



The Effect of Ethanol and Ethyl Acetate Fraction of Chayote fruit (*Sechium edule* Jacq. Swartz) on the Oxidative Stress and Insulin Resistance of Male White Rat Model Type 2 Diabetes Mellitus

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Abstract

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BACKGROUND: Oxidative stress in type 2 diabetes mellitus (T2D) causes insulin resistance and disordered insulin secretion. Pathomechanisms of T2D consist of dysfunctional pancreatic β -cell and insulin resistance caused by free radical (reactive oxygen species and reactive nitrogen species) that produced from the glucose metabolism pathway. Insulin resistance can be measured using the homeostatic model assessment of insulin resistance (HOMA-IR). Oxidative stress can measure through the activities of malondialdehyde (MDA) and superoxide dismutase (SOD).

AIM: This research aims to study the potential of chayote (*Sechium edule* Jacq. Swartz) to be used as antihyperglycemic in T2D.

MATERIALS AND METHODS: This research was conducted with a post-test randomized controlled group design. Eleven groups with four male rats each were used. Normal untreated rats were treated under *ad libitum* feeding and drinking condition. Meanwhile, the rat models were induced with the combination of 45 mg/kg b.w. streptozotocin, 110 mg/kg b.w. nicotinamide, 40.5 mg/kg b.w. metformin, high-fat diet, and/or chayote extract. The chayote extract was orally administered to the rat in the form of ethanol extract and/or ethyl acetate fraction, with three dosages of 45 mg/kg b.w., 100 mg/kg b.w., and 150 mg/kg b.w. for each extract type. The body weight, glucose level, insulin level, MDA, and SOD activities were measured. The HOMA-IR was used.

RESULTS: The lowest body weight of the rat model in week 0 was 145 ± 25.31 , founded in Group H that was treated with ethyl acetate fraction of chayote extract (45 mg/kg b.w.). The lowest blood sugar level in the group with 2 h glucose load was 112.5 ± 27.00 on average, found in Group G that was treated with chayote ethanolic extract (150 mg/kg b.w.). The highest SOD in the group treated with chayote extract was 1.27 ± 0.20 , founded in Group H treated with ethyl acetate 45 mg/kg b.w. The lowest level of MDA was 0.86 ± 0.70 in Group H treated with ethyl acetate 45 mg/kg b.w. The lowest fasting blood sugar spectrophotometer level was 150.54 ± 17.24 mg/dl in Group K with metformin treatment, followed by 155.16 ± 31.92 mg/dl in Group K treated 45 mg/kg b.w. ethanol treatment. The highest insulin level was 6.14 ± 0.71 , founded in Group F that was treated with chayote ethanolic extract 100 mg/kg b.w. The lowest measurement of HOMA-IR was 0.16 ± 0.80 in Group E treated with ethanol extract of chayote 45 mg/kg b.w.

CONCLUSION: Ethanol extract and fractionation of chayote work as an antioxidant and anti-insulin resistance.

Introduction

Chronic hyperglycemia induces the increase of oxidative stress due to the uncontrolled reactive oxygen species (ROS) and reactive nitrogen species. This condition plays an important role in tissue destruction of the patients of Type 1 diabetes mellitus (T1D) and Type 2 diabetes mellitus (T2D). There will be damage in pancreatic β -cell for T1D and insulin resistance or disordered insulin secretion for T2D patients [1], [2], [3]. To measure the ineffective performance of insulin as a result of insulin resistance, as well as to check the function of pancreatic β -cell, homeostatic model assessment of insulin resistance (HOMA-IR) can be used as a measurement [4], [5].

Two main pathophysiology mechanisms of HOMA-IR are dysfunctional pancreatic β -cell and insulin resistance [4], [6], [7], [8]. These two mechanisms are underlined by the existence of oxidative stress, the increase of ROS product by hexosamine pathway, advanced glycation end-products, fat acid free, and leptin which produces ROS. The increasing of free radicals will stimulate the formation of adipokines, cytokines, and prostanoids which then cause insulin resistance [3].

The damage of pancreatic β -cell can be modeled in a hyperglycemic animal model, using streptozotocin (STZ) and nicotinamide (NA) to induce hyperglycemia. STZ may damage the pancreatic β -cell, however, the NA application may protect pancreatic β -cell from further damage caused by the STZ effect.

