was carried out by inducing diabetes using streptozotocin, by fasting the mice for 18 hours (drinking water was still given), then injected with streptozotocin (55 mg / kg) dissolved in distilled water, then injected intraperitoneal to the mice. The mice used were those whose blood sugar levels reached> 200 mg / dl constant for 3 days of measurement. Furthermore, each group consisting of six animals was given an extract and control sample. The test was carried out for 15 days. After induction, blood was taken on days 3, 6, 9, 12, 15[17,18].

Data Analysis

The data were analyzed by using Paired Samples Test Statistic to see the significant difference between the control group and the treatment group, followed by using one-way analysis of variance (ANOVA) test with a 95% confidence level to determine the average difference between treatments. If there are differences, it is followed by using the Post Hoc Tukey test to determine the differences between treatment groups.

RESULT AND DISCUSSION

The results of glucose tolerance and antihyperglycemic tests from the ethanol extract of *Stichopushermanii* can be seen in Tables 1 and 2.

Table: 1. The Result of Glucose Tolerance

Groups	Normal ± SD (mg/dl)	M0 ± SD (mg/dl)	M30 ± SD (mg/dl)	M60 ± SD (mg/dl)	M90 ± SD (mg/dl)	M120 ± SD (mg/dl)
Negative control (CMC Na)	82.33 ± 3.51	195.33 ± 18.61	210.00 ± 49.93	216.67 ± 62.17	165.67 ± 24.95	129.67 ± 7.64
Positive Control (Metformin)	75.33 ± 5.68	110.00 ± 14.17	122.00 ± 29.13	107.33 ± 10.06	87.33 ± 9.86	79.33 ± 5.03
SHE 50 mg/kg	74.33 ± 4.16	172.33 ± 23.86	188.33 ± 13.05	161.00 ± 9.16	134.67 ± 7.37	99.67 ± 3.51
SHE 100 mg/kg	75.33 ± 6.11	177.00 ± 17.69	189.67 ± 28.43	158.00 ± 18.00	125.33 ± 15.88	98.00 ± 11.14
SHE 200 mg/kg	86.00 ± 13.75	197.00 ± 43.48	208.33 ± 39.68	168.00 ± 31.00	136.00 ± 24.57	111.00 ± 28.48
SHE 400 mg/kg	75.67 ± 11.59	164.00 ± 7.21	173.33 ± 11.50	140.33 ± 11.67	121.00 ± 8.18	93.00 ± 12.12
SHE 800 mg/kg	91.00 ± 11.79	143.67 ± 13.20	151.00 ± 20.22	135.00 ± 21.07	113.33 ± 19.73	98.33 ± 7.02*

Explanations:Normal (blood glucose level before glucose administration), M (time in minute) SHE (*Stichopushermanii* extract),*(not significantly different from positive control $p \geq 0.05$)

Table 2. The Result of Antihyperglycemic Activity of *Stichopushermanii* Ethanol Extract

Groups	Inhibition Percentage of Rats Blood Glucose Level(%) ± SD				
	Day 3	**Day6**	**Day9**	**Day12**	**Day15**
Negative Control (CMC Na)	5.41 ± 1.51	8.18 ± 1.83	9.06 ± 4.04	12.97 ± 4.21	19.44 ± 3.79
Positive Control (Metformin)	15.28 ± 4.25	30.90 ± 2.29	46.51 ± 3.35	61.16 ± 3.29	71.81 ± 4.42
SHE 50 mg/kg	4.62 ± 1.37	10.97 ± 2.32	18.95 ± 2.29	27.57 ± 4.80	35.95 ± 3.46
SHE 100 mg/kg	6.42 ± 1.17	12.59 ± 1.92	22.83 ± 1.32	33.20 ± 0.85	41.88 ± 1.99
SHE 200 mg/kg	8.05 ± 1.85	15.04 ± 2.16	26.02 ± 2.48	35.89 ± 2.05	46.22 ± 0.83
SHE 400 mg/kg	10.96 ± 1.76	20.28 ± 1.14	35.27 ± 4.69	44.79 ± 6.31	55.60 ± 3.64
SHE 800 mg/kg	13.12 ± 3.92	24.99 ± 1.66	39.55 ± 1.71	54.03 ± 2.04	65.85 ± 2.94*

Explanations :SHE (*Stichopushermanii* extract),*(not significantly different from positive control $p \geq 0.05$).

Based on table data, it shows that the provision of test samples (day 3, day 6, day 9, day 12, and day 15) of CMC Na, metformin, extract according to each dose has increased but in the metformin group experienced a more rapid increase in effect. In the EET group 800 mg / kg bw on day 14 it can be seen from the tuckey data, this group did not differ significantly from the metformin group in increasing blood glucose levels. Meanwhile, the extract groups of 50 mg / kg bw, 100 mg / kg, 200 mg / kg, and 400 mg / kg of body weight differed significantly from the metformin group.

The decrease in glucose levels is caused by the presence of bioactive compounds that can prevent the oxidation of pancreatic beta cells due to chemical induction so that damage can be minimized. The bioactive compounds contained in the extract include alkaloids[2,19]. Alkaloids work by stimulating the hypothalamus and reducing gluconeogenesis so that blood glucose levels and insulin requirements decrease. Alkaloids are also proven to have the ability to regenerate damaged pancreatic β cells, as well as saponins which can prevent complications of diabetes. Currently, GLUT 4 is often used as a variable in research on the improvement / recovery of type 2 diabetes mellitus[10,20–22].

