Antihyperglycemic effect of Patikan Kebo in the rat models of diabetic

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ARTICLE INFO

A B S T R A C T

Diabetes mellitus is a heterogeneous metabolic disorder characterized by an increase in blood sugar levels due to insulin disorders. Current treatment for diabetes mellitus focuses on controlling and lowering blood glucose. However, long-term treatment for diabetes mellitus can have side effects that cause several medical problems. Medical therapy using natural ingredients as an alternative therapy is currently being considered because of its potential and minimal side effects. Patikan kebo is a natural ingredient containing the flavonoid quercetin group. However, the antihyperglycemic effect of the patikan kebo leaves is not known with certainty. This study aimed to determine the effect of patikan kebo leaf extract (EDPK) on the levels of GDP, insulin, HOMA-IR, and HOMA-β in male rats with diabetes mellitus induced by streptozotocin (STZ) and nicotinamide (NA). The design of this study was a pre-and post-test control group design. 42 male Wistar rats were divided into 7 groups: K (normal mice), KN (DMT2 mice without intervention), KP (DMT2 mice intervened with acarbose 1.8 mg/kg BW/day), P1 (DMT2 mice intervened with EDPK dose 75 mg/kg BW/day), P2 (DMT2 mice intervened with EDPK dose 150 mg/kg BW/day), and P3 (DMT2 rats intervened with EDPK dose 300 mg/kg BW/day). The intervention was given for 7 and 15 days. The data were statistically analyzed using the Two Way ANOVA test. EDPK was proven to decrease GDP and HOMA-IR levels and increase insulin and HOMA-β in the male Wistar rat model of diabetes mellitus induced by STZ+NA. EDPK with a dose of 300 mg/kg BW/day proved to have the strongest antihyperglycemic effect.

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A B S T R A K

Diabetes melitus merupakan penyakit kelainan metabolisme heterogen yang ditandai dengan adanya peningkatan kadar gula darah akibat gangguan sekresi insulin. Pengobatan diabetes melitus saat ini berfokus pada pengendalian dan penurunan kadar glukosa darah. Akan tetapi lama terapi pengobatan pada diabetes melitus dapat memberikan efek samping yang menyebabkan beberapa masalah medis. Terapi pengobatan menggunakan bahan alami sebagai terapi alternatif saat ini lebih dipertimbangkan karena potensi serta minimalnya efek samping yang diberikan. Patikan kebo merupakan bahan alami dengan kandungan flavonoid golongan quercetin. Namun saat ini belum diketahui secara pasti efek antihiperglikemik dari daun patikan kebo tersebut. Penelitian ini bertujuan untuk mengetahui efek ekstrak daun patikan kebo (EDPK) terhadap kadar GDP, insulin, HOMA-IR, dan HOMA-β pada tikus jantan diabetes melitus yang diinduksi Streptozotocin (STZ) dan Nicotinamide (NA). Rancangan penelitian ini dengan pre and post test controlled group design. 42 ekor tikus jantan wistar dibagi menjadi 7 kelompok; K (tikus

normal), KN (tikus DMT2 tanpa intervensi), KP (tikus DMT2 diintervensi dengan acarbose 1,8 mg/kgBB/hari), P1 (tikus DMT2 diintervensi EDPK dosis 75mg/kgBB/hari), P2 (tikus DMT2 diintervensi EDPK dosis 150mg/kgBB/hari), P3 (tikus DMT2 diintervensi EDPK dosis 300mg/kgBB/hari). Intervensi diberikan selama 7 dan 15 hari. Data dianalisis statistik menggunakan uji Two Way Annova. EDPK terbukti dapat menurunkan kadar GDP dan HOMA-IR, serta meningkatkan insulin dan HOMA-β pada tikus jantan wistar model diabetes melitus yang diinduksi STZ+NA. EDPK dengan dosis 300 mg/kg BB/hari intervensi terbukti memiliki efek antihipeglikemi paling kuat.

INTRODUCTION

Diabetes mellitus is a disease known as the "silent killer" and is a big problem in the world. Globally, Indonesia is ranked seventh with the number of people with diabetes mellitus of 463 million, and this will continue to increase to 537 million in 2021, this number is predicted to continue to increase until 2045 (IDF, 2017). Diabetes mellitus in Indonesia continues to increase every year, there are 10.9% of people live with diabetes mellitus (Kesehatan, 2018). The most common type of diabetes mellitus is type 2 diabetes mellitus (DMT2), almost 90% of DM sufferers have type 2 DMT.