NA injection after giving STZ with time interval may give partial protection to the β -cell cytotoxic effect. Effective protection is attained if NA is injected 2 h after giving the STZ, and less effective protection if the interval is longer. STZ was carried to pancreatic β -cell through glucose transporter (GLUT2), which may cause DNA damage through the raising of poly(ADP-ribose) polymerase (PARP-1) activities [9], [10].

The abnormality caused by hyperglycemia can be controlled through the oral treatment of antihyperglycemic, which can alternatively be utilized from herbal medicine with anti-hypoglycemia potential and antioxidant activities. According to Siahaan *et al.* [11], the flavonoid content in the plant, as in chayote (*Sechium edule* Jacq. Swartz), can decrease the blood sugar level (BSL) and increase the antioxidant level in diabetic mice. There was also a difference in the diameter of the pancreatic β -cell between chayote-treated mice and non-treated mice [11], [12].

Materials and Methods

Study site

This study was undertaken at the Biomedical Laboratory, Faculty of Medicine, University of North Sumatra for a year.

Collection and preparation of plant materials

Chayote was collected with purposive sampling method from the yards of a citizen of Sidamanik district, Sumatera Utara, Indonesia.

Experimental design

This research is a laboratory experimental research conducted with a post-test randomized controlled group design using hyperglycemic white male Wistar rat. The design of this research has allowed the measurement of the effect of treatment to the experimental group by comparing the treated group with the untreated/control group. Nonetheless, the design does not allow the measurement of the level of the change caused by the treatment, as there was no initial measurement at the beginning of the research. The treatments to all samples were conducted at the same time and the post-test only control group design was conducted after a certain time. In this design, the sample was obtained using simple random sampling method. Therefore, the animal model, location of experiment, and the materials were homogenous.

Experimental groups

The animal models used in this research are healthy white male Wistar rats aged 2.5–3 months old, with a body weight between 150 and 220 g. The rats body weight was measured in week 0 to determine if the rats used to fit into the inclusion criteria specified. The selection of rat as a model was based on its genetic similarity with human and their ability to adapt to the laboratory environment. The number of the sample used was determined using randomization method for animal experiment by Federer equation: $(n-1)(t-1) \geq 15$, where t is the number of treatments while n is the number of animals per treatment. Eleven groups with four male rats each were then used in this research, with 44 male rats as a sample in total.

The following 11 groups of rats were used for the experiments, Group A: Normal untreated rats under *ad libitum* feeding and drinking condition (the negative control), Group B: Rats were administered with STZ 45 mg/kg b.w. + NA 110 mg/kg b.w. + high-fat diet (HFD) (the positive control), Group C: Rats were administered with STZ 45 mg/kg b.w. + HFD (the positive control), Group D: Rats were administered with NA 110 mg/kg b.w. + HFD (the positive control), Group E: Rats were administered with STZ 45 mg/kg b.w. + NA 110 mg/kg b.w. + HFD + chayote ethanol extract 45 mg/kg b.w., Group F: Rats were administered with STZ 45 mg/kg b.w. + NA 110 mg/kg b.w. + HFD + chayote ethanol extract 100 mg/kg b.w., Group G: Rats were administered with STZ 45 mg/kg b.w. + NA 110 mg/kg b.w. + HFD + chayote ethanol extract 150 mg/kg b.w., Group H: Rats were administered with STZ 45 mg/kg b.w. + NA 110 mg/kg b.w. + HFD + ethyl acetate fraction of chayote extract 45 mg/kg b.w., Group I: Rats were administered with STZ 45 mg/kg b.w. + NA 110 mg/kg b.w. + HFD + ethyl acetate fraction of chayote extract 100 mg/kg b.w., Group J: Rats were administered with STZ 45 mg/kg b.w. + NA 110 mg/kg b.w. + HFD + ethyl acetate fraction of chayote extract 150 mg/kg b.w., and Group K: Rats were administered with STZ 45 mg/kg b.w. + NA 110 mg/kg b.w. + HFD + metformin 40.5 mg/kg b.w.

