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# Test of the Effectiveness of Bilalo Sea Cucumber (Actinopyga Mauritiana) in Pancreatic Beta Cell Repair in Aloxan-Induced Mice (Mus Musculus)

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## ABSTRACT

Introduction diabetes mellitus (DM) is a degenerative disease that occurs due to abnormal carbohydrate metabolism. DM conditions occur when the pancreas is unable to produce insulin or the sensitivity of cell receptors to insulin is very low. The increase in DM occurs when the pancreatic beta cells are unable to produce insulin or the sensitivity of cell receptors to insulin is very low. Damage to pancreatic beta cells can be caused by diabetogenic substances such as alloxan. Teripang bilalo (Actinopyga mauritiana) is a marine animal that has been used as food and medicine. Contains many secondary metabolites, namely flavonoids, alkaloids, glycosides, steroids, and saponins. active compound flavonoids and saponins., research objectives to know the effectiveness of oral administration of teripang billion in reducing blood sugar levels and the ability to repair tissue in pancreatic beta cells seen from hispatology, materials, and methods is an experimental study with 25 mice as subjects. Oral administration of sea teripang bilalo ethanol extract at doses of 100, 200, and 400 mg/kg, positive control by oral administration of glibenclamide, and negative control by administration of Na-CMC then a histopathological examination of the pancreas was performed, research results in the administration of teripang bilalo ethanol extract 400 mg / kg proved to be able to reduce blood sugar levels to 179.4 mg / dl and proved to have an effect on the improvement of pancreatic histology, which is a picture of damage to peripheral islands of langerhans by 1, damage to islands of langerhans ≤50% by 2.4 and damage, conclusions administration of teripang bilalo ethanol extract is proven to reduce blood sugar levels and have an effect on improving pancreatic histology.

Keywords: Diabetes mellitus (DM); teripang bilalo (Actinopyga mauritiana); blood sugar levels; pancreatic histology

## ABSTRAK

diabetes mellitus (DM) adalah penyakit degeneratif yang terjadi karena metabolisme karbohidrat yang abnormal. Kondisi DM terjadi ketika pankreas tidak mampu memproduksi insulin atau sensitivitas reseptor sel terhadap insulin sangat rendah. Peningkatan DM terjadi ketika sel beta pankreas tidak dapat memproduksi insulin atau sensitivitas reseptor sel terhadap insulin sangat rendah. Kerusakan pada sel beta pankreas dapat disebabkan oleh zat diabetogenik seperti alloxan. Teripang bilalo (Actinopyga mauritiana) adalah hewan laut yang telah digunakan sebagai makanan dan obat. Mengandung banyak metabolit sekunder, yaitu flavonoid, alkaloid, glikosida, steroid, dan saponin. senyawa aktif flavonoid dan saponin., tujuan penelitian untuk mengetahui efektivitas pemberian oral teripang bilalo dalam menurunkan kadar gula darah dan kemampuan untuk memperbaiki jaringan di sel beta pankreas yang dilihat dari hispatologi, bahan, dan metode adalah penelitian eksperimental dengan 25 tikus sebagai subjek. Pemberian oral ekstrak etanol teripang laut bilalo dengan dosis 100, 200, dan 400 mg/kg, kontrol positif dengan pemberian oral glibenclamide, dan kontrol negatif dengan pemberian Na-CMC kemudian dilakukan pemeriksaan histopatologi pankreas, hasil penelitian dalam pemberian ekstrak etanol teripang bilalo 400 mg/kg terbukti mampu menurunkan kadar gula darah menjadi 179.4 mg/dl dan terbukti memiliki efek pada perbaikan histologi pankreas, yaitu gambaran kerusakan pulau-pulau perifer langerhans sebanyak 1, kerusakan pulau-pulau langerhans ≤50% sebanyak 2.4 dan kerusakan, kesimpulan pemberian ekstrak etanol teripang bilalo terbukti menurunkan kadar gula darah dan memiliki efek pada perbaikan histologi pankreas.

Kata kunci: Diabetes mellitus (DM); teripang bilalo (Actinopyga mauritiana); kadar gula darah; histologi pankreas

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## INTRODUCTION

Diabetes mellitus (DM) is a degenerative disease that occurs due to abnormalities in carbohydrate metabolism. This condition occurs when the pancreas is unable to produce insulin or because the sensitivity of cell receptors to insulin is very low[1]. According to the WHO (*World Health Organization*), DM is one of the four major chronic diseases, in addition to cardiovascular disease, cancer, and chronic respiratory disease[2].