Diabetes mellitus is usually characterized by the body's inability to produce insulin (IDF, 2017), and increased blood sugar levels during fasting cause the role of insulin to weaken . Type 2 diabetes mellitus is characterized by insulin resistance, where the insulin produced is not used properly, or is not as needed. Progressive decline in pancreatic β-cell function is due to apoptosis, this may be due to aging, genetics, and insulin resistance. The etiology of DMT2 is very complex and involves several factors, such as lifestyle and genetics (Unger RH & Orchi L, 2010).

Treatment of diabetes mellitus currently only focuses on controlling and reducing blood glucose levels, however, long-term treatment of diabetes mellitus with drugs can have side effects that cause several medical problems (Tran et al., 2020). Therefore, medical therapy using natural ingredients that are rich in antioxidants as an alternative therapy is more considered because of the potential and minimal side effects (Tran et al., 2020).

One of the therapeutic approaches to diabetes therapy is to slow the absorption of glucose through inhibition of the α-glucosidase enzyme found in the digestive organs. Inhibition of the α-glucosidase enzyme in the digestive organs of the small intestine will cause the rate of hydrolytic cleavage of oligosaccharides to decrease so that the process of carbohydrate digestion spreads to the lower small intestine and causes the overall rate of absorption of glucose in the blood to slow down (Shai et al., 2010).

Patikan Kebo has been widely used as a traditional herbal medicine in the treatment of diabetes. Several previous studies have reported that Patikan Kebo contains large amounts of phenolic and flavonoid components (Asha S, Deevika B & Sadiq, MA., 2014). One of the phenolics and flavonoids found in patikan kebo is quercetin. Quercetin is a compound that can control fasting blood sugar levels and postprandial hyperglycemia. The main mechanism for lowering blood sugar levels is the activity of inhibitors on the α-glucosidase enzyme, which results in decreased blood sugar absorption (Jadhav & Puchchakayala, 2011).

So far, there has been no study that has analyzed the compounds in patikan kebo leaves taken from the local soil of Gianyar Bali which have the potential to contain quercetin and antioxidant activity against indicators of DM, pancreatic cells, insulin resistance, and sensitivity. This study aims to determine the effect of patikan kebo leaf extract (EDPK) on blood sugar, insulin, HOMA-IR, and HOMA-β.

METHODS

Animals

This study used 42 male Wistar rats aged 8-10 weeks weighing ± 200 g with blood sugar levels > 150 mg/dl. Mice were adapted for 7 days and maintained in a special room under the control of 12 hours of bright light and 12 hours of the dark cycle, given Comfeed and drink ad labitum. The rat model of diabetes mellitus was achieved through intraperitoneal induction with streptozotocin (65 mg/kg BW) and nicotinamide (230 mg/kg BW) for a period of 72 hours. The number of samples was calculated using the Institutional Animal Care and Use Committee (IACUC) (2002) and divided into 7 groups: K is the normal group, KN is the T2DM group without intervention, KP is the group of T2DM rats given acarbose, and P1, P2, and P3 are the groups given EDPK intervention with doses of 75, 150, and 300 mg/kg BW, respectively, per day. Intervention is given through a gastric sonde.

Study Setting

This research is a laboratory experiment with randomized pre and post-test controlled group design. The research was conducted in April-May 2022 at the Laboratory of the Center for Food and Nutrition Studies, Gadjah Mada University (UGM), Yogyakarta, Indonesia. The research subjects used male Wistar rats with the DM model.

Preparation of Intervention

Acarbose dosage

The dose of acarbose used in this study was 1.8 mg/200 g BW/day, obtained from the daily dose of T2DM patients, namely 1 x 100 mg, and then converted to a human dose (70 kg) for mice (200 grams), and a daily dose of acarbose was found to be 0.018 (Stevani, 2016).

