JKKI
SCIENTIA EST BASIC VITAE

Jurnal Kedokteran dan Kesehatan Indonesia

Indonesian Journal of Medicine and Health

Journal homepage: https://journal.uii.ac.id/JKKI

Effects of methanol extracts of insulin leaves (*Tithonia diversifolia* (hemsl.) A. Gray) on insulin resistance and secretion of alloxan induced-obese diabetic rats

Raden Edi Fitriyanto[1]*, Sugiarto[2], Dwi Tonang Ardiyanto[2]
[1]Department of Biochemistry, Faculty of Medicine, Universitas Islam Indonesia, Yogyakarta, Indonesia
[2]Nutrition Science Postgraduate Program, Universitas Sebelas Maret, Surakarta, Indonesia

Original Article

ARTICLE INFO

Keywords:
Obesity, diabetes mellitus, Tithonia diversifolia (Hemsl.) A. Gray, Wistar rats, HOMA-IR index, HOMA-B index, insulin resistance, insulin secretion

***Corresponding author:**
edi.fitriyanto@uii.ac.id

DOI: 10.20885/JKKI.Vol11.Iss2.art11

History:
Received: March 3, 2020
Accepted: August 11, 2020
Online: August 31, 2020

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ABSTRACT

Background: Obesity can lead to insulin resistance contributing to diabetes mellitus (DM). Empirically, insulin leaves of Tithonia diversifolia (Hems.) A. Gray (Td) can be used as a traditional DM medicine.
Objective: This study aims to determine effects of Td methanol extracts on insulin resistance and secretion in DM rats induced by a high-fat diet and alloxan.
Methods: This study was a randomized pre and post-test control group. Its research subjects were divided into five groups: a normal group, an obese group with diabetes without therapy (KN), an obese group with DM treated by the Td extracts with a dose 50 mg/kg, 250 mg/kg, and 500 mg/kg. The DM obesity was induced by a high-fat diet followed by administration of alloxan with a dose of 135 mg/kg intraperitoneally. The Td extracts was administered after 7 days of DM induction by using gastric swabs. Insulin resistance in the rats was measured based on HOMA-IR index values, while their insulin secretion was based on HOMA-B values. A statistical analysis was conducted by a 95% confidence level.
Results: Blood glucose levels decreased to normal levels in all the groups administered by the Td extracts, except in the KN group. The HOMA-IR index values in all groups treated by the Td extracts did not increase except in the KN group. The HOMA-B index values in groups with doses of 50 and 250 mg/kg BW experienced a higher increase and significantly differed with the KN group.
Conclusion: The administration of the Td extracts with doses of 50 and 250 mg/ kgBW in the DM rats could decrease blood glucose levels, could prevent insulin resistance and could increase insulin secretion.

Latar Belakang: *Obesitas dapat menimbulkan resistensi insulin sehingga mengakibatkan diabetes melitus (DM). Secara empiris, daun* Tithonia diversifolia (Hems.) A. Gray (Td) *dapat digunakan sebagai obat tradisional DM.*
Tujuan: *Penelitian ini bertujuan untuk mengetahui pengaruh ekstrak metanol Td terhadap resistensi dan sekresi insulin pada tikus DM yang diinduksi diet tinggi lemak dan aloksan.*
Metode: *Penelitian ini merupakan randomized pre and post-test control group. Subjek penelitian dibagi menjadi lima kelompok yaitu; kelompok normal, kelompok obesitas DM tanpa terapi (KN), kelompok obesitas DM dengan pemberian ekstrak metanol Td 50 mg/kgbb, 250 mg/kgBB, dan 500 mg/kgBB. Obesitas DM diinduksi dengan diet tinggi lemak dilanjutkan injeksi aloksan 135 mg/kgBB secara intraperitoneal. Pemberian ekstrak Td dilakukan setelah induksi DM selama 7 hari melalui sonde lambung. Resistensi insulin*

diketahui dari nilai indeks HOMA-IR, sedangkan sekresi insulin dari nilai HOMA-B. Analisis statistik dilakukan dengan tingkat kepercayaan 95%.

