The Effect of Moringa Leaf Ethanol Extract with Glycated Albumin in Wistar Rats Type 2 Diabetes Mellitus Model

Husain Satriaanggara[1], Dewi Karita[1], Yenni Bahar[2], Yunia Annisa[3]

[1]*Department of Biochemistry Universitas Muhammadiyah Purwokerto*
[2]*Department of Herbs Medicine, Universitas Muhammadiyah Purwokerto*
[3]*Department of Internal Medicine, Universitas Muhammadiyah Purwokerto*.

***) Correspondence Author**
Dewi Karita
Department of Biochemistry Universitas Muhammadiyah Purwokerto
K.H Ahmad Dahlan PO BOX 202 Purwokerto, Indonesia
Email: dewikarita@gmail.com
Telp: +6281327041084

Abstract

Introduction: Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated plasma glucose levels. Gold standard of DM diagnosis is through HbA1c examination. The American Diabetes Association (ADA) and European Association Diabetes Study (EASD) recommend glycated albumin as an alternative biomarker in diagnosing DM. One of the DM therapies can use herbal plants, namely Moringa (*Moringa oleifera Lam*.). This study aimed to determine the effect of ethanol extract of Moringa leaf (*Moringa oleifera Lam*.) on glycated albumin levels in wistar strain white rats (*Rattus norvegicus)* model of diabetes mellitus induced by streptozotocin-nicotinamide.
Methods: This is an experimental research with post test control group method. Subjects were 36 rats divided into 6 groups. At the end of the study, rats were examined for GA levels using the ELISA method. Data were analyzed using JASP and One-Way Anova test followed by post hoc test.
Results: The lowest reduction in glycated albumin levels was found in the group of rats given 300 mg of Moringa leaf ethanol extract (34,397±2,564). One-way Anova test showed a significant difference ($p<0.05$), post hoc test obtained significant differences in each group ($p<0.05$).
Conclusions: There is an effect of moringa leaf ethanol extract on glycated albumin levels in white rats (*Rattus norvegicus*) model of diabetes mellitus induced by streptozotocin-nicotinamide.

Keywords: diabetes mellitus; glycated albumin; Moringa leaf.

INTRODUCTION

Type 2 DM occurs due to two main causal factors, including reduced insulin secretion by pancreatic cells and reduced insulin sensitivity (1). Based on International Diabetes Federation (IDF), the global prevalence of diabetes mellitus will increase to 10.9% (700 million) in 2045 (2,3). Diabetes mellitus has quadrupled in prevalence worldwide during the last three decades, and it is now the ninth leading cause of mortality. Diabetes affects approximately one in every eleven persons globally, with type 2 diabetes accounting for 90% of all cases. The majority of T2DM patients experience at least one complication, with cardiovascular problems being the predominant cause of morbidity and mortality(4).

HbA1c is the gold standard for assessing blood glucose levels. However, the accuracy of HbA1c can also be influenced by several diseases that can affect the age of erythrocytes (5). Glycated albumin (GA) is an alternative biomarker for hyperglycemia (6). In recent years, GA, a relatively new glycemic control measure that is unaffected by food intake or erythrocyte longevity, has received a lot of attention. It is represents glycemic control status for up to 2-3 weeks. (7,8)

Treatment for type 2 DM can use herbal plants, one of those is *Moringa oleifrea Lam*. Moringa oleifera (MO) has been utilized as a food source and in traditional medicine due to its potential antihyperglycemic, antioxidant, anti-inflammatory, and lipid-regulating qualities. These qualities may be explained by the abundance of phytochemicals found in the tree's leaves, fruits, roots, and oil. The evidence for the acute antihyperglycemic effects of MO extract on diabetic animal models appears to be strong, but more chronic and long-term research are required. However, the hypoglycemic effects of MO on humans are less established (9,10). High antioxidant levels in Moringa leaves can be useful for lowering glucose levels in the blood. The prior test resulted in a substantial difference in fasting blood glucose levels between the treatment and control groups. This demonstrates the effect of MO extract on fasting blood glucose levels in type 2 diabetic patients (11,12).