Preparation of chayote ethanolic extracts

The chayote ethanolic extract was obtained through the maceration process using ethanol 80%. About 10 simplicia and 75 parts of the solvent were kept in a stoppered container for 5 days with frequent agitation until the soluble matter is dissolved. The liquid phase was separated from the solid material with frequent filtration. A sufficient amount of solvent was added to solid residue until the liquid collected is 100 parts. The liquid phase was then moved to another tightly stoppered container, was protected from sunlight, and placed in cool space for 2 days. The layer of liquid

closer to the top of the container was separated from the other material with decantation. The concentrated ethanolic extract was then resulted using a rotary evaporator at 40°C set of temperature.

Preparation of ethyl acetate fraction

About 20 g of concentrated chayote ethanolic extract was mixed with 50 ml of ethanol 96% and 100 ml aquadest until homogenous. The mixture was partitioned in the separator funnel using 100 ml ethyl acetate until the liquid phase showed a negative response to FeCl₃ and ethyl acetate fraction was formed. The concentrated ethyl acetate fraction was then resulted using a rotary evaporator at 40°C set of temperature.

Hyperglycemia induction

Rats were treated with NA (110 mg/kg b.w.) with a 15 min interval to the following intraperitoneal injection of 45 mg/kg b.w. cold STZ in NaCl 0.9%. Wistar rat is categorically suffered from diabetes mellitus (DM) if the level of blood sugar is >250 mg/dl after 72 h of induction [13], [14].

Insulin resistance induction with HFD

The formula used is a modification of Listianasari *et al.* [15] formula. The rat was fed with HFD from the mixture of egg yolk, coconut oil, and butter. This diet was given using a gastric/feeding tube in the amount of 4 ml/day/rat.

Glucose loading

The rats were fasted and fed with chayote extract. The rats were 30 min later loaded with glucose 75 g (dosage for rats converted to 1.35/200 g b.w). The BSL after ½ h, 1 h, 1 ½ h, and 2 h was then measured. The rat body weight was measured using a balance and BSL was measured with a spectrophotometer. The activities of malondialdehyde (MDA), superoxide dismutase (SOD), insulin level, and HOMA-IR were measured using ELISA.

Statistical analysis

One-way analysis of variance and Kruskal–Wallis tests are used to determine if there are statistically significant differences between two or more groups of treatment in this research. Wilcoxon signed-rank test and dependent T-test are used as needed. The level of significance was set at $p < 0.05$. Statistical analyses were performed with SPSS, version 18 (SPSS Inc., Chicago, IL, USA).

Results

Body weight of rats

The result showed that the rats model has had the proper body weight relevant to the inclusion criteria. Body weight of the rats after the diabetic induction and body weight after extract treatment in the 2nd–3rd weeks are shown in Table 1.

Table 1: Body weight of rats before and after induction and extract treatment

Groups	Groups			
	Week 0	Week 1	Week 2	Week 3
A	159.33 ± 12.10	159.67 ± 12.42	162.00 ± 12.77	163.33 ± 12.34
B	178.33 ± 21.36	245.00 ± 77.97	222.67 ± 61.72	203.67 ± 22.50
C	157.67 ± 4.51	222.67 ± 10.02	215.67 ± 5.03	145.33 ± 28.02
D	195.00 ± 33.78	222.67 ± 5.86	201.33 ± 12.06	226.83 ± 66.23
E	164.75 ± 32.35	179.25 ± 27.54	178.50 ± 35.26	176.63 ± 37.24
F	178.25 ± 9.39	199.25 ± 13.55	178.50 ± 11.03	149.50 ± 47.26
G	184.50 ± 14.62	208.75 ± 24.30	207.25 ± 20.45	207.88 ± 22.23
H	145.00 ± 25.31	171.75 ± 34.36	168.00 ± 20.20	153.88 ± 8.76
I	146.25 ± 38.86	141.25 ± 34.48	144.00 ± 39.56	121.25 ± 26.39
J	170.25 ± 41.96	187.00 ± 49.09	186.50 ± 56.25	178.13 ± 57.50
K	170.75 ± 26.91	182.00 ± 23.57	177.50 ± 31.03	182.88 ± 26.05
p-value	0.361**	0.038**	0.105*	0.023*

*Analysis of variance test, ** Kruskal–Wallis test

Based on the measurement of body weight (Table 1), the lowest body weight at the beginning of the experiment is found in Group H with average weight of 145 ± 25.31g, at which rats were treated with STZ 45 mg/kg b.w., NA 110 mg/kg b.w., HFD, and ethyl acetate fraction of chayote extract 45 mg/kg b.w. Meanwhile, the highest weight is 195 ± 33.78 g in a group of positive control, at which rats were treated with NA 110 mg/kg b.w. and HFD (Group D). There is no significant difference between the groups assessed.