Hasil: *Kadar glukosa darah mengalami penurunan sampai kadar normal pada semua kelompok yang diberikan ekstrak metanol Td, kecuali kelompok KN. Nilai indeks HOMA-IR pada semua kelompok Td tidak mengalami peningkatan kecuali kelompok KN. Indeks HOMA-B pada kelompok dosis 50 dan 250 mg/kg BB mengalami peningkatkan lebih tinggi dan berbeda bermakna dengan kelompok KN.*

Kesimpulan: *Pemberian ekstrak metanol daun insulin dosis 50 dan 250 mg/kgBB pada tikus obesitas DM menurunkan kadar glukosa darah, mencegah resistensi insulin dan meningkatkan sekresi insulin.*

INTRODUCTION

Obesity is a complex disorder that involves a control of appetite and energy metabolism, causing excessive fat accumulations and health problems. It is a chronic metabolic disorder that affects public health.[1] It is clinically determined by values of body mass index (BMI), which is a result of dividing body weight (kilograms) by square of height (meters). The BMI values for Asian people can be classified as underweight (less than 18.5 kg/m^2), normal (18.5 - 24.9 kg/m^2), overweight (25.0 - 29.9 kg/m^2) and obese (more than 30 kg/m^2).[2] It also can cause metabolic disorders, leading to increasing levels of cholesterol, triglycerides and insulin resistance, and it also can increase risks of coronary heart diseases, ischemic strokes, type 2 diabetes mellitus (DM), and mortality.[3–6]

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycaemias due to abnormalities in insulin secretion, impaired insulin mechanism or both of them, that can cause chronic complications in the eyes, kidneys, nerves and blood vessels. Problems of insulin mechanism can occur due to obesity caused by an increase of free fat levels in the blood leading to inflammation causing for insulin resistance.[4,7] Insulin resistance is loss of tissue sensitivity to insulin hormones. The insulin resistance causes problems for glucose metabolism. In the first stage, the body compensates for the insulin resistance by stimulating pancreatic β cells to increase its production contributing to an increase of hormone levels. At the next stage, the pancreatic β cells will be damaged so that they cannot function properly.[1] Damages in the pancreatic β cells can be caused by environment factors.[8]

DM sufferers have increased in last recent years. In 2014 there were more than 387 million people diagnosed with DM, and it was estimated that 175 million people with DM were undiagnosed. This number is estimated to increase to 592 million sufferers in 2035. Meanwhile, data from the International Diabetes Federation (IDF) stated that in 2010 were 7 million of DM sufferers, and it was estimated that it will increase to 12 million in 2030.[9] Indonesia Basic Health Research (RISKESDAS) in 2007 found that prevalence of DM in urban societies above 15 years old was 5.7%. The prevalence of DM sufferers in 2013 was 6.9%, increasing to 8.5% in 2018. In 2018 the most prevalence was in a range 55-64 years old and 65-74 years old.[10,11]

Today some plants have been found as a medicine for DM.[12] Then some plants found can increase a treatment for insulin resistance.[13] Some studies also show hope for improvement and function of pancreatic β cells.[14,15] Meanwhile, some existing hypoglycaemic drugs also have some limitations.[16] Therefore, research on potential substances to treat DM can provide clear information about effects of the substances. Insulin leaves or Mexican sunflower (Tithonia diversifolia), also called rondho semoyo, harsaga, paik wood, and kipahit and included in the Asteraceae family, have been widely used for traditional DM treatment. This plant is known to have anti-oxidant and anti-proliferative effects.[17] This plant is easily found in various places in Indonesia as it can grow wildly and is easy to bred, so it can be a cheap alternative of DM herbal treatment.[18–20] Thus, this study is to prove effects of the insulin leaves on insulin resistance levels and secretion functions of pancreatic β cells by using parameters of the HOMA-IR index and the HOMA-B index.

METHODS

Research design

This study is a laboratory experimental research with pre and post-test control group design. Its subjects were white Wistar (*Rattus norvegicus*) with DM obesity that were treated by administering various doses of insulin leaf methanol extracts. This study examined effects of administering the extracts on the levels of insulin resistance and secretion by using the parameters of the HOMA-IR and HOMA-B indices before and after treatment.[21]

Research subject

This study used experimental animals to avoid adverse effects of the treatment for humans. The number of rats used was 25. Their sex was male and their age was approximately 2 months old. Its sample size was calculated by using a Federer formula, namely (n-1) (t-1)> 15, as (t) was the number of treatments, and (n) was the number of samples per group.22 Results of calculation of the number of the rats per treatment group were at least 5 rats.[21] Next, the rats were divided into five groups by simple randomization: Normal Rat Group (TN), Negative Control Group (KN), Treatment Group 1 (P1), Treatment Group 2 (P2) and Treatment Group 3 (P3).