Patikan Kebo Leaf Extract (EDPK)

The patikan kebo leaves used in this study were taken homogeneously in the local area from the Alam Jaya Herbal Independent Medicinal Plant Research Center, Gianyar Regency, Bali, Indonesia. The extraction of patikan kebo leaves was carried out at the Center for Food and Nutrition Studies, Gadjah Mada University (UGM) by immersing simplest in a 96% ethanol solution in the ratio of 1 g of simplest to 10 ml of ethanol for ± 48 hours while stirring regularly using a shaker at 150 rpm at room temperature (60 °C). Then the patikan kebo leaves were filtered, and the filtrate was taken using Whatman filter paper number 1. Then the filtrate was put into a rotary evaporator to obtain patikan kebo leaf extract (Tran et al., 2020).

Patikan Kebo Leaf Extract Dosage

The dose used in this study was obtained from the calculating of the quercetin content in patikan kebo namely 189,2 g (Tran et al., 2020). The doses of patikan kebo leaf extract used were 75, 150, and 300 mg/kg BW/day. The middle dose is 150 mg, and then lower and higher doses are determined by doubling or halving the moderate dose.

Measurement Method

Measurement of blood glucose levels

Measurements on blood samples taken via retroorbital were carried out quantitatively using the Enzymatic Colorimetric Test Glucose Oxidase Phenol Aminoantipyrina Peroxidase (GOD-PAP) method using mg/dL units (Subiyono et al., 2016).

Measurement of Insulin Levels

Blood samples were obtained from the retroorbital, and then insulin levels were measured using the ELISA kit method (Zenix-520 Automated Elisa Processor, PT. Sumifin, Indonesia) (Label *mouse insulin* Elisa kit merk *Fine test*).

Homeostasis Model Assessment Index

Insulin resistance was determined using the Homeostasis Model Assessment Insulin Resistance (HOMA-IR) formula (Byun AR et al., 2015), and to determine the level of pancreatic β-cell function strength using the Homeostasis Model Assessment of Pancreatic Beta Cell Function (HOMA-β) formula (Nurhidajah and Nurrahman, 2016).

Data Analysis

In this study, after the data were tested for normality and homogeneity, the results showed that the data were normally distributed and the data variations were homogeneous (p >0.05). Furthermore, the data were subjected to the *two way annova* test to determine the difference in effect between all treatment groups and continued with the Post Hoc Tukey HSD test if the data was normal and homogeneous which was intended to see differences in each dependent variable. For data distribution that is not normal, testing is done with the Friedman test. The results are declared significant or there is a difference if found (p <0.01).

RESULTS AND DISCUSSION

The average blood sugar, insulin, HOMA-IR, and HOMA-β levels are shown in Table 1

Effects of Patikan Kebo Leaf Extract on Blood Glucose

Modeling mice into DMT2 animal models using the diabetogenic agent STZ-NA. The use of STZ will inhibit the Krebs cycle and reduce the supply of oxygen to the mitochondria. Limited mitochondrial ATP production results in a drastic reduction of pancreatic beta-cell nucleotides (Chandran et al., 2016). STZ causes a decrease in insulin secretion into the blood vessels, its toxicity will cause the breakdown of insulin-containing pancreatic β cells (Delgado et al., 2013). Administration of NA early in the induction of STZ in animal models of DMT2 greatly affects pancreatic beta cells and can reduce DNA methylation (Ghasemi et al.,2014), the combination of the use of STZ and NA will provide massive damage protection to pancreatic beta cells (Szkudelski et al., 2013; Furman, 2016).

In this study, experimental animals induced by STZ-NA were shown to have hyperglycemia with blood sugar levels ≥150 mg/dl (Table 1), except for group K, which was the normal group. Normal blood sugar levels in rats range from 70–140 mg/dL (Akbarzadeh, A, 2007). Hyperglycemia is a sign of T2DM (Mufeed Jalil Ewadh et al., 2014).

The α-glucosidase enzyme is related to DM, inhibition of the α-glucosidase enzyme in the digestive organs, namely the small intestine, will cause a decrease in the rate of hydrolytic cleavage of oligosaccharides so that the process of carbohydrate digestion will spread to the lower small intestine and cause the overall rate of absorption of glucose in the blood to slow down (Shai et al., 2010). The diabetes drug acarbose belongs to the alpha-glucosidase class. Patikan kebo functions similarly to the drug acarbose as an inhibitor of the α-glucosidase enzyme. Administration of acarbose in the positive control group at a dose of 1.8 mg/kg BW/day was shown to reduce blood sugar levels to a normal limit of 126 mg/dL.

