The Effect Of *Dendrophthoe Pentandra* (L.) Miq Ethanol Extract On Malondialdehyde (MDA) In Hyperglycemic Rats

Anggun Syafitri[1], Yuandani[2*], Tri Widyawati[3]

[1]Postgraduate Programs, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
[2]Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
[3]Departement of Pharmacology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia
*Corresponding Author:
Email: yuandani@usu.ac.id

Abstract
Dendrophthoe pentandra (L.) Miq is a parasitic plant with medicinal potential found in 3000 other plant species. The duku parasite is usually considered a parasite on the duku plant and is often discarded because it is thought to interfere with the growth of the duku plant. The purpose of this study was to see the effect of the duku parasite leaf extract on the reduction of malondialdehyde (MDA) in hyperglycemic rats. MDA levels were measured by thiobarbituric acid-reactive substance (TBARS) assay with doses of duku mistletoe leaf ethanol extract of 400, 200, and 100 mg/kg BW. MDA levels were analysed using rat blood plasma, which was measured using a spectrophotometer at a wavelength of 532 nm. Oral administration of EEDBD 400, 200, and 100 mg/kg BW reduced MDA levels in hyperglycemic rats. However, only EEDBD 400 mg/kg BW showed a significant difference between the negative control (CMC-Na), but with glibenclamide and the normal groups, there was no significant difference.

Keywords: *Dendrophthoe pentandra, hyperglycemic rats and malondialdehyde (MDA).*

I. INTRODUCTION

Traditional medicine in various regions is a hereditary inheritance based on experience, empirical evidence, and further scientific evidence through preclinical and clinical trials [1]. The use of traditional medicine continues to increase and develop rapidly in society. This is supported by various factors and issues currently developing in the form of a "back to nature" attitude.Many medicinal plants have been proven to have antioxidant and antidiabetic effects, one of which is the *Dendrophthoe pentandra* leaf in the duku plant. Duku is a tropical fruit plant that is quite common in Indonesia and is often overgrown with parasites, especially from the species *Dendrophthoe pentandra* (L.) Miq and *Scurrula ferruginea* (Jack) Danser. *D. pentandra* is a parasitic plant found in 3000 other plant species with medicinal potential [2], [3]. The *D. pentandra* is usually considered a parasite on the duku plant and is often discarded because it is thought to interfere with the growth of the duku plant.

However, the leaves of the parasite, which have been considered parasites, actually have properties for human health [4].The metabolic syndrome is a collection of metabolic risk factors that contribute to an increased risk of diabetes mellitus and cardiovascular disease. Metabolic syndrome is a condition of abnormal metabolism in the body that is characterized by the presence of three or more signs and symptoms, including central obesity, hypertriglyceridemia, low HDL levels, high blood pressure, and high fasting blood glucose level [5]. Hyperglycemia tends to have an adverse effect on the body's health because high blood glucose level tend to encourage the formation of free radicals or reactive oxygen species through an oxidation-reduction mechanism by encouraging more electron donors into the electron transport chain in the mitochondria [6]. Research on the effect of the *D. pentandra* on malondialdehyde (MDA) levels has never been done in experimental animals or humans. Therefore, the present study was conducted to evaluate the effect of *D. pentandra* ethanol extract on malondialdehyde levels in hyperglycemic rats.

II. METHODS

2.1 Preparation of *D. pentandra* Samples

D. pentandra samples were taken from the duku field area, Medan Johor Village, Medan, North Sumatra Province. The fresh leaves of the *D. pentandra* are sorted, thoroughly washed, drained, and then air dried to dry. *D. pentandra* powder was extracted by the maceration method, soaking the sample in 96%

ethanol for 5 days, re-macerating for 2 days, filtering, and then concentrating with a rotary evaporator to obtain a thick extract [7], [8].

2.2 Preparation of *D. pentandra* Leaves Ethanol Extract Suspension (EEDBD)

The 100 mg/kg BW EEDBD was weighed in a watch glass, then put into a mortar, and 0.5% Na-CMC suspension was added little by little while grinding until homogeneous and then put into a 10 mL volumetric flask. The volume was made up of 0.5% Na-CMC suspension up to the marked line [9].

2.3 Preparation of Test Animals

The animals used were white male rats with a body weight of 180–200 grams, divided into 6 groups, each consisting of 4 rats. Before the experiment, they were acclimatized for 2 weeks [10].