Extract production

The extracts were made at the Research Laboratory of the Faculty of Medicine, Universitas Islam Indonesia, started by selecting healthy and undamaged leaves from sequences of 5-10 counted from the tip. The fresh leaves were washed by water to clean from dirt, and then they were dried at a room temperature (for about 2 weeks). The dried leaves were crushed into a powder and stored in a tightly closed container in a dry place. A maceration method was used to extract the insulin leaves with a ratio of 1:10 (powder and analytical methanol solvent), and they were left for 48 hours. Then filtering with filter papers was conducted to obtain filtrates. Results of the filtering were concentrated with a rotary evaporator at a temperature of 60˚C. To remove the remaining solvent, evaporation was conducted on a water bath at a temperature of 60˚C to obtain thick extracts. Evaporation and its process were maintained in optimal temperatures to avoid damages for active substances.[23] The obtained extracts were a form of sediments resulted from the filtering papers, and they were collected in a pot container. Some of the extracts were tested for pharmacognosy of the active substances in the Laboratory for Testing of Drugs, Food and Cosmetics, the Pharmacy Study Program of Universitas Islam Indonesia. The test was conducted by a chromatography method using the High-Performance Liquid Chromatography (HPLC).

Induction and treatment

The normal rat group was treated by a standard diet. The standard diet was in the following: maximum water (12%), crude protein (19%), crude fat (5%), maximum crude fibre (4.5%), maximum ash (6.5%), calcium (0.9-1.2%), phosphorus (0.7-0.9 %). Meanwhile, the negative control group and the treatment group were treated by the standard died added by 3 cc of liquid lard per day for 4 weeks. Treating by diet with high calories and fat have proved to cause obesity in monkeys, dogs, pigs, hamsters, squirrels and rats. In experimental animals, percentages of energy derived from fat in the diet has a positive correlation with body fat levels. A high-fat diet also can cause an increase in appetite (hyperphagia).[25,26]

The obesity was measured by a significant increase of body weight above normal, by assuming that all the control rats treated in the laboratory was normal. Parameter of obesity of the rats was the body mass index (BMI); with a formula: body weight (g) were divided by the rat length square (cm^2). The length of the rats was measured from the tip of the nose to the anus. Normal BMI scores of the rats ranged from 0.45 to 0.68 g/cm^2; it was considered obesity if the BMI was above normal.[27]

After the rats met the parameters of obesity, DM induction was conducted by using alloxan

monohydrate at a dose of 135 mg/kgBW intraperitoneally in the groups of P1, P2, and P3. Before the induction, the rats were treated to fasting for 10 hours. After 4 days, their fasting blood glucose levels were measured. Normal rat glucose levels were 50-135 mg/dL, and the rats were considered as DM if the fasting blood glucose levels were above normal; if hyperglycaemia did not occur, the induction was repeated with the same dose.[28-30]

The insulin extracts were administered after the DM induction was successful for 7 days by using a gastric swab with a volume of 3 cc. Before administering, the extracts were weighed by using an analytical balance according to doses per body weight of the rats, and they were diluted with enough dimethyl sulfoxide (DMSO) and added by distilled water with a volume of 3 cc for each rat. The doses of the extracts in the groups were: the P1 had a dose of 50 mg/kgBW; the P2 had a dose of 250 mg/kgBW; and the P3 had a dose of 500 mg/kgBW. In the normal rat group (TN) and the negative control (KN) group, 3 ml of distilled water was given by using a gastric swab.

Level measurement of blood glucose and insulin

After treated for 7 days, the rats were set to fasting for 10 hours, and their venous blood samples were taken. The blood samples were taken by using a haematocrit microcapillary tube inserted into the retroorbital venous with a volume of 3 cc. When taking blood, anaesthesia were injected to the rats with a volume of 0.1 cc ketamine HCl to make the rats calm during the treatment. The blood samples were collected in a tube and rotated by centrifuge at a speed of 5000 ppm for 10 minutes to obtain serums. Examination of the fasting blood glucose levels was conducted quantitatively (in unit of mg/dL) by using the enzymatic colorimetric glucose oxidase-phenol aminopheazone (GOD-PAP) method. Then quantitative examination of insulin levels was by using the ELISA method in micro units/millilitre (μU/ml).

Level measurement of insulin resistance and secretion

The results of the examination of fasting blood glucose levels and fasting blood insulin levels were used to measure an index of the homeostatic model insulin resistance assessment (HOMA-IR) and an index of homeostatic model assessment pancreatic β cells (HOMA-B). The HOMA-IR index was used as a parameter of the level of insulin resistance and secretion, while the insulin secretion level was measured based on the HOMA-B index. The maximum level of the HOMA-IR index was 1 demonstrating a state of complete insulin resistance, while the maximum levels of HOMA-B was 100% indicating a complete level of secretion.[4,31] The HOMA-IR is also associated with some risk factors for a complicating DM, such as cardiovascular disorders and decreasing functions of kidney.[32]

The measurement of the HOMA-IR and HOMA-B indices were in the following: the HOMA-IR index was FI multiplied by FG/405, in which FI (fasting insulin) was a fasting insulin level (μg/L) and FG (fasting glucose) was a fasting blood sugar level (mg/dL). The HOMA-B index was = 20 x FI/FG-63 in percentage.

