Indonesian Journal of Medicine (2021), 06(03): 239-245
Masters Program in Public Health, Universitas Sebelas Maret

Research

The Effectiveness of Ethanol Extract, Chayote (*Sechium Edule* (Jacq.) Swartz) Fraction, and Juice on Pancreatic β-Cell Diameter of Male White Rats Wistar Strain with Type 2 Diabetes Mellitus

Sanggam Bangun Hutagalung[1,2], Jekson Martiar Siahaan[3,4], Hendrika Andriana Silitonga[5,6]

[1)]Department of Anatomical Pathology, Faculty of Medicine, Universitas Methodist Indonesia, Medan
[2)]Laboratorium Biomedical, Master's Programme in Biomedical Sciences, Faculty of Medicine, Universitas Methodist Indonesia, Medan
[3)]Department of Physiology, Faculty of Medicine, Universitas Methodist Indonesia, Medan
[4)]Department of Molecular Biology, Master's Programme in Biomedical Sciences, Faculty of Medicine, Universitas Methodist Indonesia, Medan
[5)]Department of Histology, Faculty of Medicine, Universitas Methodist Indonesia, Medan
[6)]Department of phytochemistry, Master's Programme in Biomedical Sciences, Faculty of Medicine, Universitas Methodist Indonesia, Medan

ABSTRACT

Background: Diabetes Mellitus is characterrized by metabolic disturbances due to prolonged hyperglycemia causing oxidative stress which destroys pancreatic β cells. Adjuvant therapy that has antihyperglycemic effectiveness and is required to improve the diameter of pancreatic beta cells, one of which comes from *Sechium edule* (Jacq.) Swartz which has potential as an antihyperglycemia, antioxidant, anti-apoptosis, cardioprotective, insulin resistance. This study aimed to examine The effectiveness of ethanol extract, Chayote (*Sechium Edule* (Jacq.) Swartz) fraction, and juice on pancreatic β-cell diameter of male white rats wistar strain with type 2 diabetes mellitus.

Subjects and Method: This was a randomized controlled trial. Sample was *Rattus novergius* sp. 54 tails. The dependent variable was the diameter of the pancreatic β cells. The independent variables were ethanol extract, *Sechium edule* (Jacq.) Swartz fraction and juice, at a dose of 50 mg/KgBW, 100 mg/KgBW, 150 mg/KgBW. The data were analyzed by Anova test.

Results: The group of mice induced by Streptozotocin 50 mg/kgBW + nicotinamide (120 mg/ kgBW) + HFD and obtained ethanol extract of chayote fruit 150 mg / kgBW, orally, had the highest pancreatic β cell diameter compared to the other groups (Mean= 284.03; SD= 5.15).

Conclusion: *Sechium edule* (Jacq.) Swartz has potential as an anti-apoptosis which can inhibit pancreatic β cell damage

Keywords: pancreatic β cells, anti-apoptosis

Correspondence:
Sanggam Bangun Hutagalung. Department of Anatomical Pathology, Faculty of Medicine, Universitas Methodist Indonesia, Medan. Jl. Setia Budi Pasar II Tj. Sari, Medan 20132, North Sumatera. Email: sanggam1973@gmail.com.

Cite this as:
Hutagalung SB, Siahaan JM, Silitonga HA (2021). The Effectiveness of Ethanol Extract, Chayote (*Sechium Edule* (Jacq.) Swartz) Fraction, and Juice on Pancreatic β-Cell Diameter of Male White Rats Wistar Strain with Type 2 Diabetes Mellitus. Indones J Med. 06(03): 239-245. https://doi.org/10.26911/theijmed.2021.06.03.01.