2.4 Induction of Test Animals

Twenty-four male rats weighing 180-200 g fasted for 18 hours were weighed, determined to be fasting glucose blood level, and induced with nicotinamide 230 mg/kg BW intraperitoneally. After 15 minutes, they were induced with 65 mg/kg streptozotocin solution intraperitoneally [11].

2.5 Measurement of MDA Levels

MDA levels were measured using a thiobarbituric acid-reactive substance (TBARS) assay. TMP was used as a standard in this assay due to the difficulty of producing pure malondialdehyde (MDA), which is stable during storage, so that MDA can be obtained by hydrolyzing TMP by heating in an acidic environment with methanol as a by-product of the reaction [12], [13]. Based on the reaction between MDA and TBA, MDA forms a TBA-MDA bond complex that produces a pink to reddish color, and then its intensity is measured using a spectrophotometer [14].

III. RESULT AND DISCUSSION

3.1 Induction of Test Animals

The test animals were induced using streptozotocin (STZ) and nicotinamide (NA) induction to increase blood glucose level in rats. The results of the blood glucose level measurements can be seen in Table 1.

Table 1. Results of rat Blood Glucose Level measurements after induction

No	Treatment Group	Blood Glucose Level after induction (mg/dl) Mean ± SEM, n=4
1	Na-CMC	346.25 ± 23.05
2	Glibenclamide dose of 0.45 mg/kgBw	367.75 ± 16.19
3	EEDBD 100 mg/KgBW	365.50 ± 8.73
4	EEDBD 200 mg/KgBW	366.25 ± 11.05
5	EEDBD 400 mg/KgBW	367.75 ± 9.88

Table 1 above shows that after NA and STZ induction, the blood glucose value increased above the normal limit of >200 mg/dl. The results of increasing glucose blood level can be seen in Figure 1.

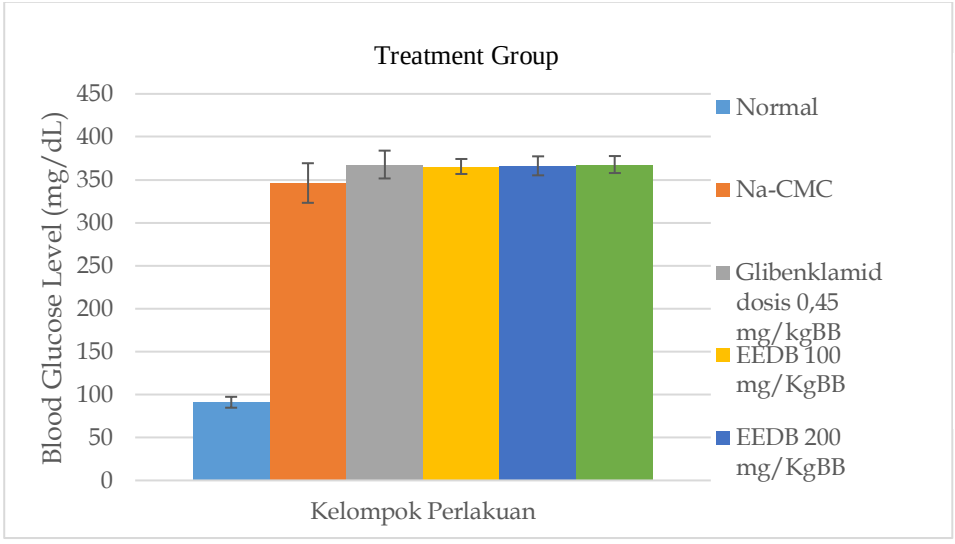

Fig 1. Blood Glucose Level in rats after induction

The figure 1 shows that the blood glucose level in rats increases due to the induction of NA and STZ. The mechanism of STZ in increasing blood glucose level in the blood is that streptozotocin can enter pancreatic cells with the help of GLUT-2 so that it is selectively toxic to specific cells. Streptozotocin can reduce GLUT-2 expression in the pancreas, as well as in the kidney and liver. The mechanism of cell death caused by streptozotocin induction is caused by DNA alkylation, the release of nitric oxide (NO), the release of reactive oxygen species (ROS), and the inhibition of O-GlcNAcse. After streptozotocin enters the cell, STZ decomposes spontaneously to form methyldiazohydroxide and isocyanate molecules. The methyldiazohydroxide molecule will decompose to form a highly reactive carbonium ion (CH3+), which is the primary key for DNA alkylation. When the cell's DNA is damaged, it will activate poly ADP-ribosylase, where poly ADP-ribosylase overstimulates, and there is a decrease in ATP and NAD+ so that in the end free radicals will form, which can damage pancreatic cells. Insulin production is inhibited and decreases due to pancreatic-cell damage, resulting in diabetes mellitus [15]–[17].