According to IDF (*International Diabetes Federation*) estimates in 2013, 382 million people are living with DM in the world. By 2035 that number is expected to rise to 592 million people. Of the 382 million people an estimated 175 million are undiagnosed, thus threatening to progressively develop into complications unnoticed and without prevention[3]. Based on the cause, DM is divided into type 1 DM, type 2 DM, Gestational diabetes, and other types of diabetes. Type 1 diabetes is caused by very little or no insulin production in the pancreas. Type 2 DM is the most common type, this condition is caused because the body does not effectively use insulin. Gestational diabetes is a condition of high blood sugar levels that occur in pregnant women. While other types of DM occur due to other diseases or due to severe infections[4].

In general, DM has symptoms such as rapid thirst (polydipsia), rapid hunger (polyphagia), lots of urination (polyuria), blurred vision, long wound recovery, easy to feel tired, and easily sleepy. DM can also cause life-threatening complications such as heart disease, stroke, hypertension, cholesterol, kidney failure, and diabetic neuropathy (nerve damage caused by diabetes). With the side effects and long-term effects of the use of oral antidiabetic drugs, WHO recommends the use of traditional medicine in the prevention and treatment of DM[5].

Traditional medicine using plants and animals has become a culture in Indonesian society. Sea cucumber is one of the remedies that has been used for hundreds of years in China and is believed to be able to cure various types of diseases. In addition to having a high nutritional content, sea cucumber also contains high bioactive ingredients and is very useful for various healing such as kidney disease, anemia, anti-cancer, anti-inflammatory, anti-thrombotic, anti-bacterial, prevention of aging of body tissues, and preventing arteriosclerosis and DM[6]. Sea cucumber has a high content or nutritional content. The content substances and nutrients of sea cucumber consist of protein, carbohydrates, fat, collagen, *fatty acids Eicosapentaenoic* acid (EPA) *and Docosahexaenoic acid* (DHA), chromium, lectins, minerals, and vitamins. From the results of the study, the nutritional content of sea cucumbers in dry conditions consists of 82% protein, 1.7% fat, and 4.8% carbohydrates[7].

The bioactive ingredients in sea cucumber are also known as antioxidants that help reduce damage to cells and body tissues. Sea cucumber extract can also play a role in fighting disorders caused by free radicals, both from metabolic products and external factors such as pollution and radiation[8]. The content of flavonoid compounds from sea cucumbers has antioxidant effects[9]. The antibacterial and antifungal content of sea cucumber can increase its ability for skin care purposes. Sea cucumber is also known to have antinociceptive and anti-inflammatory effects[6]. Based on the above background, researchers are interested in conducting research testing the effectiveness of bilalo sea cucumber in pancreatic cell repair in alloxan-induced mice. The purpose of this study was to determine the effectiveness of oral administration of bilalo sea cucumber in lowering blood sugar levels and the ability to repair tissue in pancreatic beta cells seen from it's his pathology.

## RESEARCH METHOD

This research is an experimental study using samples of bilalo sea cucumber ethanol extract (*Actinopyga mauritiana*) induced in mice tested with the ANOVA method and continued with *Duncan's Post Hoc test*. The research was conducted in March-April 2019 at the Pharmacology Laboratory and Pharmaceutics Laboratory, Faculty of Pharmacy, Tjut Nyak Dhien University, Medan. This research includes the preparation of test materials, identification/determination of test materials, making extracts from *bilalo sea cucumber (Actinopyga mauritiana) by maceration using PA ethanol solvent, making oral preparations in various concentrations of bilalo sea cucumber ethanol extract (*Actinopyga mauritiana*), and evaluation including histological tests.*

## RESULTS AND DISCUSSION

Data on phytochemical screening results of bilalo sea cucumber ethanol extract can be seen in Table 1.

**Table 1. Malaria Distribution in Sulawesi Island**

| No. | Golongan Senyawa | Examination Results |
|---|---|---|
| 1. | Alkaloid | + |
| 2. | Flavonoid | + |
| 3. | Saponin | + |
| 4. | Steroid | + |
| 5. | Glikosida | + |

Information:

(+) : Contains compounds

(-) : No contains compounds

Based on the table data above shows that phytochemical screening results for sea cucumber ethanol extract (EET) bilalo gave positive results (+) containing alkaloid compounds, flavonoids, saponins, steroids, and glycosides. The Effect of Bilalo Sea Cucumber Ethanol Extract (*Actinopyga mauritiana*) and Glibenclamide on Reducing Blood Glucose Levels.

