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Research Article

Dayak Onion Tuber (Eleutherine Bulbosa (Mill) (Urb) Extract Ethanol Test on The Histopatological Description of Pancreas Wistar Rat Inducted Streptozotocin

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Abstract.

This study aimed to determine the effect of Dayak Union tuber (Eleutherine bulbosa (Mill) Urb) ethanol extract on the regeneration of pancreatic β cells of Wistar rats induced streptozotocin. This research is laboratory experimental study by using 30 rats, they are divided into 5 groups, each group consisting of 6 rats, they are group 1 (normal control), group 2 (sick control) was given Na CMC 1 % b/v, Group 3 (positive control) was given glibenclamide at a dose of 0.25 mg / KgBW, Group 4,5 and 6 were given (*Eleutherine bulbosa* (Mill) Urb) extract at a dose of 125 mg / KgBB, 250 mg / KgBB and 500 mg / kgBW orally for 15 consecutive days. The histopathological damage level of the pancreas was observed with HE staining using a 400x magnification Olympus Cx-21 microscope. The result of the study showed that there were secondary metabolites of alkaloids, flavonoids, saponins, steroids, and tannins in the ethanol extract of Eleutherine palmifolia (Mill) Urb); The histopathological description showed that the damage to the structure of Langerhans islands and only a dose of 500 mg / KgBB the ethanol extract of Eleutherine palmifolia (Mill) Urb) showed improvement.

Keywords: Dayak Onion Tuber; histopathological; extract ethanol

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1. Introduction

Diabetes mellitus is a metabolic disease with various etiologist, characterized by chronic hyperglycaemia with metabolic disorders of carbohydrates, fats, proteins as a result of insulin function disorders (insulin resistance), decreased pancreatic function and both [1]. Diabetes Mellitus is divided into 2 types, namely type 1 and type 2 diabetes. Type 1 diabetes insulin-dependent diabetes mellitus (IDDM) is characterized by the body's immune system destroying pancreatic β cells so that β cells are unable to produce the hormone insulin which functions to reduce blood glucose levels. Type

2 diabetes non insulin dependent diabetes mellitus (NIDDM) begins with insulin resistance, which is a decrease in the sensitivity of insulin receptors in the liver, muscle tissue and adipose tissue so that the insulin hormone is not used properly. Because of the increased need for insulin, the pancreas tries to produce more insulin [2].

Diabetes mellitus can cause excessive production of free radicals known as Reactive Oxygen Species (ROS). ROS triggers oxidative stress because there are more free radicals in the body than antioxidants. Free radicals have the ability to diffuse into the lipid membrane to produce malondialdehyde (MDA). MDA is one of the end products of cell membrane lipid peroxidase by excess free radicals so that MDA is used as an index for measuring free radical activity in the body. In addition to lipid, ROS oxidation target is DNA. In DNA oxidation, guanine nucleotides are prone to ROS oxidation reactions. The oxidation of guanine in the DNA strands causes DNA to lose guanine nucleotides and is damaged so that it inhibits cell division processes in spermatogenesis and mitochondria and interferes with respiration which can disrupt cell energy [3].The pancreas produces two glands, namely endocrine and exocrine glands. The exocrine part of the pancreas produces digestive enzymes together with alkaline fluids both are excreted into the small intestine through the exocrine ducts, excretion is carried out in response to a small intestinal hormone called secretin. The endocrine part of the pancreas is made up of millions of cells that are forming a separate group known as Langerhans Island. Langerhans islands vary in shape and size, located between the cells of the exocrine part of the pancreas [4].

One of the plants that is widely used and has medicinal properties for diabetes is the Dayak bulb (EleutherineBulbosa (Mill) Urb). Studies on the phytochemical content of Dayak onion tubers show that the active compounds of alkaloids, flavonoids, glycosides, and saponins have hypoglycemic activity or lower blood glucose levels which are very useful for the treatment of diabetes mellitus. The flavonoids contained in Dayak red onions are able to regenerate pancreatic β cells on the island. Langerhans [5].Based on this, the researchers were interested in conducting further research on the effect of Dayak bulb extract (EleutherineBulbosa (Mill) Urb) on repairing damage to the pancreas by looking at the histopathological picture of the pancreas of wistar rats.The purpose of this study was to see the histopathological picture of the pancreas of the streptozotocin-induced diabetic rats after administering Dayak bulb extract (EleutherineBulbosa (Mill) Urb).