Patikan kebo leaf extract contains large amounts of phenolic and flavonoid components (Asha S, Deevika B & Sadiq, MA., 2014). One of the phenolics and flavonoids found in patikan kebo is quercetin, this compound can control fasting blood sugar levels and postprandial hyperglycemia . Patikan kebo contains 300 parts per million (ppm) of quercetin, which has an antioxidant effect (Duke, 2010).

Antioxidants are able to prevent further tissue damage and death caused by free radicals. The mechanism of quercetin can control fasting blood glucose levels and postprandial hyperglycemia through its inhibitory activity on the alpha-glucosidase enzyme so that the absorption of glucose can be slowed (Jadhav & Puchchakayala, 2011; Pereira et al., 2011). Quercetin also inhibits GLUT 2 in the intestinal mucosa so that it can reduce glucose absorption, this causes reduced absorption of glucose and fructose from the intestine so that blood sugar levels fall (Ajie, 2015).

This study is in line with Devi's (2017) which showed that after administration of patikan kebo methanol extract at doses of 200 mg/kg and 400 mg/kg, significantly reduced blood sugar levels in DM rats for 7 days of intervention. In Tran's study (2020), the results of the ethanolic extract of Euphorbia hirta Linn at a dose of 500 mg/kg for 15 days were shown to have a hypoglycemic effect or lower blood glucose levels in alloxan-induced diabetes mellitus rats, as well as

other in vitro experiments providing evidence that the extracted ethanol and ethyl acetate fraction inhibited α-glucosidase activity.

Table 1
Effect the patikan kebo leaf extract

Category	Group	Average Pretest H-0	Average Posttest H-7	Average Posttest H-15	$\triangle$ *Mean* ± SD (g)	p[134]
Fasting Blood Glucose (mg/dL)	K	70,69± 1,27	71,64 ± 1,13	72,33±1,09	1,64 ± -0,18[e]	0,001
	KN	295,48± 2,94	260,62 ± 3,21	261,51 ± 3,02	-33,97 ± 0,08[a]	0,001
	KP	262,25± 2,29	206,68 ± 6,00	126,61 ± 5,43	-135,64 ± 3,14[d]	0,001
	P1	262,92± 4,63	230,23 ± 3,59	169,87 ± 2,48	-93,05 ± -2,15[b]	0,001
	P2	260,94± 3,60	218,72 ± 5,03	144,21 ± 3,34	-116,73 ± -0,26[c]	0,001
	P3	265,37± 1,33	206,20 ± 2,51	132,34 ± 2,85	-133,03 ± 1,52[d]	0,001
Mean±SD (g)		230,2± 72,3[A]	199,01 ± 60,68[B]	151,14 ± 58,14[C]		
p[2]		0,001	0,001	0,001		
Insulin levels (pg/ml)	K	563,42±3,10	558,24±3,68	556,10±3,41	-7,32±0,31[a]	0,001
	KN	439,58±3,39	435,16±3,01	431,95±3,79	-61,63±0,4[f]	0,001
	KP	439,12±4,30	492,04±8,44	508,36±3,36	69,24±-0,94[c]	0,001
	P1	437,29±6,80	459,56±6,05	467,64±7,46	30,35±0,66[e]	0,001
	P2	439,28±7,73	460,17±9,05	488,69±4,90	49,41±-2,83[d]	0,001
	P3	445,22±6,99	490,52±9,25	526,21±5,07	80,99±-1,92[b]	0,001
Mean±SD (g)		460,65±46,88[C]	482,61 ± 40,08[B]	496,49 ± 40,8[A]		
p[2]		0,001	0,001	0,001		
HOMA-IR	K	2,95 ± 0,58	2,96 ± 0,50	2,98 ± 0,50	0,03 ± -0,08[e]	0,001
	KN	8,45 ± 0,07	8,39 ± 0,09	8,36 ± 0,12	-0,09 ± 0,05[a]	0,001
	KP	8,53 ± 0,08	7,53 ± 0,29	4,76 ± 0,22	-3,77 ± 0,14[d]	0,001
	P1	8,51 ± 0,22	7,83 ± 0,15	5,88 ± 0,11	-2,63 ± -0,11[b]	0,001
	P2	8,49 ± 0,20	7,45 ± 0,23	5,21 ± 0,13	-3,28 ± -0,07[cd]	0,001
	P3	8,75 ± 0,16	7,49 ± 0,15	5,16 ± 0,11	-3,59 ± -0,05[c]	0,001
Mean±SD (g)		7,61 ± 2,11[A]	6,94 ± 1,84[B]	5,39 ± 1,62[C]		
p[2]		0,001	0,001	0,001		
HOMA-β	K	810,08±139,07	708,03±93,16	651,77±83,58	-158,31±-55,49[a]	0,001
	KN	24,16±0,51	23,78±0,47	23,50±0,39	-0,66±-0,12[b]	0,001
	KP	23,80±0,42	37,02±1,37	86,79±6,57	62,99±6,15[b]	0,001
	P1	23,63±0,58	29,69±0,77	47,28±1,43	23,65±0,85[b]	0,001
	P2	23,97±0,55	31,94±1,13	65,07±2,65	41,1±2,1[b]	0,001
	P3	23,75±0,30	37,00±1,00	47,61±25,66	23,86±25,36[b]	0,001
Mean±SD (g)		154,90±301,29[A]	144,58±257,56[A]	159,41±226,18[A]		
p[2]		0,292	0,292	0,292		