**Table 2. Results of measuring blood glucose levels of test animals**

| Group | Blood sugar levels | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | Hari | | | | |
| | **Before** | **After** | **3** | **6** | **9** | **12** | **15** |
| Na-CMC | 80,2a | 425,6a | 414.2E | 407.6E | 397.8e | 393.4e | 384.2e |
| Glibenklamid | 81a | 412a | 212,4a | 193a | 183,8a | 166a | 146,8a |
| EAT 100 mg/kgBB | 79a | 419,6a | 396,8d | 380,4d | 370,6d | 351d | 331,4d |
| EAT 200 mg/kgBB | 76,8a | 409,4a | 303c | 275,8c | 260c | 240,6c | 215,4c |
| EAT 400 mg/kgBB | 80,6a | 408a | 233b | 218,2b | 205,2b | 192,6b | 179,4b |

**Description:** The superscript of different letters showed a marked difference in each control group and treatment group (p,0,05)$^{abcde}$

The table above shows the blood sugar levels of all treatment groups in mice before being induced with alloxane had blood sugar levels of 76-80 mg / dL. When mice have been induced with alloxane, there is an increase in blood sugar levels in each group. Increase in blood sugar levels from 408-425 mg / dL. Blood sugar levels of the Na-CMC group on the third day were 414 mg / dL and on the 15th day blood sugar levels were 384 mg / dL. There was a slight decrease in blood sugar levels in the Na-CMC group. So Na-CMC is proven to be unable to lower blood sugar levels. The blood sugar level of the glibenclamide group on the third day was 212 mg / dL and decreased blood sugar levels by 146 mg / dL on the 15th day. The decrease in blood sugar levels in the glibenclamide group reached normal blood sugar levels. Sea cucumber ethanol extracts 100 mg/kgBB on the third day blood sugar levels of 396 mg/dL and experienced a slight decrease on day 15 of 331 mg/dL showed a decrease in blood sugar levels was not significant. On the third day of EET blood sugar levels of 200 mg/kg body weight were 303 mg / dL and experienced a considerable decrease in blood sugar levels on the 15th day of 215 mg / dL but did not reach normal blood sugar levels. The EET group was 400 mg/kgBB on the third day 233 mg/dL and had a decrease in blood sugar levels on day 15 179 mg/dL. The decrease in high blood sugar levels at EET 400 mg/kg BB is almost close to glibenclamide, hereby it is stated that sea cucumber ethanol extract at a concentration of 400 mg/kg BB can reduce blood sugar levels.

**Histopathologists of the Pancreas**

During the 2-week treatment, mice were dissected then the pancreas was taken to be made into preparations which were first fixed with 10% formalin and stained with the Hematoxylin Eosin (HE) method which is a type of double staining because it uses two types of dyes to observe the general structure of tissue that has acid and alkaline properties so that tissue parts look more clearly. Conducted in the terrain veterinary hall. Histopathological images of the pancreas from the results of microscopic examination and readings with a magnification of 40 times.

**Table 3 Results of calculation of damage to the pancreas of mice**

| Treatment | Damage to the peripherals | Langerhans Island damage ≤ 50% | Langerhans Island damage ≥ 50% |
|---|---|---|---|
| Na-CMC | 3,8c | 9,2c | 10,2c |
| Glibenklamid | 0,6a | 1,2a | 1,2a |
| EAT 100 mg/kg BB | 3,2c | 8c | 6,6b |
| EET 200 mg/kg BB | 2,6b | 6,8b | 5,4b |
| EAT 400 mg/kg BB | 1a | 2,4a | 1,8a |

**Remarks:** *Superscript* of different letters showed a marked difference in each treatment group (p,0,05)$^{ab}$

The results of histopathological examination and statistical analysis of Duncan's test in the study of sea cucumber ethanol extract had a significant effect on the repair rate of Langerhans island damage in mice and showed a marked difference in the negative control group and treatment group. Peripheral damage in the Na-CMC group obtained a value of 3.8 and did not have a noticeable difference with the sea cucumber ethanol extract group 100 mg/kg BB obtained a value of 3.2 while the glycblamide test group, sea cucumber ethanol extract 200 mg/kg BB, and 400 mg/kg BB there was a real difference. Similarly, in Langerhans Island damage ≤50%, there was no real difference between the Na-CMC group

and the sea cucumber ethanol extract group of 100 mg/kg BB. Both groups have high damage rates. Langerhans island damage ≥50% in the Na-CMC group with a value of 10.2 was significantly different from the sea cucumber ethanol extract test group 100 mg/kg BB and sea cucumber ethanol extract group 200 mg/kg BB. While in the glibenclamide group and sea cucumber ethanol extract 400 mg/kg BB did not have a noticeable difference.