2. Method

2.1. Tools and Materials

The tools used in this study are maceration equipment, rotary evaporator, glassware, blender, electric balance, simplicia drying cabinet, porcelain cup, desiccator, separating funnel, furnace, a set of moisture determination tools, a scale. animals, syringes, oral swabs, animal restrainers, rat cages, glucometer, strip glucotes, and Rat Insulin ELISA Kit (Chem Cruz).The materials used in this study were Dayak onions (*Eleutherine Bulbosa* (Mill) (Urb); ethanol 96%, glibenclamide tablets (indopharma); CMC Na 0.5%; glucose 50%; streptozotocin; (Chem Cruz); concentrated sulfuric acid; Mayer and Dragendroff's reagents, chloroform, Ammonium hydroxide; anhydrous acetic acid; FeCl3; sodium hydroxide; concentrated hydrochloric acid; methanol; formalin; aquadest.

2.2. Making Ethanol Extract of Dayak Bulbs (Eleutherine Bulbosa (Mill) Urb)

The manufacture of Dayak bulb simplicia is obtained by washing and drying it in a drying cupboard, then mashing and sieving until dry powder is obtained. 2 kg of dry powder Dayak bulb is macerated with 96% ethanol for 2 days, during soaking it is stirred several times, then the simplicia is filtered by using filter paper. The simplicia is soaked 2 times, until a clear filtrate is obtained. Then the filtrate obtained is separated with a rotary evaporator to obtain a thick extract (Maksum, 2008). The thick extract that has been in the rotary evaporator is placed in a beaker glass and covered with aluminum foil and then stored in the freezer to prevent damage to the extract. The solvent used is carboxyl metal cellulose (CMC) with a concentration of 0.5% to produce the desired extract (Maksum, 2008)

2.3. Preparation of Streptozotocin Solution

The rats were conditioned to be diabetic by inducing it with Sreptozotocin (dissolved with 0.9% NaCl, 60 mg / kg) intraperitoneally.

2.4. Test Animals

The test animals used were 30 rats divided into 5 groups, each group consisting of 6 rats with group details, namely group 1 (normal control, group 2 (sick control) given 1% w /

v Na CMC suspension, group 3 (control positive) were given glibenclamide dose of 0.25 mg / KgBB, groups 4.5 and 6 were each given Dayak bulb extract (Eleutherinebulbosa (Mill) Urb) at a dose of 125 mg / KgBW, 250 mg / KgBB and 500 mg / kgBW per oral for 15 consecutive days.

2.5. Pancreatic Histology Test

The test animal was killed by means of neck dislocation where previously anesthetic was used using ether. The dead animal was placed on a fixation board with the stomach pointing upwards. The cuts were made on the skin of the stomach in a cross section until the internal organs of the mice were seen. Then the mouse pancreas was taken, then rinsed with aqueous solution and then stored in a special container containing 10% formalin. After that the sample was taken to the medical pathology anatomy laboratory, University of North Sumatra and then analyzed the histological picture of the pancreatic tissue (Tandi J, 2017).

2.6. Data analysis

The histopathological picture of pancreatic wistar rats observed in this study is the general morphological changes of Langerhans islands, namely: changes in the shape and structure of Langerhans islands and changes in cells (shape and size).Observation data were analyzed statistically using the one way ANOVA test with the Dunnet advanced test * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Histopathological preparations were observed under a microscope at 400x magnification and microscopic changes were noted (histopathological preparations were observed).

3. Result

3.1. Phytochemical Screening

Phytochemical screening for simplicia and ethanol extract showed that all secondary metabolites were found in plants. Compounds that are thought to have antidiabetic activity are flavonoids. The results of this study were also found by Niluh et al. (2016), namely alkaloids, glycosides, flavonoids, phenolics, saponins, triterpenoids, tannins, steroids and quinones [6]. The combination of the antioxidant capacity and the inhibitory ability of the alpha glucosidase enzyme found in Dayak onion bulbs shows that Dayak

bulbs have the potential as an antidiabetic which is beneficial in the prevention and protection against diabetes mellitus.