In the following week after glucose induction, the lowest body weight (141.25 ± 34.48 g) in the 1st week is found in group of rats that were treated with STZ 45 mg/kg b.w., NA 110 mg/kg b.w., HFD, and ethyl acetate fraction of chayote extract 100 mg/kg b.w. (Group I) while the highest (245 ± 77.97g) is the positive control treated with STZ 45 mg/kg b.w., NA 110 mg/kg b.w., and HFD (Group B). No significant difference is found among the group in week 1. In week 2, the lowest weight is in the group treated with STZ 45 mg/kg b.w., NA 110 mg/kg b.w., HFD, and ethyl acetate fraction of chayote extract 100 mg/kg b.w. with Group B stays in the highest weight among the group. However, there is no significant difference among the groups. In week 3, a Group I also has the lowest weight among all, while Group D that shows the highest weight in week 0 stays in the highest position in week 3. $p < 0.05$ shows that there is a difference among groups in this week.

Glucose loading

The result of the glucose load for the rats is shown in Table 2. In the 1st week of treatment, the highest fasting BSL is found in Group F with an average value of 337.50 ± 140.22. The lowest is found in Group

Table 2: Blood sugar level resulted from the glucose loading

Weeks	0 h		0.5 h		1 h		1.5 h		2 h		P
	1	2	1	2	1	2	1	2	1	2	
A	107.33 ± 12.74	124.67 ± 3.51	124.33 ± 8.74	138 ± 2	121.33 ± 8.74	132.33 ± 4.04	113.67 ± 14.05	125.67 ± 9.29	109.67 ± 14.01	118.67 ± 8.08	0.331 ^b
B	154.33 ± 36.75	143.67 ± 43.62	167.67 ± 33.08	202 ± 36.51	155 ± 31.61	192 ± 39.51	145 ± 31.95	184 ± 36.16	129 ± 36.5	145.00 ± 24.52	
C	183 ± 45.03	287 ± 77.25	229.00 ± 79.68	322 ± 92.63	225.67 ± 80.21	312.33 ± 81.82	209 ± 79.47	285 ± 97.52	199 ± 64.09	295.33 ± 95.00	
D	186.67 ± 11.02	109.33 ± 10.60	222 ± 32.51	170.67 ± 44.06	184.33 ± 22.14	146.67	131.33 ± 27.59	124.33 ± 21.55	108 ± 12.77	104.67 ± 15.53	
E	226.25 ± 109.56	150.75 ± 115.25	357 ± 241.89	386.25 ± 247.14	308.25 ± 211.33	394.25 ± 238	278.25 ± 185.86	228.75 ± 125.98	243 ± 154.31	181.75 ± 109.42	
F	337.50 ± 140.22	253.25 ± 148.36	218.50 ± 64.10	449.25 ± 175.84	331.50 ± 180.34	449 ± 206.28	254.5 ± 109.71	329 ± 135.96	160 ± 29.36	269.25 ± 157.13	
G	238.75 ± 168.20	99.75 ± 23.43	336.50 ± 163.93	270 ± 32.54	262 ± 151.45	247 ± 18.24	221 ± 123.97	180.75 ± 52	208 ± 126.47	112.5 ± 27.00	
H	210 ± 63.04	283.75 ± 210.18	449.50 ± 180.78	574.75 ± 23.17	417.5 ± 211.64	547.5 ± 63.00	357.5 ± 196.86	554.25 ± 42.93	284 ± 136.77	499.75 ± 64.46	
I	261.5 ± 69.52	101.25 ± 9.54	507.75 ± 184.50	517.25 ± 69.17	513.75 ± 172.50	522.5 ± 90.02	424.75 ± 138.68	312 ± 138.92	386 ± 131.71	115.75 ± 1.70	
J	207.50 ± 223	163.25 ± 94.91	299.75 ± 210.47	309.75 ± 193.74	329.5 ± 189.80	302.75 ± 205.10	205.5 ± 84.10	222 ± 176.16	169.75 ± 64.63	175.75 ± 95.95	
K	143 ± 30.39	130.25 ± 22.02	159.25 ± 33.43	171.75 ± 26.35	164.75 ± 36.39	144.25 ± 10.53	140.75 ± 28.5	139 ± 12.94	117.5 ± 29.26	126.25 ± 16.66	
Nilai P.	0.065*	0.032*	0.018*	0.003*	0.010*	<0.001**	0.028*	0.005*	0.008**	<0.001**	