**Table 4 Results of day 15 blood glucose measurements of test animals and damage to the pancreas of mice**

| Treatment | Blood sugar levels (mg/dl) day 15 | Damage to the peripherals | Langerhans Island damage ≤ 50% | Langerhans Island damage ≥ 50% |
|---|---|---|---|---|
| Na-CMC | 384.2e | 3,8c | 9,2c | 10,2c |
| Glibenklamid | 146,8a | 0,6a | 1,2a | 1,2a |
| EAT 100 mg/kg BB | 331,4d | 3,2c | 8c | 6,6b |
| EET 200 mg/kg BB | 215,4c | 2,6b | 6,8b | 5,4b |
| EAT 400 mg/kg/BB | 179,4b | 1a | 2,4a | 1,8a |

Table 4 can be seen in the negative treatment with Na-CMC administration obtained KGD day 15 of 384.2 mg/dl with a description of damage to the peripheral island of Langerhans, damage to the island of Langerhans ≤ 50% and damage to the island of Langerhans ≥ 50% respectively 3.8, 9.2 and 10.2. This treatment showed high blood sugar levels accompanied by a picture of damage to the island of Langerhans that was heaviest. In treatment with glibenclamide, administration obtained KGD day 15 amounted to 146.8 mg/dl with a picture of damage to the peripheral island of Langerhans, damage to the island of Langerhans ≤ 50%, and damage to the island of Langerhans ≥ 50% respectively 0.6, 1.2 and 1.2. This treatment shows normal blood sugar levels accompanied by improved features in the peripheral and pancreatic islets of Langerhans. In treatment with EET administration of 100 mg/kg, bb obtained KGD day 15 amounted to 331.4 mg/dl with a description of damage to the peripheral island of Langerhans, damage to the island of Langerhans ≤ 50% and damage to the island of Langerhans ≥ 50% respectively of 3.2, 8 and 6.6. This treatment shows high blood sugar levels accompanied by a picture of damage and there has been no improvement in the peripheral and pancreatic islets of Langerhans. In treatment with EET 200 mmg / kgbb obtained KDG day 15 of 215.4 mg/dl with a description of damage to the peripheral island of Langerhans, damage to the island of Langerhans ≤ 50%, and damage to the island of Langerhans ≥ 50% respectively of 2.6, 6.8 and 5.4. In this treatment, blood sugar levels have decreased but are not yet normal accompanied by damage to the peripheral parts of Langerhans island so the improvement is not perfect. While in the treatment with EET 400 mmg / kgbb obtained KDG day 15 of 179.4 mg/dl with an overview of damage to the periphery of Langerhans island of 1, damage to Langerhans island ≤ 50% of 2.4 and damage to Langerhans island ≥ 50% of 1.8. This treatment shows a normal KGD accompanied by repairing damage to the peripheral parts of Langerhans Island so that Langerhans Island is visible even though there is still damage.

**Hasil Skrining Fitokimia**

Based on the table data above shows that phytochemical screening results for sea cucumber ethanol extract (EET) bilalo gave positive results (+) containing alkaloid compounds, flavonoids, saponins, steroids, and glycosides. These secondary metabolite compounds have benefits in treatment, namely: Alkaloids in the field of health alkaloids have an effect as a trigger for the nervous system, raise blood pressure, reduce pain, antimicrobials, sedatives, and heart disease drugs[10]. Flavonoids are antibacterial, anti-inflammatory, treatment of diabetes mellitus, allergic diseases, cancer, infections caused by viruses, peptic ulcers, cardiovascular, and osteoporosis11. Saponins are active compounds that inhibit glucose absorption and prevent glucose from rising in the blood, so they can be used to treat diabetes[12]. Corticosteroids are known as contraceptive drugs and can prevent inflammation and rheumatism[11]. Glycosides have an effect as a heart drug, diuretic, and expectorant[10].