CONCLUSSION

The ethanol extract of *Stichopushermanii* has antihyperglycemic activity and is able to reduce blood glucose levels from glucose tolerance and streptozotocin induction methods. Activity to reduce glucose levels was seen at the 120th minute after glucose consumption and the 15th day after streptozotocin induction at a dose of 800 mg / kg BW.

CONFLICT OF INTEREST

All author have no to declare.

REFERENCES

1. Janakarim NB, Mohamed A, Rao C V. Sea Cucumbers Metabolites as Potent Anti-Cancer Agents. Mar Drugs. 2015;13(5):2909–23.
2. Prawitasari DS, Safitri I, Notopuro H. Effects of Golden Sea Cucumber Extract (Stichopus hermanii) on Fasting Blood Glucose, Plasma Insulin, and MDA Level of Male rats (Rattus novergicus) Induced with Streptozotocin. Fol Med Indones. 2019;55(2):107–11.
3. Windari HAS, Tarman K, Safithri M, Setyaningsih I. Antioxidant Activity of Spirulina platensis and Sea Cucumber Stichopus hermanii in Streptozotocin-Induced Diabetic Rats. Trop Life Sci Res. 2019;30(2):119–29.
4. Nayareh P, Hasan MK. Effect of Chromium Supplementation on Blood Glucose, Hemoglobin A1C, Lipid Profile and Lipid Peroxidation in Type 2 Diabetic Patients. Iran J Diabetes Obes. 2012;4(4):178–84.
5. Slater MJ, Lassudrie M, Jeffs AG. Method for Determining Apparent Digestibility of Carbohydrate and Protein Sources for Artificial Diets for Juvenile Sea Cucumber, Australostichopus mollis. J World Aquac Soc. 2011;42(5):714–25.
6. Kew PL, Don MM. Jewel of the Seabed: Sea Cucumbers as Nutritional and Drug Candidates. Int J Food Sci Nutr.

2012;63(5):616–36.

7. Xu C, Zhang R, Wen Z. Bioactive Compounds and Biological Functions of Sea Cucumbers as Potential Functional Foods. J Funct Food. 2018;49:73–84.
8. Khotimchenko Y. Pharmacological Potential of Sea Cucumbers. Int J Mol Sci. 2018;19(5):1342.
9. Meliani N, El Amine Dib M, Allali H, Tabti B. Hypoglycaemic Effect of Berberis vulgaris L. in Normal and Streptozotocin-Induced Diabetic Rats. Asian Pac J Trop Biomed. 2011;1(6):468–71.
10. Elekofehinti OO, Kamdem JP, Kade IJ, Adanlawo IG, Rocha JBT. Saponnins from Solanum anguivi Lam. Fruit Exhibit In Vitro and In Vivo Antioxidant Activities in Alloxan-Induced Oxidative Stress. Asian J Pharm Clin Res. 2013;6(2):249–54.
11. Husni A, Ismed F, Afriyandi D. Standardization Study of Simplicia and Extract of Calamondin (Citrus microcarpa Bunge) Peel, Quantification of Hesperidin and Antibacterial Assay. Pharmacogn J. 2020;12(4):777–83.
12. Yulianingtyas A, Kusmartono B. Optimization of Solvent Volume and Maceration Time on Extraction of Flavonoids from Averrhoa bilimbi Leaves. J Tek Kim. 2016;10(2):58–64.
13. Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants. 2015;4(196):412–2167.
14. Capdevilla S, Giral M, Ruiz de la Torre L. Acclimatization of Rats after Ground Transportation to a New Animal Facility. Lab Anim. 2007;41:255–61.
15. Langley SC, Browne RF, Jackson AA. Altered Glucose Tolerance in Rats Exposed to Maternal Low Protein Diets in Utero. Comp Biochem Physiol Part A Physiol. 1994;109(2):223–9.
16. Islam A, Akhtar A, Khan RI, Hossain S, Alam K, Ibne Wahid I, et al. Oral Glucose Tolerance Test (OGTT) in Normal Control and Glucose Induced Hyperglycemic Rats with Coccinia cordifolia L. and Catharanthus roseus L. Pak J Pharm Sci. 2009;22(4):402–4.
17. Muruganadan S, Srinivasan K, Gupta S, Gupta PK, Lal J. Effect of Mangiferin on Hyperglycemia and Atherogenicity in Streptozotocin Diabetic Rats. J Ethnopharmacol. 2005;97(3):497–501.
18. Rasch R. Prevention of Diabetic Glomerulopathy in Streptozotocin Diabetic Rats by Insulin Treatment. Diabetologia. 1979;16:319–24.
19. Pringgenies D, Rudiyanti S, Yudiati E. Exploration of Sea Cucumbers Stichopus hermanii from Karimunjawa Islands as Production of Marine Biological Resources. In: IOP Conference Series: Earth and Environmental Science. 2018. p. 1–8.
20. Arulselvan P, Subramanian SP. Beneficial Effects of Murraya koenigii Leaves on Antioxidant Defense System and Ultra Structural Changes of Pancreatic β-cells in Experimental Diabetes in Rats. Chem Biol Interact. 2007;165(2):155–64.
21. Tiong SH, Looi CY, Hazni H, Arya A, Paydar M, Wong WF, et al. Antidiabetic and Antioxidant Properties of Alkaloids from Catharanthus roseus (L.) G. Don. Molecules. 2013;18(8):9770–84.
22. Elekofehinti OO. Saponins: Anti-Diabetic Principles from Medicinal Plants – A Review. Pathophysiology. 2015;22(2):95–103.