Notes: K (normal), KN (negative control), KP (positive control/drug acarbose 1.8 mg/kg BW), P1 (Patikan kebo leaf extract 75 mg/kg BW), P2 (Patikan kebo leaf extract 150 mg/kg BW), and P3 (Patikan kebo leaf extract 300 mg/kg BW).
$\triangle$: difference in variable values (blood sugar, insulin levels, HOMA-IR, and HOMA-β) on day 0 and day 15
[1234]) Statistical results using the Two Way Anova test
[1]) significant effect of dose (group)
[2]) the significance of the effect of the duration of administration
[3]) the significance of the interaction effect of dose and duration of administration
[4]) the significance of the effect of the change in value (intercept)
[abc]) significant effect between doses (Tukey HSD post hoc test)
[ABC]) the significance of the difference in effect between the lengths of administration (Tukey HSD post hoc test)

Effects of Patikan Kebo Leaf Extract on Insulin Levels

Intervention for 7 and 15 days showed significant differences between groups. The group with the acarbose and EDPK interventions had the effect of decreasing insulin levels. The main pathophysiology that underlies the occurrence of type 2 diabetes mellitus genetically, namely defects in pancreatic beta cell function and insulin resistance. When caloric intake is excessive and unbalanced with energy expenditure, it can trigger mitochondrial NADH (mNADH) and reactive oxygen species (ROS). Excessive ROS production will cause the activity of pancreatic β cells and other cells to decrease, and at the same time, hyperglycemia will trigger ROS signals and stimulate insulin (Pitocco et al., 2013).

Modeling DMT2 with mice using the diabetogenic agent STZ-NA. STZ induction will damage insulin-producing pancreatic β cells, the STZ mechanism begins by producing free radicals, which will damage β cells by damaging the pancreatic mitochondrial system and inhibiting O-GIcNAcase through DNA alkylation (Goud, 2015). Administration of nicotinamide protects pancreatic cells from massive damage and only causes partial damage to pancreatic cells, whereas those induced solely by streptozotocin result in experimental animals with β-cell necrosis (Szkudelski et al., 2013). So that the DM condition produced by STZ-NA results

in impaired insulin secretion and insulin resistance (Szkudelski et al., 2013).

In this study, the KN group, which was induced by STZ-NA and was only given standard feed without any intervention, experienced a decrease in insulin levels, while the KP and P1-P3 group, which was induced by STZ-NA experienced an increase in insulin levels. Patikan kebo leaf extract can inhibit and reduce the effects of free radicals generated from STZ so that insulin secretion continues and increases insulin receptor sensitivity in cells that have insulin receptors. This study is in line with the research of Sheliya et al., (2015) which proved that patikan kebo contains a flavonoid of the quercetin group which indicates the presence of an α-glucosidase inhibitor that is comparable to the drug acarbose, this is what is suspected of causing increased insulin secretion and also increased insulin sensitivity. where compounds that act as antioxidants to increase insulin secretion in the calcium pathway are flavonoids (Al-Ishaq et al., 2019).