*Wilcoxon test, ^bdependent t-test, ^cindependent t-test, ^dKruskal-Wallis

A with an average value of 107.33 ± 12.74. There is no significant difference between each group.

The highest BSL of the rats loaded with glucose, 30 min after the treatment, is around 507.75 mg/dl founded in Group I while the lowest average value is 124.33 ± 8.74 mg/dl in Group A. p < 0.05 shows that there is the difference in the results of the groups. From the measurement 1 h after glucose load, the highest BSL is found in Group I (513.75 ± 172.50 mg/dl) while the lowest in Group A (121.33 ± 8.74 mg/dl). p < 0.05 means that it is significantly different in groups. From the measurement 2 h after glucose load, the highest is in Group I with the average value of BSL 386 ± 131.71 mg/dl while the lowest group founded in Group D with average 104.67 ± 15.53 mg/dl. From the treated group, the average of the lowest BSL is in Group K 117.5 ± 29.26. p < 0.01 shows that there is a difference in groups. In the 2nd week, the fasting BSL of the rats was measured. The highest is in Group C (287 mg/dl) while the lowest is Group G (99.75 mg/dl). p < 0.05 shows that there is a difference in groups. From the measurement 30 min after glucose load, the group with the highest BSL is Group H (574.75 mg/dl) while the lowest level is in Group A (138 mg/dl). p < 0.01 means that there is a difference among the groups.

The highest average of BSL in the measurement of 1 h afterload is in Group H with the level of 547.5 ± 63.00 mg/dl, while the lowest average is in Group A with BSL 132.33 ± 4.04. p < 0.001 means that there is a difference in groups. The highest average BSL in 1.5 h is in Group H with 554.25 ± 42.93 mg/dl, while the lowest BSL average is in Group A 125.67 ± 9.29 mg/dl. p < 0.01 means that there is a difference in groups. From the measurement 2 h after glucose load, the checking glucose load in 2 h, the highest average value in Group H is 499.75 ± 64.46 mg/dl, meanwhile, the lowest average is in Group A 118.67 ± 8.08. For the therapy group, the lowest BSL average value is in Group G 112.5 ± 27.00 mg/dl. p < 0.001 means that there is a difference in groups.

If we compare the average of fasting BSL between the 1st and 2nd weeks, the highest average value is found in week 1 Group F with average value 337.50 ± 140.22 mg/dl. Meanwhile, the lowest average found in week 2 Group I with average value BSL 101.25 ± 9.54 mg/dl. p < 0.01 means that there is a difference between each group.

From the average glucose loaded in 30 min after oral glucose load, the lowest BSL is found in the treated Group K in week 1 with an average value of 159.25 ± 33.43 mg/dl. Meanwhile, the highest BSL is found in week 2 Group H with average BSL 574.75 ± 23.17 mg/dl. The p-value shows no difference among the groups. However, Group K shows the ability of metformin in decreasing the BSL in 30 min after giving the glucose, followed by Group F treated with extract ethanol 100 mg/kg b.w. with BSL average 218.50 ± 64.10 mg/dl.

From the average glucose loaded in 1 h after oral glucose load, the group that has the lowest average of BSL is also Group K in the 2nd week with an average value of 144.25 ± 10.53 mg/dl. The highest average value is also in Group F the 2nd week with average BSL 449 ± 206.28 mg/dl. There is no significant difference showed among the groups. However, Group K also shows the metformin ability in decreasing BSL in 1 h after giving the glucose. It is also followed by Group F that was treated with ethanol extract 150 mg/kg b.w. with the average BSL 247 ± 18.24 mg/dl.

