The comparison on antihyperglycemic activity between *gedong gincu* mango leaf (*Mangifera indica var. gedong gincu*) and metformin in streptozotocin-induced diabetic rats

Q Aqyun, A F M Z Zein*, V Meidianawaty

1 Faculty of Medicine, Swadaya Gunung Jati University, Jl. Terusan Pemuda, No.1A, Cirebon, West Java, Indonesia 45132

*Corresponding author: fariz_zein_dr@yahoo.com

Abstract. Pharmacologic treatment is one of important modalities in the comprehensive management in type 2 diabetes mellitus, whereas metformin is the first line drug of choice in initiating oral antihyperglycemic agent. Mangiferin, quersetin, tannins, and flavonoid have blood-glucose lowering properties. They are available in mango leaf. *Gedong gincu* mango is widely available in Cirebon, West Jawa, Indonesia, but the study enrolling its leaf in antihyperglycemic effect is not provided yet. This study was aimed to compare antihyperglycemic activity between *gedong gincu* mango leaf (*Mangifera indica var. gedong gincu*) and metformin on streptozotocin-induced diabetic rats. This study was experimental research with pre- and post-test control group design enrolling 30 wistar streptozotocin-induced rats divided into 5 groups of treatment consists of 1 negative control group, and 4 positive control group using metformin 9mg/200gr and 3 groups given gedong gincu mango leaf extract with doses of 150 (P1), 300 (P2) and 600 (P3) mg/200 gr for 7 days. There is significant decrease in blood glucose level in P1, P2, and P3 (96.24mg/dl, 116.62 mg/dl, and 136.21mg/dl). We concluded that *gedong gincu* mango leaf has antihyperglycemic activity in dose-dependent response. The 600 mg/200g of it is not inferior to 9mg/200g of metformin in antihyperglycemic activity.

1. Introduction
A report of Riset Kesehatan Dasar (RISKESDAS) by Departemen Kesehatan Indonesia in 2007 showed that the mean of Diabetes Mellitus (DM) prevalence in urban area, particularly for above 15-year-old inhabitants, was 5.7%. The lowest prevalence was 1.7% in Papua province while the highest one was 11.1% in North Maluku and West Kalimantan province. On the other side, the prevalence of Impaired Glucose Tolerance (IGT) was around 4.0% in Jambi province until 21.8% in West Papua province with the mean as much as 10.2% [1].

Diabetes is a group of metabolic disease with the characteristic of hyperglycemia due to the defect of insulin secretion, insulin resistance, or both. The clinical manifestation of hyperglycemia is usually the occurrence of clinical disorder from its vascular disease. A patient with light impaired glucose tolerance (impaired fasting glucose) still has a risk of having the diabetic metabolic complication [2]. The pharmacologic treatment for diabetes mellitus can be conducted by oral antidiabetic agent or insulin. The first drug of choice in pharmacologic treatment for DM is metformin. Metformin inhibits
hepatic glucogenesis. In other side, it may induce nausea as unfavorable common side effect. Further, it is contraindicated in patients with kidney function impairment due to lactic acidosis. Thus, alternative treatment is necessary. One of the plants believed as antidiabetic is mango [3].

Mango is known containing phenol, flavonoid, and tannin after conducting phytochemical screening to all parts of mango. By giving the ethanol extract of arummanis mango leaf with the high-rise dose on rats, it was gained the significant result from the negative control. Four other studies using infusion of golek mango leaf to rats also showed the different significant blood glucose level from the negative control [5].

Indonesia has many mango varieties, and one of them is gedong gincu mango. It is the leading variety from Cirebon. It has not only a good taste, but also the contents which is as antidiabetic. However, there has not been a study which reveals that the extract of gedong gincu mango leaf can also reduce blood glucose level yet. Thus, we conducted a study to investigate the comparison of the antihyperglycemic activity between gedong gincu mango and metformin in diabetic rats.

2. Materials and Methods

This study was an experimental research with pre- and post-test control group design. The samples were 30 Wistar rats. The technique of sampling used in this study was simple random sampling. This study was conducted in November 2017. Simplisia making was conducted in Mundu, Cirebon while the extracting process in Lansida, Yogyakarta. Both -adaptation and experiment- were conducted at PAU UGM, Yogyakarta.

Tools used in this study were the stable for experimental animal, feeding tube, capillary micro tube, test tube rack, centrifuge tube, small graduated cylinder, oven, stirring spoon, filter, disposable syringe, beaker glass, rotary evaporator, balance, vortex (Stardust FC FC15) and spectrometry UV-VIs. Materials used in this study were the extract of Gedong Gincu mango leaf, male Wistar rats weighing as much as 200-250gram, the standard woof, aqua, ethanol 75% as solvent, Metformin and material for measuring blood glucose level, Glucose Oxidase Phenol 4-Aminoantipirin (GOD-PAP) from Diagnose Systems International (DSI).