Statistical analysis

All obtained data in this study were average with a confidence level of 95%. Data normality test of this study used a Shapiro-Wilk test. Its normally distributed data, difference test before and after treatment, used a paired-t test; meanwhile, its mean difference test between groups applied One-Way ANOVA followed by a post hoc test (Tukey HSD) if necessary. Data test that were not normally distributed, difference test before and after treatment, applied a Wilcoxon test; meanwhile, mean difference test between treatment groups used a Kruskal Wallis test that can be followed by a Mann Whitney test to identify different groups if needed.[21,22]

RESULTS

Pharmacognosy test on Td extracts indicated that the Td extracts contained flavonoids, alkaloids, saponins and polyphenols, but they did not contain terpenoids (Table 1). Measurement results of the mean BMI of the rats at randomization showed that all groups were within normal limits. The mean BMI after 4 weeks after administering additional liquid of lard in the KN, P1, P2, and P3 groups revealed that all the rats met the criteria of obesity (more than 0.68 g/cm^2), and the mean BMI of the KN group treated by the standard diet was within normal limits (Table 2)

Table 1. Results of Pharmacognosy Test on Extracts of Insulin Leaf Methanol

Compound Identification	Result
Flavonoid	+
Alkaloid	+
Saponin	+
Polyphenol	+
Terpenoid	-

Table 2. Body Mass Indices of the Rats

Group	Mean BMI of the Rats + SEM (g/cm^2)	
	BMI at randomization	BMI after obesity induction
TN	0.56+0.05	0.57+0.01
KN	0.57+0.03	0.81+0.04
P1	0.54+0.03	0.77+0.02
P2	0.54+0.03	0.76+0.02
P3	0.56+0.02	0.78+0.02

Note: TN: normal rat group; KN: negative control group; P1: group with a dose of 50 mg/kg BB of insulin extracts; P2: group with a dose of 250 mg/kg BB of insulin extracts; P3: group with a dose of 500 mg/kg BB of insulin extracts.

The results of examining fasting serum glucose and insulin levels before and after administering the Td extracts can be seen in Table 3. Statistical tests on the data above indicated a significant decrease in the mean fasting blood glucose levels in the KN, P1, P2, and P3 groups. In the KN group that was without treatment, decreases of glucose levels were still within criteria of DM; meanwhile, in the P1, P2, and P3 groups the levels decreased into normal limits. The results of the Mann Whitney test found that the decrease of the glucose levels due to the Td extracts with doses of 50 and 250 mg/dl was more significant than the KN group. The mean of fasting blood insulin levels after treatment did not change in the normal control group but increased significantly in the negative control and treatment groups. ANOVA test results demonstrated a significant difference between the normal control group and the DM group. Then the increase of insulin levels of the DM groups with or without of the Td extracts had no difference.

Table 3. Levels of Fasting Glucose and Insulin of the Rats before and after the Treatment.

Group	N	Mean glucose levels fasting blood + SEM (mg/dL)		p*	p**	Mean insulin levels fasting blood + SEM (mg/dL)		p*	p***
		Before treatment	After treatment			Before treatment	After treatment		
TN	5	96.60+1.50	96.60+1.72	1.00		2.16+0.14	1.84+0.06	0.145	
KN	5	174.00+17.25	132.00+4.03	0.046		1.44+0.22	2.80+0.32	0.004	
P1	5	201.80+4.28	112.80+8.41	0.001	0.004	1.52+0.32	2.86+0.39	0.001	0.000
P2	5	205.40+5.40	103.20+11.18	0.001		1.38+0.25	2.74+0.24	0.001	
P3	5	180.20+18.51	115.20+7.90	0.010		1.76+0.61	2.48+0.61	0.001	

Note: : TN: normal rat group; KN: negative control group; P1: group with a dose of 50 mg/kg BB of insulin extracts; P2: group with a dose of 250 mg/kg BB of insulin extracts; P3: group with a dose of 500 mg/kg BB of insulin extracts; *: Paired-t test; **: kruskal wallis test; ***: Anova test

Measurement results of the HOMA-IR index before and after treatment pointed out that the DM group treated with the Td extracts did not increase the insulin resistance. On the other hand, the DM group without treatment indicated a significant increase of insulin resistance (almost full; approaching level 1). The difference test for changes in the HOMA-IR values by using the Kruskal Wallis test found a significant difference between the negative control group and the other groups (Table 4).