From the measurement of glucose loaded in 1.5 h after glucose was given, the highest BSL is in the 2nd week in Group E with the average value of 554.25 ± 42.93 mg/dl. Meanwhile, the lowest BSL is in the 2nd week in Group K 139 ± 12.94 , followed by Group G for the 2nd week with BSL average 180.75 ± 52 mg/dl. $p = 0.144$ shows the difference among the groups even though Groups K and G show the decreasing of clinical BSL.

From the measurement of glucose loaded in 2 h after glucose was given, the highest BSL is in week 2 in Group H with an average value of 499.75 ± 64.46 mg/dl. Meanwhile, the lowest BSL is found in week 2 Group G with an average value of 112.5 ± 27.00 . There is no significant difference showed in the 2 h glucose load. The average BSL in Group G is, however, clinically lower compared with Group K which was treated with metformin. It is even lower than the healthy or untreated group.

Measurement of SOD, MDA, BSL, and HOMA-AIR

Data of the measurement of SOD, MDA, BSL, insulin, and HOMA-IR on the 3rd week are shown in Table 3.

Table 3: Data from the analysis of SOD, MDA, BSL, insulin, and HOMA-IR

Kelompok	SOD	MDA	BSL	Insulin	HOMA-IR
A	1.09 ± 0.26	0.689 ± 0.03	186.38 ± 9.70	4.60 ± 1.15	2.12 ± 0.59
B	0.26 ± 0.40	0.79 ± 0.10	215.57 ± 93.18	2.53 ± 2.30	1.69 ± 1.73
C	0.88 ± 0.77	0.89 ± 0.08	413.09 ± 200.63	4.64 ± 3.80	5.87 ± 4.97
D	1.74 ± 0.29	1.11 ± 0.14	276.69 ± 62.34	5.02 ± 4.15	3.79 ± 3.24
E	1.10 ± 0.75	1.59 ± 0.69	155.16 ± 31.92	0.44 ± 0.24	0.16 ± 0.08
F	0.96 ± 0.83	1.40 ± 0.23	167.06 ± 24.34	6.88 ± 1.00	2.83 ± 0.59
G	1.00 ± 0.82	1.19 ± 0.27	187.18 ± 54.57	3.88 ± 2.42	1.68 ± 1.09
H	1.27 ± 0.20	0.86 ± 0.07	229.58 ± 29.93	6.14 ± 0.71	3.44 ± 0.26
I	1.10 ± 0.74	1.00 ± 0.28	163.12 ± 14.62	3.78 ± 2.41	1.46 ± 0.89
J	1.05 ± 1.26	1.50 ± 0.18	278.08 ± 56.74	4.71 ± 3.39	3.32 ± 2.83
K	0.47 ± 0.85	1.24 ± 0.10	150.54 ± 17.24	4.58 ± 3.70	1.75 ± 1.51
P-value	0.525**	0.001*	0.001*	0.188**	0.071*

*Analysis of variance, **Kruskal-Wallis

The highest SOD level in the extract treated group is found in Group H with ethyl acetate 45 mg/kg b.w. treatment. The average level is 1.27 ± 0.20 . Meanwhile, the group with the lowest level of SOD is Group F which was treated with 100 mg ethanol extract/kg b.w. resulted SOD 0.96 ± 0.83 . There were no significant differences between groups, however, the treatment of extracts in Group H was able to increase SOD antioxidants higher than other groups.

The highest MDA level is in Group E with 45 mg/kg b.w. ethanol extract treatment, which is 1.59 ± 0.69 . The lowest is 0.86 ± 0.07 founded in Group H with 45 mg/kg b.w. ethyl acetate treatment. $p = 0.001$ means that there are significant differences between groups. Group H extract can reduce oxidative stress better than other groups.