BACKGROUND

Diabetes mellitus type 2 (T2DM) is also called non-insulin dependent DM is a metabolic disorder due to hyperglycemia characterized by peripheral insulin resistance and reduced pancreatic β cell mass

(Jörns et al., 2020; Lartey et al., 2020). The number of pancreatic β cells ranges from 50-80%, more dominant than other cells in pancreatic islet cells. In adult humans the diameter ranges from 0.5-4 million with a size of 30-40 to 400-500 µm, with a weight of 0.6-2.1g, insulin secretion 50-250 ug / g (Marchetti and Ferrannini, 2015; Marchetti, 2020). A study by Siahaan (2017) found normal pancreatic cell diameter in male white mice (*Mus musculus L.*) strain of DH Webster hyperglycemia nomal 100-400 µm, whereas those induced by streptozotocin (STZ) had a diameter of 68.79µm (Siahaan, 2017).

The decrease in pancreatic β cell mass in T2DM is due to insulin resistance resulting in glucotoxicity, increased lipidotoxic fat, induces oxidative stress and proinflammatory cytokines (Jezek et al., 2018; Iftikhar et al., 2020; Lee et al., 2020). This situation can be modeled in experimental animals by giving the Streptozotocin-Nicotinamide-High Fatt Diet (STZ-NA-HFD). Streptozotocin induces oxidative stress that destroys pancreatic β cells, but to avoid damage that can cause experimental animals to become type 1 diabetes mellitus (DMT1), nicotinamide is needed as a protective agent against pancreatic β cells which is antioxidant while giving HFD so that the experimental animals are in a state of insulin resistance (Siahaan, 2019).

The decrease in pancreatic β cell mass due to this metabolic disorder can be prevented by the presence of potential active ingredients derived from plants such as flavonoids which have antihyperglycemic potential, one of which can be found in the chayote plant *Sechium edule* (Jacq.) Swartz. The bioactive compound components contained in this plant are as many as 8 flavonoids, namely 3 C-glycosyl and 5 O-glycosyl flavones. Flavonoids work as antihyperglycemia by disrupting the metabolism and absorption of carbohydrates in the small intestine, namely through inhibition of the action of the α amylase and α glucosidase enzymes, inhibiting carbohydrate absorption in the small intestine, stimulating glucose transport in peripheral tissues, are insulin mimetic, which works like insulin to stimulate synthesis glycongen and is an insulin secretagogue, which stimulates insulin production and has a protective effect on pancreatic β cells (Siahaan, 2020). The purpose of this study was to determine the effectiveness of ethanol extract, fraction and chayote juice (sechium edule (jacq.) Swartz) on the diameter of pancreatic β cells of male white rats Wistar strain of diabetes mellitus type 2 induced by STZ-NA-HFD.

SUBJECTS AND METHOD

1. Study Design

This was a randomized controlled trial using the post-test intervention and control groups.

2. Population and Sample

The experimental animals were healthy white male Wistar rats, aged 2.5 - 3 months, body weight 180-220 grams. The selection of mice as experimental animals is based on the consideration that genetically, mice have similarities to humans and have the ability to adapt to the laboratory environment. The sample allocation (grouping) of experimental animals is using simple random sampling. The sample size was estimated using Federer's formula, each group using 3 male white rats Wistar (*Rattus novergius sp.*) With 18 groups of treatment groups so that the total sample of the study was 54. Siamese pumpkin is a purposive sampling method, which is taken from the residents' yards of Sidamanik.

3. Study Variables

The dependent variable was the diameter of pancreatic β cells, while the independent

variable was the variation in the dose of ethanol extract, *Sechium edule* (Jacq.) Swartz fraction and juice.

4. Operational Definition of Variables

Pancreatic β cell diameter was the result of histological examination of pancreatic preparations using Hematoxylin-Eosin (HE) staining.

5. Study Instruments

The examination of the diameter of the pancreatic β cells was carried out in the Anatomical Pathology laboratory, Faculty of Medicine, Methodist University of Indonesia

6. Data Analysis

Pancreatic β cell diameter was analyzed by using Anova test.

RESULTS

The diameter of pancreatic β cells was measured after 21 days of administration of *Sechium edule* (Jacq.) Swartz fraction and juice between groups can be seen in Table 1.

Table 1. Diameter of pancreatic β cells in male white rat Wistar strain (*Rattus novergius sp*.)