Table 4. Index Differences of HOMA-IR of the Rats before or after Treatment.

Group	N	Mean indices of HOMA-IR + SEM (mg/dL)		p*	p**
		Before treatment	After treatment		
TN	5	0.52+0.04	0.45+0.01	0.147	
KN	5	0.59+0.07	0.91+0.09	0.001	
P1	5	0.77+0.17	0.83+0.17	0.265	0.001
P2	5	0.70+0.14	0.72+0.14	0.078	
P3	5	0.74+0.25	0.69+0.15	0.600	

Note: HOMA-IR: homeostatic model assessment insulin resistance; * Paired-t test; ** Kruskal-Wallis test.

After the treatment, all the groups with or without treatment showed an increase of the HOMA-B values except in the normal control group. However, the highest change occurred in the group administered by the Td extracts with a dose of 250mg/kgBW, followed the group with a dose of 50mg/kgBB. Based on the Mann Whitney test, the increase of HOMA-B index values indicated that the insulin secretion was significantly different from the group without treatment.

Table 5. Difference of HOMA-B Indices of the Rats before and after Treatment

Group	N	Mean indices of HOMA-IR + SEM (mg/dL)		p*	p**
		Before treatment	After treatment		
TN	5	23.10+0.73;(0.761)	20.04+1.60;(0.199)	0.249	
KN	5	5.72+1.82;(0,284)	14.96+2.03;(0,791)	0.007	
P1	5	3.89+0.76;(0.293)	21.25+0.91;(0.870)	0.001	0.003
P2	5	3.50+0,61;(0.152)	30.47+6.74;(0.681)	0.019	
P3	5	6.12+2.20;(0.491)	12.97+5.80;(0.324)	0.026	

Note:
Paired-t test, significant different if p**< 0,05
Kruskal-Wallis test after treatment, difference of HOMA-B before treatment and after treatment.

DISCUSSION

Induction using lard and a high calorie diet were successful in making the rats in this study obese as indicated by an increase of their BMI. Treating a high-calorie diet aimed to increase the insulin resistance closely related to occurrences of DM.[33] Wistar rats are experimental animals that are often used because a high calorie and fat diet can increase their weight easily. Most of the rats tend to get fat with this diet. Outbred rat models, including Sprague-Dawley and Wistar, are strains commonly used to study obesity because their weight is easy to increase.[25] Obesity is also a risk factor for insulin resistance and type 2 DM. Fat tissue in obesity produces various compounds, including hormones, cytokines, and free fatty acids (FFA).[34,35] A chronic mild inflammatory process contributing to an increase of insulin resistance also occurs in the obesity.[36]

Induction with alloxan monohydrate after obesity in this study was successful to increase the blood glucose levels. Alloxan (2,4,5,6-tetraoxyprimidine; 5,6-dioxyuracil) is a hydrophilic and unstable compound that can be administered intravenously, intraperitoneally and subcutaneously. A dose of intravenous alloxan injection which is effective for the induction of DM starts from 65 mg/kg, and the dose is two to three times higher when it is administered intraperitoneally or subcutaneously.[30] Alloxan has high activity against compounds containing SH, GSH, cysteine and sulfhydryl compounds. Alloxan is reduced to hyaluric acids and gets re-oxidation to alloxan; this redox reaction produces superoxide radicals. The superoxide radicals are spontaneously dismutated into hydrogen peroxide helped by enzyme superoxide dismutase. Pancreatic β cells experience DNA damages caused by a poly ADP-ribosylation stimulation, a process in DNA repair. Alloxan also releases ferritin ions and reduces them to ferrous ions, together with hydrogen peroxide that are highly reactive hydroxy compounds.[30]

Administration of the Td extracts, especially with doses of 50 and 250 mg/kgBW for 7 days in the DM rats, was proven to significantly reduce the glucose levels. A pharmacognosy test on the Td extracts demonstrated compounds of flavonoids, alkaloids, saponins and polyphenols. Another research on insulin leaf flowers also showed compounds of phenols, tannins, flavonoids, alkaloids, and saponins.[24] Flavonoids are polyphenolic compounds that have antioxidative characteristics and can modulate pancreatic insulin secretion so that they can cause hypoglycaemic effects.[19,37] The hypoglycaemic effects after 4 hours of administration of the Td extracts with a dose of 500 mg/kg in KK-Ay rat models significantly decreased the blood glucose levels. The blood glucose levels decreased significantly after 7 hours of administration with a dose of 500 mg/

kg and 1500 mg/kg. Measurement results 2 and 3 weeks after treatment also found that glucose levels decreased significantly.[19] Doses of 500 mg/kgBW of aqueous extracts of the insulin leaves were also shown to significantly reduce blood glucose levels of rats in a minute of 30, 60, 120 and 180 compared to the control group.[37]