In the beginning step of the extraction process, it was conducted a determination of plant gained from Mundu, Cirebon. From the result of the determination, it was true that gedong gincu mango was used (Mangifera indica var. gedong gincu), belonging to the family of Acardiaceae. The next step was simplisia making. The fresh mango leaves were washed until they were really clean and then dried. After the process of drainage for 7 days, it would be gained 900 gram simplisia from 3.5kg fresh gedong gincu mangos.

Simplisia from the mango leaves was 100gram extracted by 200mL ethanol 75%. Ethanol 75% was used in order to make polar and non-polar compound extracted. The extraction took time for 11-12 hours, so that the liquid could be transparent. Then, the liquid extract gained was evaporated by rotary vacuum evaporator at the temperature of 50°C, which is below the ethanol boiling, so the heating below the boiling point of solvent can protect the compound contained in solvent. The evaporation was continued by the water heater until the thick extract was gained.

The 30 Wistar rats were divided to 5 groups consisting 6 rats for each. Rats were firstly adapted with the room condition of the experiment for 7 days. Rats which had been adapted would get fasting for a night. Then, rats were injected by Streptozotosin (STZ) – Nicotinamide (NA) with the dose of 110 i.p for NA and after 15minutes, given the dose of 45 i.p STZ. After 72 hours, the blood glucose was checked to know whether the rats had been diabetic or not. If there was the increasing of rats’ blood glucose level until 200mg/dL, then rats was diabetic[15, 16].

This study used 5 groups; 2 control groups and 3 experimental groups. They were:

a. Group 1 as the negative control group given the standard woof and induced by STZ and NA.

b. Group 2 as the positive control group induced by STZ and NA given the experiment of metformin as much as 9mg/200gr.

c. Group 3 as the experimental group 1 induced by STZ and NA and given the Mango leaf extract with the dose of 150mg/200gr.
d. Group 4 as the experimental group 2 induced by STZ and NA and given the Mango leaf extract with the dose of 300mg/200gr.

e. Group 5 as the experimental group 3 induced by STZ and NA and given the Mango leaf extract with the dose of 600mg/200gr.

The data gained were analyzed statistically by using normality test, Shapiro-Wilk test, because the sample used in this study were 30 rats (<50). Then, they were analyzed by data homogeneity test by using Levene test. The next analysis was One Way Anova to test the hypothesis and then Post-hoc test by using Least Significant Different (LSD) to know which groups were different. The hypothesis test was to know how the influence of giving gedong gincu mango extract and conducted by using parametric statistics test, Paired-T Test.

3. Results and Discussion

The research result of the effectiveness comparison of gedong gincu mango (Mangifera indica var. Gedong Gincu) leaf extract to Wistar rats induced by streptozotocin and nicotinamide can be seen in Table 1. The mean result of reducing blood glucose level on Wistar rats of each group is displayed in Figure 1.

Table 1. The means of white rats’ blood glucose level

| Experimental Groups | Means of White Rats’ Blood Glucose Level | Means of Blood Glucose Level Reduction |
|---------------------|----------------------------------------|---------------------------------------|
|                     | Pre-test | Post-test |                                     |
| K-                  | 248.7900 | 250.2367 | -1.45                                |
| K+                  | 250.6633 | 101.5367 | 149.13                               |
| P1                  | 248.2483 | 152.0100 | 96.24                                |
| P2                  | 247.9467 | 131.3250 | 116.62                               |
| P3                  | 249.2733 | 113.0617 | 136.21                               |
| Total               | 219.1719 | 136.4667 | 82.71                                |

The negative control group, which was not given the extract or metformin, did not even have the reduction. The experimental group 1 given the gedong gincu mango leaf extract with the dose of 150mg/200gr had the blood glucose reduction as much as 96.24mg/dl. The experimental group 2 given the gedong gincu mango leaf extract with the dose of 300mg/200gr had the blood glucose reduction as much as 116.62mg/dl. The experimental group 3 given the gedong gincu mango leaf extract with the dose of 600mg/200gr had the blood glucose reduction up to 54.64% while the dose of 300mg/200gr could only reduce up to 46.91%, and the lowest reduction was on the dose of 150mg/200gr which had the reduction as much as 38.68% (Figure 1).

It can be seen that among three doses examined, the highest blood glucose level reduction was the mango leaf extract 600mg/200gr, which could reduce blood glucose level up to 54.64% while the dose of 300mg/200gr could only reduce up to 46.91%, and the lowest reduction was on the dose of 150mg/200gr which had the reduction as much as 38.68% (Figure 1).