Effect of Patikan Kebo Leaf Extract on Biomarker Sensitivity Values

HOMA-IR and HOMA-β values are indicators to see insulin sensitivity and the strength of pancreatic beta cell function. In this study, it was found that there was no significant difference in the HOMA-IR values between the KP and P2 groups compared to the K and KN groups, as well as P2 and group P3. This shows that the doses in the P2 and P3 groups have the same ability as the acarbose drug used as a comparison. The P2 and P3 groups showed the most effective dose in reducing the HOMA-IR index value compared to the P1 group during the 7 and 15 days of intervention, and the duration of administration for 15 days was effective in increasing insulin sensitivity based on the HOMA-IR index value. The decrease in the HOMA-IR value is related to the content contained in patikan kebo leaves, namely the quercetin class of flavonoids, which are protective against damage to pancreatic beta cells as insulin producers and can increase insulin sensitivity (Ajie, 2015).

The higher the HOMA-IR value, the higher the degree of insulin resistance. Insulin resistance is measured using the HOMA-IR index based on fasting blood sugar levels and insulin levels, normal values for HOMA-IR < 4.0 and > 4.0 mean insulin resistance occurs (Kurniawati et al., 2017). The HOMA-β value has a significant effect in increasing the HOMA-β value, but it is known that there is no significant difference in both interventions for 7 days and 15 days. These results indicate that the administration of EDPK when compared to the acarbose positive control group has the same effect in reducing HOMA-IR values and increasing HOMA-β values. HOMA-β is an indicator to measure the level of strength of pancreatic β cells in producing insulin (Nugroho, 2017), the greater the value of HOMA-β, the better the strength of β cells. HOMA-β is said to be normal if it is at a value of > 50% (Omiya et al., 2015).

Administration of patikan kebo and acarbose leaf extracts for 7 and 15 days of intervention resulted in an increase in HOMA-β values. The increase in HOMA-β value is thought to be due to the role of bioactive compounds contained in patikan kebo leaves which stimulate GLP-1 hormone secretion and inhibit DPP-4 enzymes such as the role of alkaloids and tannins (Zahra et al., 2017). An increase in the HOMA-β index is also associated with an improvement in pancreatic β-cell dysfunction, where this improvement is mediated by the ability of IRS to increase GLP-1 hormone secretion. The secretion of this hormone is biphasic, with the second phase being induced by the presence of undigested nutrient components in the intestinal lumen. GLP-1 activity can stimulate an increase in pancreatic β-cell mass and pancreatic insulin content (Shen et al., 2011).

LIMITATION OF THE STUDY

In this study, increases in insulin levels, sensitivity, and function of pancreatic beta cells in DMT2-DM model mice treated with EDPK for 7 and 15 days have been biochemically proven, but pancreatic tissue histopathology is unknown.

CONCLUSION AND SUGGESTION

Based on EDPK doses (150 and 300 mg/kg BW/day) during the 15-day intervention, it had the same effect as the drug acarbose in reducing FGB, HOMA-IR, and increasing HOMA-β values, and insulin levels in STZ-NA-induced T2DM rats. While at a dose of 75 mg/kg BW/day, it has an effect, but its effectiveness is not the same as that of the diabetes drug acarbose. It is necessary to carry out further tests with pancreatic histopathological observations to see the picture of improved beta cell function in the pancreas organ after being given the intervention of patikan kebo leaf extract.

Acknowledgment

ETHICAL CONSIDERATIONS

This study was approved by the Research Ethics Committee of the Faculty of Medicine, Sebelas Maret University (No. 13/UN27.06.11/KEP/EC/2022).

Funding Statement

Conflict of Interest Statement

All authors have no conflict of interest in this article.

REFERENCES

Ajie, R. B. (2015). White Dragon Fruit *(Hylocereus undatus)* Potential As Diabetes Melitus Treatment. *Journal Majority*, *4*(1), 69–72.

Akbarzadeh, A. (2007). Induction of Diabetes by Streptozoticin in Rats. *Indian Journal*, *22*(2), 60–64.