3.2 Measurement of MDA levels

In this study, MDA levels were measured using the Thiobarbituric Acid Reactive Substances (TBARS) method, in which two molecules of thiobarbituric acid (TBA) react with two carbonyl groups from one molecule of MDA to form a colored MDA-TBA2 complex that can be measured using a spectrophotometer.UV-Vis. The standard is TMP, 1, 1, 3, or 4 tetra methoxy propane or malondialdehyde bis. MDA levels were analysed using rat blood plasma, which was measured using a spectrophotometer at a wavelength of 532 nm.MDA levels for the treatment results were obtained by measuring the absorbance in the presence of the addition of a standard solution of 96, 48, 24, 12, 6, and 3 pg/ml. The absorbance value of each concentration can be seen in Table 2 below.

Table 2.MDA standard absorbance.

Concentration standard (nmol/ml)	Absorbance (532 nm)
0.3	0.016
2	0.034
10	0.076
25	0.132
35	0.199
42	0.251

Based on the table, a standard curve is obtained, as shown in Figure 2 below. The standard curve is obtained from the relationship between various standard concentrations and the absorbance formed. From this calibration curve, the value of R^2 is obtained. The R^2 value ranges from 0 to 1, which indicates how closely the estimated values for the regression analysis represent the actual data. The regression line equation $y = 0.0053x + 0.0172$ with an $R^2 = 0.9878$ is derived from the standard curve.

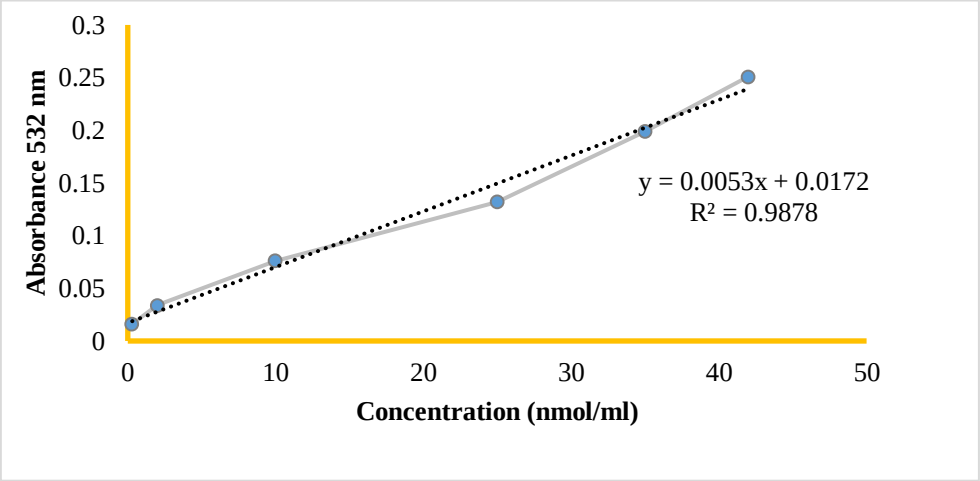

Fig 2. MDA Standard Curve

MDA activity was calculated by substituting the sample's absorbance value (y) at a wavelength of 532 nm into the linear regression line equation y = 0.0053x + 0.0172, which was obtained from the standard curve so that the MDA content value (x) was obtained. The results of MDA concentration were then carried

out with statistical analysis using One Way Analysis of Variant (ANOVA) to obtain significant differences in measurement results ($p < 0.05$) between treatment groups. The results of the MDA test on rat plasma can be seen in Table 3 below.

Table 3. MDA Levels in Rat's Plasma.