Three out of five groups given the gedong gincu mango leaf extract had 6 rats for each and the different doses. This study showed that that all experiments, except the negative control one, had the blood glucose reduction. The gedong gincu mango leaf extract reduces blood glucose level due to it contains several compounds with antihyperglycemic activity. There are several mechanisms of blood glucose reduction, such as increasing plasma insulin level, reducing oxidative stress, and inhibiting the activity of carbohydrate hydrolysis enzyme by its bioactive compound [17]. The compounds in mango leaves are mangiferin, catechin, quercetin, kaempferol, rhamnetin, anthocyanin, Gallic, and ellagic acids, propyl, and methyl gallate, benzoic acid, and protocatechuic acid [18].

Mangiferin reduces blood glucose level by two main mechanisms. First, mangiferin inhibits sucrose-glucosidase enzyme, iso-maltase, and maltase from the rats involved in the digestion of carbohydrate to monosaccharide in intestine, so the glucose absorption in intestine can be inhibited.
Another study found that *mangiferin* can also reduce triglyceride significantly. The triglyceride reduction can indirectly contribute to the antihyperglycemic activity through the mechanism of glucose-fat acid cycle. Based on Randle glucose-fatty acid cycle, the increasing of triglyceride in plasma can be as a source of increasing free fatty acid content and oxidation which inhibit insulin and glucose metabolism, then hyperglycemia develops. Thus, the triglyceride reduction which happens after giving the mango extract containing *mangiferin* can facilitate oxidizing glucose and then reducing blood glucose level [17]. Second, the *mangiferin* bioactivity, particularly on its capacity as an agent of preventing radical and producing the protection in cells.

![Figure 1. The blood glucose level reduction](image_url)

Blood glucose level reduction from *gedong gincu* mango leaf extract does not only focus on the regenerative activity of beta cell. The *gedong gincu* mango leaf extract also has the activity of reducing blood glucose level by increasing the use of peripheral glucose, increasing hepatic and muscle glycogen level, promoting the cell repair and regeneration, increasing peptide C level, being antioxidant to protect cells from oxidative stress, reducing the glissaded hemoglobin level, having the normalization of microalbuminuria and lipid profile modulation. As a result, the blood glucose level reduction on diabetic rats can be maximized.

In Table 1, it can be seen the different means of blood glucose level in every dose. It can be caused by the fact that the higher dose of the extract is given, the more active essences will be (i.e. *mangiferin*). *Mangiferin* is one of the compounds mostly contained in mango leaves. *Mangiferin* functions as anti-oxidant in the different level.

By giving metformin, it is gained the highest result. Metformin is the first drug of choice in pharmacologic treatment of type 2 DM. Its mechanism reducing blood glucose level on the diabetic rats is increasing the secretion of endorphin from adrenal gland to stimulate the connection between opioid receptors, which leads to the increasing of GLUT-4 and *phosphoenolpyruvate carboxykinase* (PEPCK) excretions. Besides, metformin activates 5′AMP-activated protein kinase (AMPK) in hepatocyte, so it reduces the activity of acetyl-CoA carboxylase and the excretion of lipogenic...
transcription factor and also inhibits liver gluconeogenesis [16,17].

In this study, it can be seen that metformin is more effective in reducing rats’ blood glucose level with the higher mean than giving gedong gincu mango leaf extract with the dose of 150, 300, and 600mg/200gram. Metformin has the reduction up to 59.49% or 149.13mg/dl if compared with giving gedong gincu mango leaf extract. However, the gedong gincu mango leaf extract with the dose of 600mg/dl, which can reduce as well as 54.64% or 136.21mg/dl, is not inferior to metformin.

4. Conclusions

Gedong gincu mango leaf has antihyperglycemic activity in dose-dependent response. The 600 mg/200g of it is not inferior to 9mg/200g of metformin in antihyperglycemic activity. Further studies are needed to develop gedong gincu mango leaf as alternative therapy in managing diabetes mellitus.