This study aimed to determine the effect of ethanol extract of Moringa leaves on glycated albumin levels in wistar rats model of diabetes mellitus induced by streptozotocin-nicotinamide.

METHODS

This is a true experimental post test only with control group design with a simple random sampling technique. The implementation of this research has been approved by the Medical and Health Research Ethics Commission of the Faculty of Medicine, Muhammadiyah University. The maintenance and treatment of wistar rats, will be carried out at the Pharmacology Laboratory, Faculty of Medicine,University of Muhammadiyah Purwokerto. Measurement of glycated albumin levels will be carried out at the Biochemistry Laboratory, Faculty of Medicine, Jendral Soedirman University. Inclusion criteria: Male rats are healthy, active and have normal behavior, body weight is 200-300 grams, 3 months old, exclusion criteria is rats were sick (nephrotic syndrome, cirrhosis), dropout criteria rats die during treatment.

Rats were acclimated for 7 days. Nicotinamide induction 230 mg/kgBW then after 15 minutes continued with streptozotocin induction 65 mg/kgBW. It was found that the minimum number of research samples for each group was 4 rats in each treatment group. The total number of samples required is 30 samples (24 rats as main samples and 6 rats as backup) with the following procedures:

a. Group I (KI): DM model rats were given ethanol extract of *Moringa oleifera lam*. leaves at a dose of 100 mg/kgBW.
b. Group II (KII): DM model rats were given ethanol extract of *Moringa oleifera lam*. leaves at a dose of 200 mg/kgBW.
c. Group III (KIII): DM model rats e were given ethanol extract of *Moringa oleifera lam*. leaves at a dose of 300 mg/kgBW.
d. Group IV (KIV): DM model rats as positive controls were given metformin at a dose of 9 mg.
e. Group V (KV): DM model rats as negative controls were given distilled water and standard feed.
f. Group VI (KVI): the normal control group was only given standard feed.

Glycated albumin (GA) levels were measured using the ELISA method. Data were analyzed using Saphiro-wilk for normality, then use One Way Anova and continued by post hoc test. Data analysis using JASP statistical programs.

RESULTS

Saphiro wilk test was carried out and all groups had $p>0.05$ then continued with One Way Anova test. Post hoc test was used to analyze differences in GA levels in each group. Post hoc test was carried out after the One Way Anova test. Post Hoc test results are declared significant ($p<0.05$). The results can be seen in table 1.

Table 1. Glycated albumin levels in each group

Group	Mean±SD	P
KI	43,818±2,467	
KII	39,408±2,304	
KIII	34,397±2,564	<0.001
KIV	32,963±1,239	
KV	44,949±3,215	
KVI	32,861±2,562	

DISCUSSION

Based on the results, it was found that *Moringa oleifera Lam*. leaf extract had an effect in reducing glycated albumin (GA) levels in wistar rats. This can be seen from the research results which show a decrease in GA levels in KI, KII, KIII as a group treated with *Moringa oleifera Lam*. leaf extract when compared with the negative control group which was only induced by Streptozotocin-Nicotinamide.

From three groups, it can be seen that the *Moringa oleifera Lam*. leaf extract treatment give the lowest glycated albumin in group III which was given a dose of 300 mg/kgBW. This is in line with research by Aju which states that the effective dose for reducing serum glucose and glycated hemoglobin (HbA1c) levels is 300 mg/kgBW (13). A decrease in serum glucose levels will be in line with a decrease in glycated albumin, this can make glycated albumin levels an alternative biomarker of short-term glucose control. Research conducted by Olurishe also stated that the effective dose of ethanol extract of Moringa leaves in reducing blood glucose in mice was 300 mg/kgBW (14). In Mithiyane's, it was reported that doses of ethanol extract from Moringa leaves varying between 100-300 mg/kgBW effectively reduced hyperglycemia within 2 weeks (15).

The decrease in glycated albumin levels was due to the quercetin content which can be an antioxidant agent in inhibiting the formation of advanced protein glycation end products, this of course will have an impact on inhibiting the formation of glycated albumin (16). Moringa leaf extract can increase insulin sensitivity and reduce inflammation in rat kidneys induced by streptozotocin-nicotinamide (17). A decrease in blood glucose can occur because moringa leaf extract contains antioxidants and quercetin which can inhibit Glucose Transport type 2 (GLUT-2) in the intestine so that it can inhibit glucose absorption (18).