No	Treatment Group	MDA Average (nm/mol) ± SEM	*P value*
1	Normal	6.53 ± 4.26	0.000[a] 0.760 -
2	Na-CMC	38.25 ± 6.62	- 0.000[b] 0.000[c]
3	Glibenclamide dose of 0.45 mg/kgBW	8.22 ± 2.79	0.000[a] - 0.760
4	EEDBD 100 mg/KgBW	24.42 ± 13.39	0.020[a] 0.008[b] 0.004[c]
5	EEDBD 200 mg/KgBW	18.06 ± 2.60	0.002[a] 0.048[b] 0.087
6	EEDBD 400 mg/KgBW	11.98 ± 3.15	0.000[a] 0.497 0.329

Information:
SEM (Standard Error Mean)
[a] significantly different from the Na-CMC control group
[b] significantly different from the glibenclamide comparison group
[c] significantly different from the normal group

Based on data on average plasma MDA levels in DM rats given EEDBD after being tested for normality with the Kolmogorov-Smirnov test, the significance of $p > 0.05$ was determined. This shows that the data is normally distributed. Then a homogeneity test was carried out with a significance result of $p > 0.05$, indicating that the data obtained was homogeneous, so the requirements for using parametric analysis with one-way ANOVA were fulfilled [18]. The ANOVA analysis results showed significant differences between treatment groups in MDA concentrations (nmol/ml), with a significance value of $p < 0.05$. The CMC-Na negative control group showed an increase in MDA compared to the normal group. Meanwhile, there was no significant difference between the normal and positive control groups or EEDBD 400 mg/Kg BW ($p > 0.05$). This shows that the administration of EEDBD can significantly affect MDA levels in DM rats, as shown in Figure 3.

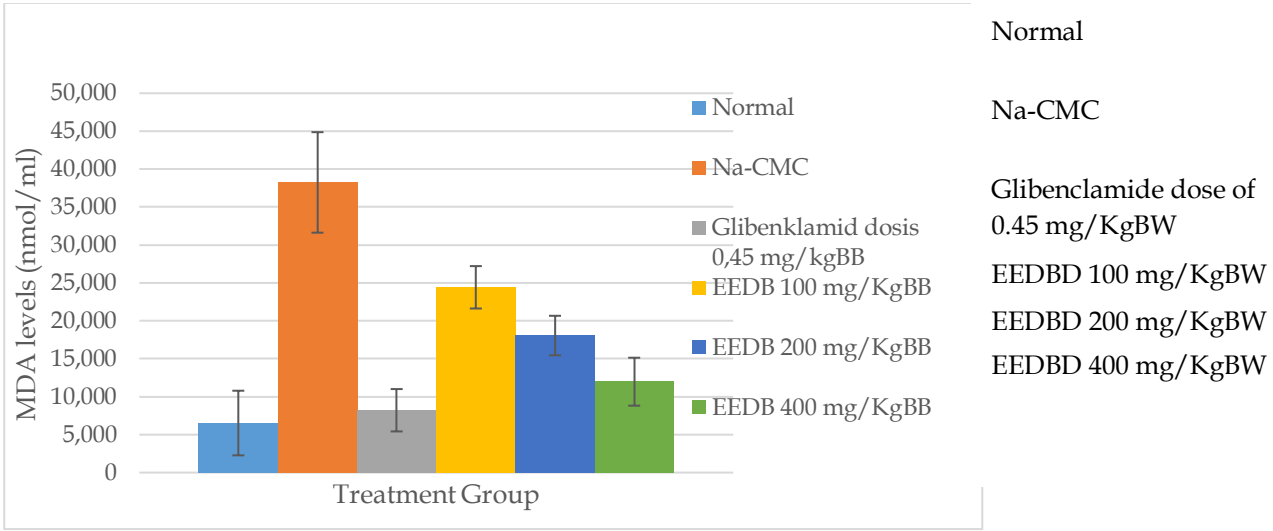

Fig 3. Effect of treatment group on MDA levels

Oral administration of EEDBD 400, 200, and 100 mg/kg BW reduced MDA levels in hyperglycemic rats. However, only EEDBD 400 mg/kg BW showed a significant difference between the glibenclamide and the control groups. The measurement results of high MDA levels indicate oxidative stress in DM-2 rats [19]. Oxidative stress in DM-2 rats can activate several pathways, including the formation of advanced glycation end-products (AGEs) and PKC1 [20]. Hyperglycemia conditions can induce oxidative stress by several mechanisms, such as glucose auto-oxidation, the polyol pathway, and the formation of AGE and PKC1/2 kinase, as seen in Figure 3 above [21], [22]. After oxidative stress, the next stage of MDA formation occurs through a non-enzymatic process with bicyclic endoperoxides produced through lipid peroxidation [23].