The hypoglycaemic effects of the extracts in this study was also supported by examination results of malondialdehyde (MDA), a lipid peroxide product as a marker of decreasing oxidative activities in the treatment group. This indicated that the aqueous extract of insulin leaves inhibited the lipid peroxidation chain reaction in the hypoglycaemic activity.[37,38] The hypoglycaemic effects of the insulin leaves was caused by an improvement of insulin sensitivity and agonist activity of peroxisome proliferator activated receptors containing terpenoid derivatives on insulin leaves20,39. The antioxidative activities of insulin flavonoid extracts of the insulin leaves also contributed to hypolipidemic effects. Examination results of total phenolic compounds and total antioxidant capacity of the aqueous extracts of insulin leaves were 55.92 ± 4.45 GAE mg/g dry weight (93.09 ± 37.91 uM TEAC / mg dry weight).[13,37,39]

In this study, the HOMA-IR index values of the DM rat group without treatment had an increase of insulin resistance, but there was no an increase of insulin resistance in the group administered by the Td extracts with a dose of 50 mg/kg, 250 mg/kg body weight or 500 mg/kg body weight.[44,13] The increase of the HOMA-IR index values was a parameter of the insulin resistance. Although insulin levels in the negative control group also increased due to compensation of high glucose levels, the insulin was less able to enter the glucose into cells (resistant). This was indicated by the high blood glucose levels of the negative control group. The insulin resistance can be caused by obesity. There was a strong relationship between intramuscular fat levels and insulin resistance in humans. Accumulations of fatty acids and their metabolites in cells activated serine/threonine kinase pathways by activation of protein kinase C-θ (PKCθ) which inhibited phosphorylation of the thyronine groups as in normal insulin mechanism. As a result, there was no translocation of GLUT4 to membranes so that glucose remains in the extra cells.[40,41] Obesity also increased production of various proinflammatory cytokines leading to insulin resistance.[42] Increased calorie intakes contributing to an increase of blood glucose levels also played roles in the insulin resistance, with an increase of ROS or a decrease of antioxidant GSH in the body.[34,36] In addition, the increase of insulin resistance could also be influenced by other factors, such as cadmium (Cd).[43]

The secretion function of pancreatic β cells can be measured according the HOMA-B index values reflecting percentages of healthy pancreatic β cells. In the treatment group, all index values increased significantly. A strong and significant correlation was found in the P1 and P2 groups with a dose of 50 mg/kg and 250 mg/kg, and they was significantly different with the increase of HOMA-B in the negative control group. The increase of this index values indicated an improvement of pancreatic β cell secretion after the alloxan induction. This indicated a functional repair of pancreatic β cells. In another study, the Td extracts was proven to reduce cell death and release of LDH from the cytosol in cytotoxicity induction with exposures to hydrogen peroxide in vitro compared to vitamin C administration.[45] It was possible that pancreatic β cell repair could occur in the rats through either recovery or regeneration processes. This study found that the function of pancreatic β cells was found in alloxan-induced DM rats receiving gastrin (G) hormone treatment and epidermal growth factor (EGF) for 7 days. In an in vitro experiment it was found that one of the factors that increase the repair of pancreatic tissue was neogenesis and β14 cell proliferation.[14] Another study stated that there was a β cell repair process in alloxan-induced rats and streptozotocin-induced DM rats.[14,15]

CONCLUSION

The administration of Td extracts in to DM rats with a dose of 50 and 250 mg/kgBW could decrease blood glucose levels and did not cause an increase of the HOMA-IR; however, the Td extracts increased the HOMA-B and significantly differed from the control group.

CONFLICT OF INTEREST

There was no conflict of interest in this study.

ACKNOWLEDGEMENT

None declare.