In this study, rats were induced with streptozotocin-nicotinamide to create conditions for type 2 DM. Streptozotocin will increase damage to pancreatic β cells and will result in disruption of insulin production, causing hyperglycemia. Streptozotocin will trigger an inflammatory process in the β cells by binding to GLUT-2 which will cause streptozotocin to enter the cytoplasm of the pancreatic β cells so that it will cause depolarization of the mitochondria which is followed by excessive energy use in the cells which will ultimately cause insulin deficiency. When the body experiences hyperglycemia or a situation where glucose levels in the body are very high in the body it can cause ROS, this can cause the death of pancreatic β cells which can cause a decrease in insulin secretion and hyperglycemia occurs which if it occurs for years can result in type 2 DM. , the flavonoid content which functions as an antioxidant can have a protective effect against damage to pancreatic β cells and can increase insulin sensitivity (19). Bule reported that the flavonoid content in the form of quercetin which has antioxidant properties can also reduce glucoside activity in vitro and stimulate the AMP-Activated Protein Kinase (AMPK) complex, so that it will increase the transcription and translation of GLUT-4 which will result in increased absorption of glucose by insulin so that glucose levels in the blood will decrease (20).

The average GA levels in KV (positive control group) had a significant difference with KI, KII. However, it was not significant when compared with KIII or the group given *Moringa oleifera Lam*. ethanol extract of 300 mg/kgBW. This is in line with research conducted by Tandi who reported that ingredients in the water ethanol extract of Moringa leaves such as flavonoids can increase insulin secretion and repair damaged pancreatic β cells (22). The flavonoid content has the same mechanism as metformin so that it can reduce glucose production in the liver (gluconeogenesis) and can regenerate

peripheral glucose intake which can reduce blood glucose. The mean GA levels in the negative control group (KV) given distilled water were significantly different when compared to the normal control group (KVI). This is because distilled water does not have a reducing effect on GA levels. This research still has limitation, there were 6 rats that died during this study

CONCLUSIONS There is an effect of moringa leaf ethanol extract on glycated albumin levels in white rats (*Rattus norvegicus*) model of diabetes mellitus induced by streptozotocin-nicotinmaide

Conflict of Interest: author declare no conflict of interest

ACKNOWLEDGEMENT:

REFERENCES

1. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of type 2 diabetes mellitus. Int J Mol Sci. 2020;21(17):1–34.
2. IDF. https://diabetesatlas.org/2022-reports/. 2022.
3. Kementrian kesehatan republik indonesia. Tetap Produktif, Cegah Dan Atasi Diabetes Mellitus. pusat data dan informasi kementrian kesehatan RI. 2020.
4. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol [Internet]. 2018;14(2):88–98. Available from: http://dx.doi.org/10.1038/nrendo.2017.151
5. Kohzuma T, Tao X, Koga M. Glycated albumin as biomarker: Evidence and its outcomes. J Diabetes Complications [Internet]. 2021;35(11):108040. Available from: https://doi.org/10.1016/j.jdiacomp.2021.108040
6. Desouza C V., Holcomb RG, Rosenstock J, Frias JP, Hsia SH, Klein EJ, et al. Results of a Study Comparing Glycated Albumin to Other Glycemic Indices. J Clin Endocrinol Metab. 2020;105(3):677–87.
7. Bomholt T, Adrian T, Nørgaard K, Ranjan AG, Almdal T, Larsson A, et al. The Use of HbA1c, Glycated Albumin and Continuous Glucose Monitoring to Assess Glucose Control in the Chronic Kidney Disease Population including Dialysis. Nephron. 2021;145(1):14–9.
8. Giglio RV, Sasso B Lo, Agnello L, Bivona G, Maniscalco R, Ligi D, et al. Recent updates and advances in the use of glycated albumin for the diagnosis and monitoring of diabetes and renal, cerebro-and cardio-metabolic diseases. J Clin Med. 2020;9(11):1–17.
9. Sutriono S. Pengaruh Ekstrak Daun Kelor (Moringa oleifera) Terhadap Kadar Glukosa Darah Dari Histopatologi Limpa Tikus Wistar (Rattus norvegicus) 2021;(December). Available from: http://repository.radenfatah.ac.id/8449/%0Ahttp://repository.radenfatah.ac.id/8449/2/bab5.pdf
10. Vargas-Sánchez K, Garay-Jaramillo E, González-Reyes RE. Effects of moringa oleifera on glycaemia and insulin levels: A review of animal and human studies. Nutrients. 2019;11(12):1–19.
11. Mursito, S. MS, Lilies B, Mawaddah H. Ekstrak Daun Kelor (Moringa Oleifera Lamk) dalam Menurunkan Kadar Glukosa Darah pada Tikus (Rattus novergicus). Bionature. 2020;21(1):6–12.
12. Lakshita N. Anak Aktif Bebas Diabetes. 2017;8(Dm):11.
13. Aju BY, Rajalakshmi R, Mini S. Protective role of Moringa oleifera leaf extract on cardiac antioxidant status and lipid peroxidation in streptozotocin induced diabetic rats. Heliyon. 2019;5(12):e02935.
14. Olurishe C, Kwanashie H, Zezi A, Danjuma N, Mohammed B. Chronic administration of