IV. CONCLUSION

Oral administration of EEDBD at doses of 400, 200, and 100 mg/kg BW reduced MDA levels in hyperglycemic rats. However, only EEDBD 400 mg/kg BW showed a significant difference between the negative control (CMC-Na), but with glibenclamide and the normal groups, there was no significant difference.

REFERENCES

[1] A. Dalimunthe, D. Pertiwi, M. Muhmmad, V. E. Kaban, N. Nasri, and D. Satria, 'The effect of extraction methods towards antioxidant activity of ethanol extract of Picria fel-terrae Lour. Herbs', in *IOP Conference Series: Earth and Environmental Science*, 2022, vol. 1115, no. 1, p. 012040.

[2] M. F. Lubis, P. A. Z. Hasibuan, H. Syahputra, J. M. Keliat, V. E. Kaban, and R. Astyka, 'Duku (Lansium domesticum) Leaves Extract Induces Cell Cycle Arrest and Apoptosis of HepG2 Cells via PI3K/Akt Pathways', *Trends in Sciences*, vol. 20, no. 2, pp. 6437–6437, 2023.

[3] M. F. Lubis, P. A. Hasibuan, H. Syahputra, and R. Astyka, 'A Review on Phytochemicals and Pharmacological Activities as Ethnomedicinal Uses of Duku (Lansium domesticum Corr.)', ***Open Access Macedonian Journal of Medical Sciences,*** vol. 10, no. F, pp. 57–65, 2022.

[4] E. A. Abdel-Sattar, A. A. Elberry, F. M. Harraz, S. A. Ghareib, A. A. Nagy, and S. A. Gabr, 'Antihyperglycemic and hypolipidaemic effects of the methanolic extract of Saudi mistletoe (Viscum schimperi Engl.)', ***Journal of Advanced Research,*** vol. 2, no. 2, pp. 171–177, 2011.

[5] S. P. AP and A. Syauqy, 'Pengaruh Pemberian Pisang Kepok (Musa Paradisiaca Forma Typical) Terhadap Kadar Malondialdehyde (MDA) Tikus Sprague Dawley Pra-Sindrom Metabolik',***Journal of Nutrition College,*** vol. 4, no. 4, pp. 314–322, 2015.

[6] M.Brownlee,'Biochemistry and molecular cell biology of diabetic complications',*Nature*,vol. 414, no.6865,pp. 813–820,2001.

[7] H. Sari, V. E. Kaban, F. R. Situmorang, and F. Fahdi,'UJI EFEKTIVITAS ANTIDIABETES KOMBINASI EKSTRAK DAUN MENIRAN (Phyllanthus niruri L.)Dan KELOPAK BUNGA ROSELLA (Hibiscus sabdariffa L.) PADA TIKUS JANTAN PUTIH', ***Jurnal Penelitian Farmasi & Herbal***, vol. 2, no. 1, pp. 24–28, 2019.

[8] M. Muhammad, N. Nasri, V. E. Kaban, D. Satria, and H. Cintya, 'Antibacterial Potential Ethanol Extract of Papaya Leaves (Carica papaya Linn.) Towards Salmonella typhi', ***BEST Journal (Biology Education, Sains and Technology)***, vol. 5, no. 2, pp. 265–270, 2022.

[9] E. E. Ginting, C. F. Sari, L. Leny, P. Parhan, and P. Ginting, 'ANALISA SENYAWA METABOLIT SEKUNDER DAN PENGARUH PEMBERIAN SERBUK SEMUT JEPANG TERHADAP KADAR KOLESTEROL PADA TIKUS PUTIH JANTAN', ***Jurnal Ilmiah Ibnu Sina***, vol. 7, no. 1, pp. 56–65, 2022.

[10] N. Nasri, U. Harahap, J. Silalahi, and D. Satria, 'Antidiarrheal Effect of Lactobacillus fermentum Isolated from Dengke Naniura on Escherichia coli-induced Rats', ***Indonesian Journal of Pharmaceutical and Clinical Research***, vol. 5, no. 1, Art. no. 1, Apr. 2022, doi: 10.32734/idjpcr.v5i1.6478.