Al-Ishaq, R. K., Abotaleb, M., Kubatka, P., Kajo, K., & Büsselberg, D. (2019). Flavonoids and their anti-diabetic effects: Cellular mechanisms and effects to improve blood sugar levels. *Biomolecules*, *9*(9). https://doi.org/10.3390/biom9090430

Asha S, Deevika B & Sadiq, MA. (2014). Euphorbia Hirta Linn- A Review On Traditional Uses. *World Journal Phytochemistry*

and Pharmacology. 3(4), 180–205. https://doi.org/10.11648/j.ijde.20170203.11

Byun A R, Lee WA, Lee HS, Shim KW. (2015). What is the most appropriate lipid profile ratio predictor for insulin resistance in each sex? A cross-sectional study in Korean populations (The Fifth Korea National Health and Nutrition Examination Survey). Diabetology and metabolic syndrome, 28(7):59.

Chandran, R., Primelazhagan, T., Shanmugam, S., & Thankarajan, S. (2016). Antidiabetic activity of Syzygium calophyllifolium in Streptozotocin-Nicotinamide induced Type-2 diabetic rats. Biomedicine and Pharmacotherapy, 82, 547–554. https://doi.org/10.1016/j.biopha.2016.05.036

Delgado, G. T. C., Tamashiro, W. M. da S. C., Junior, M. R. M., & Pastore, G. M. (2013). Yacon (Smallanthus sonchifolius): A Functional Food. Plant Foods for Human Nutrition, 68(3), 222–228. https://doi.org/10.1007/s11130-013-0362-0

Devi, S., & Kumar, M. (2017). To cite this article: Sangeeta Devi, Muneesh Kumar. In-vivo Antidiabetic Activity of Methanolic Extract of Euphorbia hirta L. International Journal of Diabetes and Endocrinology, 2(3), 36–39. https://doi.org/10.11648/j.ijde.20170203.11

Furman BL. 2015. Streptozotocin Induced Diabetic Models in Mice and Rats. Current Protocols in Pharmacology., Vol. 70, p. 5.47.1-5.47.20. https://doi.org/10.1002/0471141755.ph0547s70.

Ghasemi, A., Khalifi, S., & Jedi, S. (2014). Streptozotocin-nicotinamide-induced rat model of type 2 diabetes (review). 101(4), 408–420. https://doi.org/10.1002/0471141755.ph0547s70.

Goud, B. J. (2015). Streptozotocin–A Diabetogenic Agent in Animal Models. International Journal of Pharmacy & Pharmaceuticals Research. 3(1): 253–269

IDF. (2017). Diabetes Atlas Five edition, 5th ed. International Diabetes Federation (IDF).

Jadhav, R., & Puchchakayala, G. (2011). Hypoglicemic and Antidiabetic Activity Of Flavonoids: Boswellic Acid, Ellagic Acid, Quercetin, Rutin On STZ-NA Induced Type 2 Diabetic Rats. International Journal Pharm Pharmaceut Sci ., 2 (2) : 231-256. https://www.researchgate.net/publication/279675150_Hypoglycemic_and_antidiabetic_activity_of_flavonoids_Boswellic_acid_Ellagic_acid_Quercetin_Rutin_on_streptozotocin-nicotinamide_induced_type_2_diabetic_rats

Kemenkes Republik Indonesia. (2018). Riset Kesehatan Dasar (Riskesdas) 2018. Kementrian Kesehatan Indonesia.

Kemenkes Republik Indonesia. 2017. Gizi Dalam Daur Kehidupan. Jakarta: Kemenkes Republik Indonesia.

Kurniawati, D. M., Dharmana, E., Rachmawati, B., & Pemayun, T. G. D. (2017). Pengaruh pemberian ekstrak kedelai hitam (Glycine soja.) berbagai dosis terhadap kadar glukosa darah, kadar insulin, dan HOMA-IR. The Indonesian Journal of Nutrition, 6(1), 44–50. https://doi.org/10.14710/jgi.6.1.44-50

Nugroho, W. (2017). Efek Pemberian Ekstrak Etanol Daun Jarak Merah Terhadap HOMA-IR, HOMA-β, dan aktivitas Pparγ SEl Adiposit Model Tikus DM Tipe 2. Thesis. Universitas Airlangga.