**The Effect of Bilalo Sea Cucumber Ethanol Extract (Actinopyga mauritiana) and Glibenclamide on Reducing Blood Glucose Levels**

Based on the results of the study, there were glibenclamide group blood sugar levels on the third day of 212 mg / dL and decreased blood sugar levels of 146 mg / dL on the 15th day. The decrease in blood sugar levels in the glibenclamide group reached normal blood sugar levels. It is known that glibenclamide is an oral antidiabetic drug class of sulfonylureas that works actively to reduce blood sugar levels and is most often used. The use of antidiabetic drugs can stimulate insulin secretion from the pancreas, but glibenclamide has side effects such as allergies, nausea, vomiting, diarrhea, and disorders of the central nervous system and eyes. In addition, long-term consumption of glibenclamide has the potential to cause hypoglycemia, which is a condition where blood glucose levels are below normal.

According to the above results, each test group showed a decrease in blood glucose levels. The Na-CMC group showed the highest blood sugar levels compared to the other groups. This is because Na-CMC does not have active substances to lower blood sugar levels. The decrease in blood sugar levels of the sea cucumber ethanol extract group

400 mg/kg BB is not much different from the glibenclamide group, because glibenclamide is indeed a chemical drug that can lower blood sugar levels. EET 400 mg/kgBB can lower blood sugar levels close to glibenclamide because it contains flavonoids and saponins. Flavonoids work as antioxidants to suppress the formation of ROS (*Reactive Oxygen Species*) by inhibiting enzymes in the formation of ROS and increasing regulation and protection from antioxidants. Flavonoids are alpha-glucosidases that function to delay the absorption of carbohydrates so that blood glucose levels will decrease. Saponins can inhibit glucose absorption from the intestine, stimulate insulin production renew beta cells, and increase glucose intake into body tissues.

**Histopathologists of the Pancreas**

The pancreas is an important glandular organ in the body. Beta cells produce the hormone insulin and play a role in lowering blood glucose levels. Histopathological changes in the islets of Langerhans can occur quantitatively, such as reduction in number or size, or qualitatively, such as necrosis (cell death), atrophy (cell reduction), and fibrinosis (damaged cell tissues). Cells damaged by chemicals can cause inflammation. Aloxane administration can cause quantitative damage to pancreatic β cells. Aloxane can generate *reactive oxygen species* (ROS) through a reaction cycle whose reduction results are in the form of diluted acid. This slurbed acid will undergo a redox cycle and the result of this reaction is hydroxyl radicals. It is these hydroxyl radicals that cause damage to pancreatic β cells. Based on research on the negative group (A), namely the alloxan-induced group and given Na-CMC, was the test group that suffered the most damage so that the islets of Langerhans were not visible and in the positive group (B) the test group given glibenclamide, it was seen in the picture that the test group improved, it was seen that the cells in the islets of Langerhans were compact and solidified.

Giving sea cucumber ethanol extract dose of 400 mg/kg BB can repair damage to pancreatic structure. The improvement of pancreatic structure is thought to be due to the presence of antioxidants in bilalo sea cucumber (*Actinopyga Mauritiana*). Flavonoid compounds which are a group of polyphenols can slow down the rate of autooxidation. The ability to regenerate pancreatic beta cells in hyperglycemic mice depends on the regulation of normal blood glucose levels This shows that the administration of sea cucumber ethanol extract affects improving pancreatic structure after alloxane exposure. From these results, it can be seen that EET 400 mg/kg bb provides the highest reduction in blood sugar levels and is almost close to the value of blood sugar levels in treatment with glibenclamide administration. This was followed by significant improvement of Langerhans island damage both peripherally and to Langerhans island which was close to the value in treatment with glibenclamide administration.

## CONCLUSIONS AND SUGGESTIONS

Based on the results of the research conducted, it can be concluded that the results of research that have been carried out that the administration of sea cucumber ethanol extract 400 mg / kgbb is proven to reduce blood sugar levels in mice that have been induced alloxane to 179.4 mg/dl on day 15. Giving sea cucumber ethanol extract 400 mg/kg bb was proven to affect the improvement of pancreatic histology, namely improvement in peripheral, ≤ 50% Langerhans Island and 50% ≥ Langerhans Island damage of 1, 2.4, and 1.8 respectively. It is recommended for future researchers to isolate the active compound from bilalo sea cucumber (Actinopyga mauritiana) which is efficacious as an antidiabetic.

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