Group	Pancreatic β Cell Diameter		p
	Mean	SD	
1	211.97	24.91	
2	74.4	18.59	
3	177.83	18.17	
4	136.43	11.78	
5	107.76	104.70	
6	120.50	14.23	
7	**284.03**	**5.15**	
8	96.07	5.96	
9	86.57	10.21	<0.001
10	80.80	9.24	
11	124.40	7.18	
12	107.93	9.76	
13	94.37	5.95	
14	172.77	4.72	
15	219.37	6.35	
16	238.40	9.72	
17	136.40	13.46	
18	278.63	7.42	

Note:

a. Group 1, negative control (normal) was not given any treatment, like normal rats in general who were given excessive food and drink (ad libitum) in their cages.
b. group 2, positive control, induced Streptozotocin 50 mg / kgBW + HFD
c. group 3, Positive control, induced nicotinamide (120 mg / kg) + HFD
d. group 4, Positive control, induced Streptozotocin 50 mg / kgBW + nicotinamide (120 mg / kg) + HFD
e. group 5, the treatment group induced by Streptozotocin 50 mg / kgBW + nicotinamide (120 mg / kg) + HFD, with ethanol extract of chayote fruit 50 mg / kgBW, p.o.
f. group 6, the treatment group that was induced by Streptozotocin 50 mg / kgBW + nicotinamide (120 mg / kg) + HFD, with ethanol extract of chayote fruit 100 mg / kgBW, p.o.
g. group 7, the treatment group which was induced by Streptozotocin 50 mg / kgBW + nicotinamide (120 mg / kg) + HFD, with ethanol extract of chayote fruit 150 mg / kgBB, p.o.
h. group 8, the treatment group that was induced by Streptozotocin 50 mg / kg + nicotinamide (120 mg / kg) + HFD, with the ethyl acetate fraction of chayote 50 mg / kg, p.o.
i. group 9, the treatment group that was induced by Streptozotocin 50 mg / kgBW +

nicotinamide (120 mg / kg) + HFD, with the ethyl acetate fraction of chayote 100 mg / kgBB, p.o.

j. group 10, the treatment group that was induced by Streptozotocin 50 mg / kgBW + nicotinamide (120 mg / kg) + HFD, with the ethyl acetate fraction of chayote 150 mg / kg, p.o.
k. group 11, the treatment group that was induced by Streptozotocin 50 mg / kgBW + nicotinamide (120 mg / kg) + HFD, with the n fraction of chayote's hexane 50 mg / kgBB, p.o.
l. group 12, the treatment group that was induced by Streptozotocin 50 mg / kgBW + nicotinamide (120 mg / kg) + HFD, with fraction n hexane of chayote fruit 100 mg / kgBW, p.o.
m. group 13, the treatment group that was induced by Streptozotocin 50 mg / kgBW + nicotinamide (120 mg / kg) + HFD, with n hexane fraction of chayote fruit 150 mg / kg, p.o.
n. group 14, the treatment group that was induced by Streptozotocin 50 mg / kgBW + nicotinamide (120 mg / kg) + HFD, with chayote juice 50 mg / KgBW p.o
o. group 15, the treatment group that was induced by Streptozotocin 50 mg / kgBW + nicotinamide (120 mg / kg) + HFD, with chayote juice 100 mg / KgBW p.o
p. group 16, the treatment group that was induced by Streptozotocin 50 mg / kgBW + nicotinamide (120 mg / kg) + HFD, with chayote juice 150 mg / KgBW p.o
q. group 17, the treatment group that was induced by Streptozotocin 50 mg / kg + nicotinamide (120 mg / kg) + HFD, with metformin 500 mg / KgBW p.o
r. group 18, the treatment group that was induced by Streptozotocin 50 mg / kg + nicotinamide (120 mg / kg) + HFD, with simvastatin 500 mg / KgBW p.o

From Table 1, it was found that the highest pancreatic β cell diameter was found in group G which was given the ethanol extract of chayote fruit 150 mg/ kg, po, while the lowest pancreatic β cell diameter was found in group B as a positive control with a p value of 0.000, indicating the existence of meaningful relationship.