Nurhidajah and Nurrahman. (2016). Hypoglycemic Effect of Red Rice Germ on Insulin Levels, HOMA-IR and HOMA β Index of STZ-NA Induced Rats. Agritech 36(4), 433–439. http://dx.doi.org/10.22146/agritech.16767

Omiya, K., Minami, K., Sato, Y., Takai, M., Takahashi, E., Hayashi, A., Yamauchi, M., Suzuki, K., Akashi, Y. J., Osada, N., Izawa, K.

P., & Watanabe, S. (2015). Impaired β-cell function attenuates training effects by reducing the increase in heart rate reserve in patients with myocardial infarction. Journal of Cardiology, 65(2), 128–133. https://doi.org/10.1016/j.jjcc.2014.04.012

Pereira DF, Cazarolli LH, Lavado C, Mengatto V, Figueiredo MS, Guedes A, Pizzolatti MG, Silva FR. Effects Of Flavonoids On α-Glucosidase Activity: Potencial Targets Of Glucose Homeostatis. Nutrition, 27:1161-7. https://doi.org/10.1016/j.nut.2011.01.008

Pitocco, D., Tesauro, M., Alessandro, R., Ghirlanda, G., & Cardillo, C. (2013). Oxidative Stress in Diabetes: Implications for Vascular and Other Complications. International Journal of Molecular Sciences, 14(11), 21525–21550. https://doi.org/10.3390/ijms141121525

Shai, L. J., Masoko, P., Mokgotho, M. P., Magano, S. R., Mogale, A. M., Boaduo, N., & Eloff, J. N. (2010). Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. South African Journal of Botany, 76(3), 465–470. https://doi.org/10.1016/j.sajb.2010.03.002

Sheliya, M. A., Rayhana, B., Ali, A., Pillai, K. K., Aeri, V., Sharma, M., & Mir, S. R. (2015). Inhibition of α-glucosidase by new prenylated flavonoids from euphorbia hirta L. herb. Journal of Ethnopharmacology, 176, 1–8. https://doi.org/10.1016/j.jep.2015.10.018

Shen, L., Keenan, M. J., Raggio, A., Williams, C., & Martin, R. J. (2011). Dietary-resistant starch improves maternal glycemic control in Goto-Kakizaki rat. Molecular Nutrition and Food Research, 55(10), 1499–1508. https://doi.org/10.1002/mnfr.201000605

Stevani, H. (2016). Praktikum Farmakologi 1st ed. Pusdik SDM Kesehatan.

Subiyono, Martsiningsih M.A, D. G. S. (2016). Gambaran Kadar Glukosa Darah Metode GOD-PAP (Glucose Oxsidase-Peroxidase Aminoantypirin) Sampel Serum dan Plasma EDTA (Ethylen Diamin Terta Acetat) (Vol. 5, Issue 1, pp. 2338–5634). Gambaran Kadar Glukosa Darah. www.teknolabjournal.com

Szkudelski, T., Zywert, A., & Szkudelska, K. (2013). Metabolic disturbances and defects in insulin secretion in rats with streptozotocin-nicotinamide-induced diabetes. Physiological Research, 62(6), 663–670. https://doi.org/10.33549/physiolres.932509

Tran N., Nguye M, T. Q. & L. L. (2020). Screening of antibacterial activity, antioxidant activity, and anticancer activity of euphorbia hirta linn. Extracts. Applied Sciences (Switzerland), 10(23), 1–15. https://doi.org/10.3390/app10238408

Tran, N., Tran, M., Truong, H., & Le, L. (2020). Spray-Drying Microencapsulation of High Concentration of Bioactive Compounds Fragments from Euphorbia hirta L. Extract and Their Effect on Diabetes Mellitus. Foods, 9(7). https://doi.org/10.3390/foods9070881

Unger RH & Orchi L. (2010). Paracrinology of islets and the paracrinopathy of diabetes. Proceedings of the National Academy of Sciences, 107(37): 16009–16012. https://doi.org/10.1073/pnas.1006639107

Zahra, F., Budhiarta, A. A. G., & Pangkahila, W. (2017). Pemberian ekstrak daun cincau (Mesona palustris BL) oral meningkatkan jumlah sel β pankreas dan menurunkan gula darah puasa pada tikus putih (Rattus norvegicus) jantan galur Wistar diabetes. In Jurnal e-Biomedik (eBm) (Vol. 5, Issue 1). https://doi.org/10.35790/ebm.v5i1.15034