[11] P. Masiello *et al.*, 'Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide', *Diabetes*, vol. 47, no. 2, pp. 224–229, 1998.

[12] U. A. Siti,'Pengaruh Pemberian Mesenchymal Stem Cell Wharton's Jelly Terhadap Kadar Malondialdehid (MDA) Serum Tikus Model Osteoartritis',Universitas Andalas, 2020.

[13] D. Helianti and Y. Widiastuti, 'Aktifitas Fisik Berat Menyebabkan Degenerasi Sel Hepatosit melalui Mekanisme Stres Oksidatif', ***Jurnal kedokteran Brawijaya,*** vol. 27, no. 2, pp. 61–65, 2012.

[14] A. Wijaya, L. H. Nurani, and N. Nurkhasa, 'Aktivitas Antioksidan Sediaan Nanopartikel Kitosan Ekstrak Etanol Kelopak Rosela (Hibiscus sabdariffa L) Pada Tikus Hiperkolesterol: Pengukuran Kadar Malondialdehid (MDA)', *Kartika:* ***Jurnal Ilmiah Farmasi,*** vol. 2, no. 1, pp. 1–6, 2015.

[15] N. E. Fitriani, S. A. Akhmad, and W. Lestariana, 'Efek Kuersetin Terhadap Kadar Glukosa Darah Puasa Pada Tikus Diabetes Melitus Tipe 2 Yang Diinduksi Dengan Streptozotocin-Nicotinamide',***JKKI: Jurnal Kedokteran dan Kesehatan Indonesia,*** pp. 103–110, 2014.

[16] C. Surbakti, P. Sitorus, and D. Satria, 'Effect of Saurauia vulcani Korth. leaves on superoxide dismutase, HbA1c levels and insulin expression in hyperglycemic rats',***Open Access Macedonian Journal of Medical Science****s*, vol. 7, no. 22, p. 3741, 2019.

[17] P. Sitorus and D. Satria, 'Hypoglycemic activity of ethanolic extract of Saurauia vulcani korth', *LEAVES*. ***Asian J. Pharm. Clin. Res***, vol. 11, pp. 35–36, 2018.

[18] R. K. Patel, M. M. Patel, M. P. Patel, N. R. Kanzaria, K. R. Vaghela, and N. J. Patel, 'Hepatoprotective activity of Moringa oleifera Lam. Fruit on isolated rat hepatocytes', *Pharmacogn Mag*, vol. 4, pp. 118–123, 2008.

[19] O. O. Daniel, A. O. Adeoye, J. Ojowu, and O. O. Olorunsogo, 'Inhibition of liver mitochondrial membrane permeability transition pore opening by quercetin and vitamin E in streptozotocin-induced diabetic rats', *Biochemical and biophysical research communications*, vol. 504, no. 2, pp. 460–469, 2018.

[20] P. A. Z. HASIBUAN, P. SITORUS, and D. SATRIA, 'ANTICANCER ACTIVITY OF B-SITOSTEROL FROM PLECTRANTHUS AMBOINICUS (LOUR. SPRENG.) LEAVES: IN VITRO AND IN SILICO STUDIES', ***Asian J Pharm Clin Res,*** vol. 10, no. 5, pp. 306–308, 2017.

[21] R. Aninditha Rachmah, U. Harahap, and P. A. Z. Hasibuan, 'Pengaruh ekstrak etanol kulit buah nanas (Ananas comosus (L.) Merr.) terhadap glukosa darah pada mencit hiperglikemia secara in vivo', ***Jurnal'Aisyiyah Medika,*** vol. 2, no. 1, 2018.

[22] P. Sitorus, U. Harahap, M. Pandapotan, and T. Barus, 'Isolation of β-sitosterol from n-hexane extract of Picria fel-terrae Lour. leave and study of its antidiabetic effect in alloxan induced diabetic mice.', ***International Journal of PharmTech Research,*** vol. 6, no. 1, pp. 137–141, 2014.

[23] S. Syahara, U. Harahap, and T. Widyawati, 'Activity of Muntingia calabura leaves ethanolic extract on glucose and insulin blood levels in streptozotocin-induced rat', ***Asian Journal of Pharmaceutical Research and Development,*** vol. 7, no. 4, pp. 8–11, 2019.