DISCUSSION

This study showed that the ethanol extract of chayote Sechium edule (Jacq.) Swartz 150 mg/ kgBW proved to be better at improving the diameter of pancreatic β cells. This is because the ethanol extract has the ability to attract polar and non-polar bioactive compounds in the Sechium edule (Jacq.) Swartz chayote.such as alkaloids, flavonoids, glycosides, saponins, tannins and triterfen/ steroids even though the fractionation method contains more specific bioactive compounds (Saleh, 2016; Da'I et al., 2020; Siahaan, 2020). This study is in line with Siahaan (2017), which stated that ethanol extract has antiapoptotic ability, the only drawback of this study is that the histopathological results are representative of each sample group so that they only describe the histopathological picture and are not tested statistically, whether the ethanol extract of Sechium edule chayote (Jacq.) Swartz 200 mg/ kgBW has a statistically significant effect, although clinically it shows that the ethanol extract dose of Sechium edule (Jacq.) Chayote fruit ethanol extract 200 mg/ kgBW is better than the ethanol extract of Sechium edule chayote (Jacq.) Swartz 150 mg / KgBB, besides that this study did not compare various kinds of solvents using the fractionation method (Siahaan, 2017)

The effectiveness of alkaloid bioactive compounds is as antidiabetic by increasing insulin sensitivity by inhibiting glucogenolysis, accelerating the transport of glucose to peripheral tissues (Dizaye and Aziz, 2019). Alkaloids are also proven to be antioxidants that can inhibit DM complications (Zhang et al., 2015). In addition, the inhibitory effect of the c-Jun N-terminal kinase (JNK) signaling pathway and pancreatic β cell apoptosis (Tian et al., 2020) also

regulates the GPR40/cAMP/Ca2+/ IRS2/ PI3K/Akt signaling pathway and assists GSIS via GPR40. / cAMP / Ca2 + / CaMKII signaling (Du et al., 2019).

The mechanism of bioactive glycolsides has potential as antihyperglycemia by reducing oxidative stress, increasing lipid metabolism, reducing mitochondrial damage and reducing the activation of the apoptotic pathway induced by mitochondria, thereby inhibiting pancreatic β cell apoptosis and increasing the function of these pancreatic β cells. Apoptosis regulation has also decreased, mediated by Fas / FasL (Cheng et al., 2019). Giving glycosides acutely can stimulate insulin secretion whereas if given chronic for 48 hours it will inhibit tissue proliferation and induce apoptosis (Kittl et al., 2016).

The effectiveness of saponins as antiapoptosis by downregulating the expression of Fas and Caspase-3 genes (Chen, 2012). Its antidiabetic effect is by inhibiting α-amylase and α-glucosidase activity (Kunyanga et al., 2011). In addition, its antidiabetic potential can also be through the mechanism of activating glycogen synthesis, modulating insulin signaling, regenerating insulin activity, and suppressing gluconeogenesis (Barky et al., 2017).

Tannin is able to reduce blood sugar levels by inhibiting α-amylase and α-glucosidase activity (Sieniawska, 2015). It also inhibits glucose absorption and increases insulin sensitivity (Lu et al., 2019).

AUTHOR CONTRIBUTION

Sanggam Bangun Hutagalung, Jekson Martiar Siahaan, Hendrika Andriana Silitonga played a role in sample preparation, histopathological examination, data interpretation and article preparation.

CONFLICT OF INTEREST

There is no conflict of interest in this study.

FUNDING AND SPONSORSHIP

This research is fully funded by DRPM DIKTI Beginner Lecturer Research Grant (PDP) for the 2019-2020 fiscal year.

ACKNOWLEDGEMENT

We would like to thank DRPM DIKTI, LLDIKTI region 1, LP3M Methodist University of Indonesia for their trust, which gave researchers the opportunity to participate in Competitive Grants for Beginner Lecturer Research Higher Education (PDP).

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