Table 1: Phytochemical Screening of Simplicia Powder and Dayak Bulbs Extract.

Compound	Reactor	Reagent Results	Results	
			Simplicia	Extract
Alkaloid	Meyer, Bouchardat, Dragendrof	-White or yellow clumping deposits are formed - Forms of brown to blackish deposits -A yellow-orange precipitate is formed	+	+
Flavonoid	Mg dan HCl 0,5M	Appears pink or purple	+	+
Saponin	HCl 2N	Forms foam for not less than 10 minutes	+	-
Steroid/ Triterpenoid	Liebermann Bouchardat	The appearance of purple and red then turns green	+	+
Tanin	$FeCl_3$10%	The appearance of a green or blue color	+	+

Information :
(+): the presence of an identified substance component
(-): absence of identified substance components

3.2. EEBD Effect on Langerhans Structure Overview

The results showed that there was an improvement in the pancreatic organ of Wistar rats in the glibenclamide group, the EEBD group with a dose of 125 mg / kgbb, the EEBD group 250 mg / kgbb and the EEBD group at a dose of 500 mg / kgbb, while in the CMC-Na group the area of the pancreatic langerhans island decreased. The following is a histopathological description of the islets of the pancreatic lagerhans of wistar rats after 15 days of treatment, as shown in Figures 4.5.1 - 4.5.6

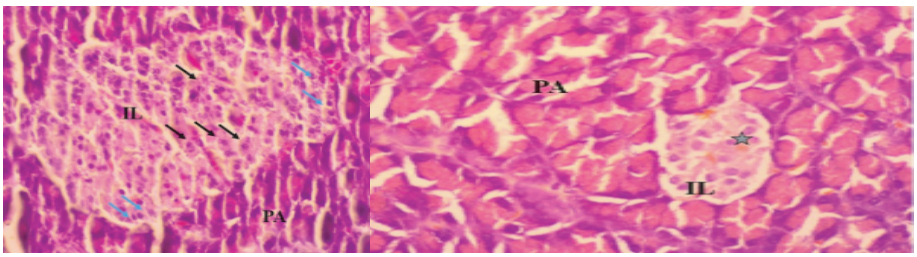

Figure 1: Normal Group Figure ??.5.2 CMC Group.

Information:PA = Pancreas, IL = Langerhans Island, blue arrow = alpha cells, black arrow = beta cells, 400x magnification, HE stain.

Normal Group. Pancreas shows pancreatic acinar cells (PA) and Langerhans cells (IL). Langerhans cells appear to be regular cell membranes, alpha cells are located on the edge with a small solid spherical nucleus, the cytoplasm is few (blue arrows) while beta

cells are located in the center with a larger nucleus, brighter eosinophilic cytoplasm (black arrow). HE, 400x.

CMC Group. A view of Langerhans cells with a smaller surface area with a smaller number of cells. It appears that the cells in the central (middle) part are experiencing hydrophic degeneration (edema) and there is intercellular bleeding (*), HE, 400x.

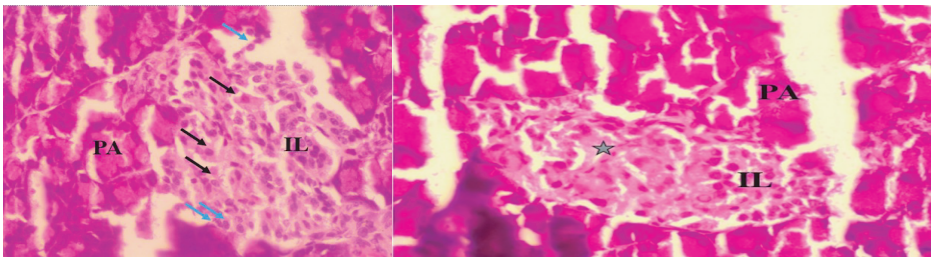

Figure 2: Glibenclamide Group Figure ??.5.4 Grub BD125.