The lowest spectrophotometer of fasting BSL is in K group treated with metformin with the level of 150.54 ± 17.24 mg/dl, followed by Group E treated with 45 mg/kg b.w. ethanol, with the level of 155.16 ± 31.92 mg/dl. $p = 0.001$ means that there are significant differences between groups although the group given the extract showed a decrease in BSL, the decrease in BSL was better in K group. Metformin has a strong ability of hypoglycemia compared to the other groups.

The highest level of insulin is in Group F which is 6.14 ± 0.71 , the lowest level is in Group E which is 0.44 ± 0.24 with $p = 0.188$ it's mean that there is no significant difference between groups, but extract in Group F could increase insulin secretion higher than with other groups. The highest HOMA-IR in Group H is 3.44 ± 0.26 , while the lowest is in Group E 0.16 ± 0.08 with no significant difference between groups. Nonetheless, Group E resistance insulin was decreased compared to other groups who received extracts or oral hypoglycemia.

Discussion

The group treated with extract and fractionation has better weight loss in Group I both at days 1, 2, and 3, compared with metformin. Fractionation of ethyl acetate chayote 100 mg/kg b.w. reduced the mass of adipose tissue in the body due to fractionation that inhibits mobilization of fat from peripheral adipose tissue to plasma [16]. It proves that ethyl acetate fraction can inhibit the performance of the pancreatic lipase enzyme as an enzyme that plays an important role in fat metabolism and fat absorption. Moreover, ethyl acetate fraction also plays a role in inhibiting α -glucosidase, thus reducing the absorption of carbohydrate [17], [18], [19].

Nevertheless, research conducted by Olaokun *et al.* [19] shows the different results with this study. The ethyl acetate fraction used did not reduce weight and the mechanism of the failure of ethyl acetate fraction in losing weight could not be fully explained. They assumed that the fraction ethyl acetate failed to inhibit the lipase enzyme while the ethyl acetate fraction was able to inhibit α -glucosidase *in vitro* [19].

Group G had a higher average body weight than the other groups in every week. However, the increase in body weight did not indicate clinical deterioration from the glucose loading. This group

had the lowest reduction in a mean of BSL (112.5 ± 27.00 mg/dl). Meanwhile, Group G which was treated with ethanol extract 150 mg/kg b.w. was able to withstand weight loss. This condition happened due to the presence of saponins which acted like insulin that stimulating glucose absorption with Glut4 expression raise, contributing in the store of glucose in adipocyte cells in the form of glycogen through stimulation of glycogenesis in glycogen synthetase, and inhibiting the enzyme gluconeogenesis of glycogen phosphorylase [20], [21], [22], [23].

Weight loss can also be an indicator of improved insulin sensitivity and well-controlled glycemic. However, while there was weight loss in Group C at week 3, weight gain occurs in the control Groups A, B, C, and D induced by STZ-NA-HFD which could also be caused by the impaired insulin performance [22]. In DM, glycogen synthesis in rat liver and skeletal muscle is disrupted. The regulation of glycogen metabolism occurs by the enzymes glycogen synthase and glycogen phosphorylase that play a major role in glycogen metabolism. The decreasing of experimental muscle glycogen storage of diabetic rats has been associated with reduced glycogen synthase activity and increased glycogen phosphorylase activity [21]. Weight loss in Group C was caused by the poor insulin performance in which Group C had the highest average insulin resistance level of 5.87 ± 4.97 . Therefore, there are indications of beta-cell damage resulting from hypoinsulinemia, hyperglycemia, and followed by excessive breakdown of muscle protein and fat to meet the energy requirements that are lacking due to the inadequacy of carbohydrates as an energy supplier. There was also a decrease in the rest of the extract treated groups with a value below the range of Group I [15], [24], [25], [26].

A glucose load test was performed to determine the body's ability to use glucose as the main source [26]. In all groups in this study, the mean of BSL 30 min after loading was increased, reaching a peak at 1 h after loading and going back down at 1.5 h and 2 h after glucose loading. Groups that have a good ability to use glucose from 30 min to 2 h after loading were the group with metformin treatment, Group K while the group with the lowest mean of BSL was in Group I with a value of 115.75 ± 1.70 mg/dl. Even though metformin rapidly reduced the mean of BSL starting from 30 min, the effectiveness of hypoglycemia in 2 h after loading was not better when compared with Group I who received 100 mg/kg b.w. ethyl acetate fractionation. The effects of hypoglycemia fractionation are caused by flavonoids, alkaloids, triterpene, glycosides, and saponins which result in the accumulation of glycogen through glycogen synthetase which is stimulated by insulin secretion [26].