REFERENCES

1. Skyler JS, Bakris GL, Bonifacio E, Darsow T, Eckel RH, Groop L, et al. Differentiation of diabetes by pathophysiology, natural history, and prognosis. Diabetes. 2017;66:241–55.
2. World Health Organization. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. The Lancet. 2004;363.
3. Organization WH. Global health observatory (GHO) data. 2015.
4. Abel ED, O'Shea KM, Ramasamy R. Insulin resistance: Metabolic mechanisms and consequences in the heart. Arteriosclerosis, Thrombosis, and Vascular Biology. 2012;32(9):2068–2076.
5. Malik VS, Willett WC, Hu FB. Global obesity: trends, risk factors and policy implications. Nature Reviews Endocrinology. 2012;9:13–27.
6. Gadde KM, Martin CK, Berthoud H-R, Heymsfield SB. Obesity, pathophysiology and management. Journal of The American College of Cardiology [Internet]. 2018;71. Available from: https://doi.org/10.1016/j.jacc.2017.11.011
7. Czech MP. Insulin action and resistance in obesity and type 2 diabetes. Nature Medicine. 2017;23:804–14.
8. Hectors TLM, Vanparys C, Ven K van der, Martens GA, Jorens PG, Gaal LF Van, et al. Environmental pollutants and type 2 diabetes: a review of mechanisms that can disrupt beta cell function. Diabetologia. 2011;54:1273–129.
9. International Diabetes Federation (IDF). Diabetes Atlas. 2014.
10. BPP Kementerian Kesehatan. Riset kesehatan dasar tahun. 2013.
11. Kementerian Kesehatan BP dan P. Riset kesehatan dasar tahun 2018. 2018.
12. Preethi PJ. Herbal medicine for diabetes mellitus: A review. International Journal of Phytopharmacy. 2013;3.
13. Mohamed Eddouks, Amina Bidi, Bachir El Bouhali, Lhoussain Hajji NAZ. Antidiabetic plants improving insulin sensitivity. .Journal of Pharmacy and Pharmacology. 2014;66:1297-1214.
14. Song I, Patel O, Himpe E, Muller CJF, Bouwens L. Beta cell mass restoration in alloxan-diabetic mice treated with EGF and gastrin. PLoS ONE. 2015;10(10).
15. N.I. El-Desouki, G.A. Tabl, K.K. Abdel-Aziz EISN. Improvement in beta-islets of Langerhansin alloxan-induced diabetic rats by erythropoietinand spirulina. The Journal of Basic & Applied Zoology. 2015;71:20–31.
16. FuenteValerón P, Pablos-Velasco PL d. Limitations of insulin-dependent drugs in the treatment of type 2 diabetes mellitus. Medicina Clínica. 2013;141:20–5.
17. Hiransai P, Tangpong J, Kumbuar C, Hoonheang N, Rodpech O, Sangsuk P, et al. Anti-nitric oxide production, anti-proliferation and antioxidant effects of the aqueous extract from Tithonia diversifolia. Asian Pacific Journal of Tropical Biomedicine. 2016;6(11):950–6.
18. Suryo A, Wahyuningsih S, Syarif R, Suharmi S, Murini T, Saputra F. Selektivitas ekstrak terpurifikasi daun Tithonia diversifolia (Hemsley) A.Gray) Terhadap sel hela. Tradistional Medicine Journal. 2013;18:22–8.
19. Miura T, Nosaka K, Ishii H, Ishida T. The herb Tithonia diversifolia, in KK-Ay diabetic mice. Biological and Pharmaceutical Bulletin Journal. 2003;28:2152–4.
20. Lin H. Sesquiterpene lactones from Tithonia diversifolia act as peroxisome