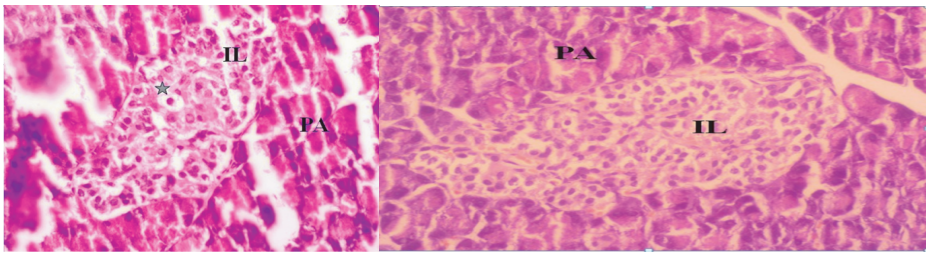

Figure 3: BD250 Group Figure ??.5.6 BD500 Group.

Information:PA = Pancreas, IL = Langerhans Island, blue arrow = alpha cells, black arrow = beta cells, 400x magnification, HE stain.

Gli Group. PA and IL. Lanherhans cells with slightly irregular membranes appear. It appears that the pancreatic cells, especially in the central (middle) area, are proliferative. (HE, 400x). BD Group 125. You can see that Langerhans cells show the cells in the central (middle) part experiencing hydrophic degeneration (edem) (*), HE, 400x. Grub BD250. Irregular surface, Langerhans cells appear. The cells in the central (middle) section undergo hydrophic degeneration (edema) and intercellular bleeding is found (*), HE, 400x. BD500 Group. Langerhans cells appear with a regular surface. The cells in the center (center) are proliferative. Cell morphology is closer to the picture of cells in the normal group, HE, 400x.

4. Discussion

Based on the picture above, a histological picture with the area of Langerhans Island is different for each treatment. In the normal group, it was seen that there was an orderly

arrangement of endocrine cells that were evenly distributed on Langerhans Island with various cell shapes, the cells did not experience necrosis and the cell nucleus looked very dense and there were no cells that experienced edema or swelling. This indicates that Langerhans Island is in normal condition or there is no damage. Whereas in the negative control group Langerhans Island had the smallest size compared to the other treatment groups, it was seen that there was degeneration of endocrine cells leading to cell necrose. Endocrine cell degeneration is seen in essence, which changes shape to be polymorph (not diverse). The changes that occur are described in the form of changes in the endocrine cell nucleus to become smaller (pycnosis) and even begin to disappear, only visible empty and enlarged cytoplasm without a nucleus. This proves that administration of STZ can damage endocrine pancreatic cells, especially β cells so that insulin secretion into blood vessels decreased.

The pancreatic histology of the positive control group, it appears that the condition of Langerhans islands is better than the test preparation group, this is seen from the wider Langerhans island area. It can be seen that the endocrine cells are still evenly distributed, the cell boundaries are still clearly visible, although there are still some endocrine cells that have degeneration but the number is not more than the group of test preparation. This is in line with the KGD data in the positive control group showing a faster decrease than the test preparation. Hyperglycemia will cause the production of free radicals to increase, decrease in KGD will reduce the risk of occidative stress due to free radicals in cells and tissues, so that it will reduce the occurrence of β cell damage, this is what underlies the provision of glibenclamide can inhibit further downturn due to STZ induction [7].Glibenclamide works primarily in increasing insulin secretion. The working mechanism of glibenclamide is to stimulate the secretion of the hormone insulin from the granules of Langerhans pancreatic β cells. Its interaction with ATP-sensitive K channels on the membrane of β cells causes membrane depolarization and this will open the Ca channel. after the formation of Ca channels, the Ca2 + ions will enter β cells then stimulate granules containing insulin and insulin secretion will occur [8]. The flavonoids present in the Dayak bulb extract are able to restore the function of the pancreatic tissue by increasing the release of insulin by β cells, which reduces blood sugar levels and can also improve the sensitivity of peripheral cells to insulin [9]. Flavonoids are reported to have antidiabetic activity which is capable regenerating cells on Langerhans islands [10].