ethanol leaf extract of Moringa oleifera Lam. (Moringaceae) may compromise glycaemic efficacy of Sitagliptin with no significant effect in retinopathy in a diabetic rat model. J Ethnopharmacol [Internet]. 2016;194:895–903. Available from: http://dx.doi.org/10.1016/j.jep.2016.10.065

15. Mthiyane FT, Dludla P V., Ziqubu K, Mthembu SXH, Muvhulawa N, Hlengwa N, et al. A Review on the Antidiabetic Properties of Moringa oleifera Extracts: Focusing on Oxidative Stress and Inflammation as Main Therapeutic Targets. Front Pharmacol. 2022;13(July):1–17.

16. Magaji UF, Sacan O, Yanardag R. Alpha amylase, alpha glucosidase and glycation inhibitory activity of Moringa oleifera extracts. South African J Bot [Internet]. 2020;128:225–30. Available from: https://doi.org/10.1016/j.sajb.2019.11.024

17. Lam M, Kou X, Li B, Olayanju JB, Drake JM, Chen N. Nutraceutical or Pharmacological Potential of Moringa oleifera Lam. Nutrients. 2018;1–12.

18. Bobaya SJ, Latuconsina VZ, Kailola N, Darah KG. Molucca Medica ISSN 1979-6358 (print) ISSN 25970246X (online) Artikel Penelitian Molucca Medica ISSN 2023;16(April):88–97.

19. Nurmalasari Y, Rafie R, Putri DF, Rahma SA. Pengaruh Pemberian Ekstrak Daun Kelor (Moringa olifera) Terhadap Kadar Glukosa Darah Tikus Putih (Rattus novergicus) Galur Wistar Jantan Yamg Diinsuksi Aloksan Sebagai Upaya Preventif Hiperglikemia. Prepotif J Kesehatan Masy. 2021;5(1):472–83.

20. Bule M, Abdurahman A, Nikfar S, Abdollahi M, Amini M. Antidiabetic effect of quercetin: A systematic review and meta-analysis of animal studies. Food Chem Toxicol. 2019;125(November 2018):494–502.

21. Nunthanawanich P, Sompong W, Sirikwanpong S, Mäkynen K, Adisakwattana S, Dahlan W, et al. Moringa oleifera aqueous leaf extract inhibits reducing monosaccharide-induced protein glycation and oxidation of bovine serum albumin. Springerplus. 2016;5(1).

22. Tandi J, Yanti Palinggi I, Tonapa Rammang S, Wahyu Handayani T. Uji Efektivitas Antihiperglikemia Daun Kelor (Moringa Oleifera Lam.) dan Gambaran Histopatologi Pankreas Tikus Putih Jantan (Rattus Norvegicus) yang Diinduksi Streptozotocin. J Jamu Indones.