Phytochemical in fruits and vegetables has an important role in reducing oxidative stress. The content of non-enzymatic antioxidants such as A, C, E, and enzymatic antioxidants such as SOD and CAT in fruits and vegetables is active compounds for

reducing oxidative stress [27]. In this study, the highest enzymatic antioxidant SOD was in Group H treated with ethyl acetate 45 mg/kg b.w. Ethyl acetate fraction can increase SOD which acts as a first-line antioxidant that is able to withstand ROS. The SOD protects cells from ROS by reducing oxygen radicals, which damages membranes and biological structures. SOD can catalyze disposal oxygen radicals to H_2O_2 which then deactivated to H_2O by CAT. The SOD can act as a major defense against ROS and prevent further production of free radicals. The SOD activity was found to be lower in diabetic subjects due to inactivation by H_2O_2 or by enzyme glycation, which occurs in diabetes. CAT is a protein heme, which is present in almost all mammalian cells and is responsible for reducing H_2O_2 to protect cells from highly reactive OH radicals. The reduced CAT activity can also be caused by inactivation by the glycation enzyme. Any combination with antioxidant properties can contribute to the reduction of partial or total oxidative damage. As a result, eliminating oxygen radicals and hydroxyl radicals are perhaps one of the most effective defenses against disease. This research shows that treatment with ethyl fraction 45 mg/kg b.w. can increase SOD activity as a compensatory mechanism against high oxidative stress precursors such as superoxide, hydroxyl radicals, and hydrogen peroxide, which are free radicals that are most often found in hyperglycemia conditions [27], [28].

Flavonoids have been proven to be good antioxidants in the treatment of oxidative stress induced by STZ [21]. Oxidative stress levels assessed by MDA markers were lower in the H group given ethyl acetate 45 mg/kg b.w. This was due to the high antioxidant SOD that could reduce free radicals so that oxidative stress decreased in this group [27], [28].

Liver plays a role in regulating glucose homeostasis through insulin that regulates glucose homeostasis between incoming glucose (glucose uptake and glycogen synthesis) and glucose out (glycogenolysis and gluconeogenesis). Insulin mediates physiological processes through different mechanisms involving increasing levels of plasma membrane glucose transporter 4 (GLUT4); hexokinase stimulation and glycogen synthase kinase 3 (GSK3); inhibition of glycogen phosphorylase; phosphoenolpyruvate carboxykinase (PEPCK); and glucose-6-phosphatase (G6Pase). The decrease in liver glycogen may be caused by the decrement of GLUT4 regulation, hexokinase, and GSK3 due to the absence of insulin [29]. In this research, the lowest insulin levels were in Group E which received ethanol extract 45 mg/kg b.w. with insulin levels 0.44 ± 0.24 . Although insulin levels were low in Group E, insulin resistance was also low in 0.16 ± 0.08 . The level of fasting BSL in the spectrophotometry showed mean levels of 155.16 ± 31.92 . This situation shows that ethanol extract 45 mg/kg b.w. did not stimulate insulin but increases insulin sensitivity.

Conclusion

Ethyl acetate fraction, even the lowest dose, was better in reducing the body weight of rat compared to ethyl fraction 100 mg/kg b.w. and 150 mg/kg b.w.; and even better than other treated and control groups. Ethanol extract 150 mg/kg b.w. was able to increase body weight better than ethanol 45 mg/kg b.w. nor 150 mg/kg b.w. nor with ethyl acetate fraction. Ethyl acetate fraction 100 mg/kg b.w. was able to reduce BSL in 2 h loading better than other treatment groups. Ethyl acetate fraction 45 mg/kg b.w. could increase the endogenous antioxidant activity of SOD while reducing oxidative stress. Ethanol extract 45 mg/kg b.w. was unable to stimulate insulin secretion while having hypoglycemia capacity through the raise of insulin sensitivity. Ethanol extract 45 mg/kg b.w. could decrease insulin sensitivity.

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