The pancreatic histology of the EEBD group at the dose of 500 mg / kg BW was better than the EEBD dose of 125 and 250 mg / kg BW. There were changes in endocrine cells that began to regenerate to their normal form, but there were still endocrine

cells that experienced necrosis.The histology of EEBD 125 mg / kg BW shows the size of Langerhans islands which is the smallest compared to other EEBD, showing necrosis, unclear cell boundaries and regular cell shape. This is consistent with the KGD data which did not show a decrease in KGD to normal levels on day 15, whereas the histological picture of EEBD at a dose of 250 mg / kg BW was better than that of 125 mg / kg BW.The improvement in histology of Langerhans pancreatic islet cells in the EEBD group was thought to be due to the presence of bioactive compounds in EEBD, namely flavonoids, alkaloids, and tannins. Flavonoids and tannins are known to play a role in capturing free radicals or function as natural antioxidants [11]. Antioxidants are involved in the repair process of damaged cells. Cell damage caused by the presence of free radicals can be overcome with the presence of antioxidants which function to reduce oxidants before damaging cells so that cell damage can be reduced. Antioxidants can suppress β cell apoptosis without changing the proliferation of pancreatic β cells, so that they can regenerate damaged β cells[12]. Alkaloids also have the ability to regenerate damaged pancreatic β cells and increase insulin production, so that KGD in the blood decreases [13].

5. Conclusion

Based on histological observations, it can be concluded that EEBD can improve the morphology of Langerhans islands. With the improvement of the pancreatic tissue, especially on Langerhans Island, there is an increase in the amount of insulin, so that glucose will enter the cells and there will be a decrease in blood glucose in the body.

References

[1] Kusmiati M, Nurpalah R, Mardiana U, Nugraha RW. The effectiveness of “X” kefir brand towards glucose level on post prandial pre-diabetes male mice (Mus Musculus) Swiss Webster strains orally. Journal of Physics: Conference Series. IOP Publishing. 2019;12135.

[2] Babu PVA, Liu D, Gilbert ER. Recent advances in understanding the anti-diabetic actions of dietary flavonoids. The Journal of nutritional biochemistry. 2013;24(11):1777-1789.

[3] American Diabetes Association (ADA). Herbs, supplements and alternative medicine. 2012. Diabetes.org. Available from:http://www. diabetes. org/ living - with-diabetes/treatment - and care/ medication /herbs - supplements – alternative -

medicines/.

[4] Aguilara FZA, Ramos RR, Guiterrez SP et al. Studi of antihyperglaicemic effect of plant used as anti diabetics. J. Page. 1998;101-110.

[5] Apriani N, Suhartono E, Izaak ZA et al. Korelasi kadar glukosa darah dengan kadar advanced oxidation protein produc (AOPP) Tulang pada tikus putih model hiperglikemia. 2011.

[6] American Diabetes Assosiacion (ADA). Diagnosis and classification of diabetes mellitus diabetes care USA. ADA; 2010.

[7] Ali RA, Dahiya A, Kushoor A, Rohilla. Streptozotocin induced diabetes mechanism of induction. International Journal of Pharmaceutical Antioxidan Activity Of Moringaoleifera. International Journal Molecular. 2011:6607-6088.

[8] Akbarzadeh A, Norouzian D, Mehrabi MR et al. Induction of diabetes by streptozotocin in rats. Indian Journal of Clinical Biochemistri. 2007;60-64.

[9] Dewi LK, Widyarti S, Rifai M. Pengaruh pemberian ekstrak etanol daun sirsak (annoramuricata linn) terhadap peningkatan jumlah sel cd4+ dan cd8+ pada timus mencit (musmulucus). Biology Journal. 2013.

[10] Ridwan A. Astrian, tanita, radendan barlian anggreani. pengukuran efek antidiabetes polifenol (Polihenon 60) berdasarkan kadar glukosa darah dan histologi pankreas mencit (Musmusculus L) S. W. Jantan Yang Dikondisikan Diabetes Mellitus. 2012.

[11] Tandi J, Roem M, Yuliet. Efek nefropati kombinasi ekstrak daun gedi merah dan kumis kucing pada tikus induksi etilen glikol. STIFA Pelita Mas. Jurnal Trop Pharmacy Cemistry. 2017;4(1):27-34.

[12] Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Poznan: University of Agriculture; 2017.

[13] Dewi NP, Allia R, Sabang SM. Uji efektivitas antidiabetes (Eleutherine bulbosa (MILL) URB. terhadap penurunan kadar glukosa darah tikus obesitas. Jurnal Farmasi, Sekolah Tinggi Farmasi Pelita Mas Palu (STIFA), Samarinda. 2016.