proliferator-activated receptor agonists. Bioorganic & Medical Chemistry Letters. 2012;22:2954–8.
21. Murti B. Desain dan ukuran sampel untuk penelitian kuantitatif dan kualitatif di bidang kesehatan. Yogyakarta: Gadjah Mada University Press; 2013.
22. Supriyadi. Statistik kesehatan. Jakarta: Salemba Medika; 2014.
23. Ballesteros LF, Teixeira JA, Mussatto SI. Selection of the solvent and extraction conditions for maximum recovery of antioxidant phenolic compounds from coffee silverskin. Food and Bioprocess Technology. 2014;7:1322–1332.
24. Gama, Da RM, Guimarães, Abreu LC de, Marcelo, Armando-Juniora, et al. Phytochemical screening and antioxidant activity of ethanol extract of Tithonia diversifolia (Hemsl) A. Gray dry flowers. Asian Pacific Journal of Tropical Biomedicine. 2014;4(9):740–2.
25. Gajda A. High fat diets for diet-induced obesity models: A brief review of the scientific literature. www. ResearchDiets. com. 2008.
26. Hariri N, Thibault L. High-fat diet-induced obesity in animal models. Nutrition Research Reviews. 2010;23:270–299.
27. Novelli E, Diniz Y, Galhardi C, Ebaid G, Rodrigues H, Mani F, et al. Anthropometrical parameters and markers of obesity in rats. Laboratory Animals Journal. 2007;41:111–119.
28. Kusumawati D. Bersahabat dengan Hewan Coba. Yogyakarta: Gabjah Mada University Press; 2004.
29. Ighodaro OM, Adeosun AM, Akinloye OA. Alloxan-induced diabetes, a common model for evaluatingthe glycemic-control potential of therapeutic compoundsand plants extracts in experimental studies. Medicina. 2017;53:365–74.
30. Nugroho A. Hewan Percobaan Diabetes Melitus: Patologi dan Mekanisme Aksi Diabetogenik. Biodiversitas. 2006;7:378–82.
31. Ki-Chul Sung, Gerald M. Reaven SHK. Utility of homeostasis model assessment of beta-cell function in predicting diabetes in 12,925 healthy Korean. Diabetic Care Journal. 2013;33:2000–2.
32. Yun Li, Lihua Zhang, Yong Gu, Chuanming Hao TZ. Insulin resistance as a predictor of cardiovascular disease in patients on peritoneal dialysis. Sage [Internet]. 2013;33(4). Available from: https://doi.org/10.3747%2Fpdi.2012.00037
33. Petersen MC, Shulman GI. Mechanisms of Insulin Action and Insulin Resistance. Physiological Reviews [Internet]. 2018;98:2133–2223. Available from: https://doi.org/10.1152/physrev.00063.2017
34. Musi N, Guardado-Mendoza R. Adipose Tissue as an Endocrine Organ. In: Cellular Endocrinology in Health and Disease. 2014. p. 229–37.
35. Shoelson SE, Herrero L NA. Obesity, inflammation, and insulin resistance. Gastroenterology. 2007;132:2169–2180.
36. Ye J. Mechanisms of insulin resistance in obesity. Frontiers of Medicine [Internet]. 2013;7(1):14–24. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3936017/pdf/nihms556133.pdf
37. Thongsom M, Chunglok W, Kuanchuea R, Tangpong J. Antioxidant and hypoglycemic effect of Tithonia diversifolia aqueous leaves extract in alloxan-induced diabetic mice. Advance in Environmental Biology. 2013;7:2116–25.
38. Claudia Di Giacomo, Luca Vanella, Valeria Sorrenti, Rosa Santangelo, Ignazio Barbagallo, Giovanna Calabrese, Carlo Genovese, Silvana Mastrojeni, Salvatore Ragusa RA. Effects of Tithonia diversifolia (Hemsl.) A. Gray extract on edipocyte differentiation of human mesenchymal stem cells. Plos One. 2015;
39. Olukunle JO, Okediran BS, Sogebi EA, Jacobs EB. Hypoglycaemic and hypolipidaemic effects of the aqueous leaf ekstract of tithonia diversifolia. Annual Research and Review in Biology. 2016;4:2656–62.
40. Savage DB, Petersen KF, Shulman GI. Mechanisms of insulin resistance in humans and possible links with inflammation. Vol.

45, Hypertension. 2005. p. 828–33.
41. Samuel VT, Shulman. GI. Integrating mechanisms for insulin resistance: Common threads and missing links. Cell. 2012;148:852–871.
42. Hotamisligil G. Inflammatory pathways and insulin action. International Journal of Obesity Relation to Metabolic Disorder. 2003;27:S53–S55.
43. Samuel Treviñoa, Michael P.Waalkes, José Angel Flores Hernández, Bertha Alicia León-Chavez, Patricia Aguilar-Alonso EB. Chronic cadmium exposure in rats produces pancreatic impairment and insulin resistance in multiple peripheral tissues. https://www.sciencedirect.com/science/journal/00039861 [Internet]. 2015;27–35. Available from: Author links open overlay panelSamuelTreviñoaMichael P.WaalkesbJosé AngelFlores Hernándezc-Bertha AliciaLeón-ChavezaPatriciaAguilar-AlonsoaEduardoBrambilaa
44. Owoyele VB, Wuraola CO, Soladoye AO, Olaleye SB. Studies on the antiinflammatory and analgesic properties of tithonia diversifolia leaf exstract. Journal of Ethnopharmacology. 2004;90:314–21.
45. Chi-Long Juang, Fei Shish Yang, Ming Shuang Hsieh, Hu-Yi Tseng, Su-Chiu Chen H-CW. Investigation of anti-oxidative stress in vitro and water apparent diffusion coefficient in MRI on rat after spinal cord injury in vivo with Tithonia diversifolia ethanolic extracts (TDE) Treatment. BMC Complement Alternative Medicine. 2014;14:447.