References
[1] Perkeni 2015 Pengelolaan Dan Pencegahan Diabetes Melitus Tipe 2 Di Indonesia
[2] Ard S W 2014 Buku Ajar Ilmu Penyakit Dalam. Interna Publishing
[3] Handayani F W and Muhtadi A 2013 Beberapa Tumbuhan Di Indonesia Berpotensi Sebagai Alternatif Obat Obat Antidiabetes. 4 pp 1-15
[4] Syah M I, Suwendar and Mulqie L 2015 Prosiding Penelit Sivitas Akad Unisba. pp 297-303
[5] Emelda A, Rahman S and Rahmah A S 2015 J Sains Dan Kesehatan. 1 (3):111-113
[6] Shah K A, Patel M B, Patel R J and Parmar P K 2010 Pharmacogn Rev. 4 (7): 42–4(Mi)
[7] Tanaya V and Retnowati R. Kim J. 2015
[8] Mathalaimutoo A 2010 Aktivitas Antidiabetes Ekstrak Etanol Daun Mangga Bapang (Mangifera Indica L. Var. Bapang) pada tukis Galur Wistar Yang Diinduksi Aloksan
[9] Fitriani N E and Akhmad S A L W 2014 Jkki. 6 (2):104-111
[10] Tobat S R, Dharma S and Rahmi A 2015 Uji Efektivitas Pemberian Terapi Insulin Long Acting Dan Rapid Acting Terhadap Penurunan Glukosa Darah Mencit Diabetes Mellitus 5 (1):23-28
[11] Hallow and Guyton 2014 A Buku Fisiologi Kedokteran Indonesian:EGC
[12] Anonymus 2017 J Clin Appl Res Educ p 40 (DOI:10.2337/Dc14-9014)
[13] Kristin E 2016 Jmedsci. 48 (2):119-130
[14] Luman A 2015 Peran Inhibitor Sodium Pada Terapi Diabetes Melitus. 42 (7):498-503
[15] Ghasemi A Acta Physiol Hung. 2014 (DOI:10.1556/Aphysiol.101.2014.4.2)
[16] Pal P, Sinha K and Sil P C 2014 Plos One Journal 9 (9) (DOI:10.1371/Journal.Pone.0107220)
[17] Gondi M, Prasada and Rao U J S 2015 J Food Sci Technol. 52 (12):7883-7893. (DOI:10.1007/S13197-015-1963-4)
[18] Masibo M and He Q 2008 Compr Rev Food Sci Food Saf. 7 (4):309-319
[19] Rajeshkar M and Rajeshkar J 2013 Journal Nat Remedies 14 (1):33-40. (http://Ojms.Cloudapp.Net/Index/Php/Int/Article/View/47403.)
[20] Kemasari P, Sangeetha S and Venkatalakshmi P 2011 J Chem Pharm Res. 3 (5):653-659
[21] Madhuri A S and Mohanvelu R 2017 Evaluation Of Antidiabetic Activity Of Aqueous Extract Of Mangifera Indica Leaves In Alloxan Induced Diabetic Rats. 10 (2):1029-1035 (DOI:10.13005/Bpj/1200)
[22] Jadhav R and Puchakayala G 2012 Int J Pharm Sci. 4 (2):251-256
[23] Parvez G M M. 2016 Pharmacological Activities Of Mango (Mangifera Indica): A Review. 5 (3):1-7
[24] Mudaw M M E, Mohamed A M and Haggaz 2013 Elsevier Cover J. 4 (5):778-785
[25] Zhang Y, Li J, Wu Z, et al. 2014 Acute And Long-Term Toxicity Of Mango Leaves Extract In Mice And Rats pp :1-9
[26] Bhowmik A, Khan L A, A Akhter and Rokeya B 2009 J Pharmacol. 4 (2):110-114. (DOI:10.3329/Bjp.V4i2.2488)
[27] Yogisha S, Raveesha K A 2010 *J Nat Prod.* 1 pp 36-45
[28] Luka C and Mohammed A. 2012 *Journal Nat Prod Plant Resource.* 2 (2):239-243
[29] Dokter P, Fakultas Gigi K. Disusun Sebagai Salah Satu Syarat Menyelesaikan Program Studi Strata I Pada Jurusan. 2016 pp 1-11
[30] Kementrian Kesehatan RI. *Infodatin Diabetes.* Presented 2014
[31] Dyah A O 2016 Efek Ekstrak Kulit Mangga Arumanis Terhadap Penurunan Edema Kaki Mencit Putih Jantan Yang Diinduksi Karagenin
[32] Perkeni. Petunjuk Praktis Terapi Insulin Pada Pasien Diabetes Melitus 2011 *Perkumpulan Endokrinol Indonesia.* pp 1-37
[33] Prasetyo A, Denashurya T G, Putri W S and In’am M 2016 Perbandingan Efek Hipoglikemik Infusa Daun Kembang Bulan (*Tithonia Diversifolia* (Hamsley) A. Gray) Dan Metformin Pada Tikus Yang Diinduksi Aloksan *43* (2):91-94
[34] Tobat S R, Dharma S, Rahmi A, *et. al* 2015 Efek Hipoglikemik Infusa Daun Kembang Bulan (*Tithonia Diversifolia* ( Hamsley ) A . Gray) dan Metformin Pada Tikus Yang Diinduksi Aloksan. *5* (1):23-28