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EFFECT OF MORINGA OLEIFERA LEAF (*MORINGA OLEIFERA* L.) ETHANOL EXTRACT APPLICATION ON CITTVALUE AND MALONDIALDEHYDE LEVELIN DIABETIC MICE

SUSILAWATI E*, IDAR, YUNIARTI E, PERMATASARI P AND MUHSININ S

Department of Pharmacology, Faculty of Pharmacy, Bhakti Kencana University

Jl. Soekarno Hatta 754 Bandung, Indonesia

***Corresponding Author: Elis Susilawati: E Mail: elis.susilawati@bku.ac.id**

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ABSTRACT

Diabetes mellitus is a disease with a component of oxidative stress. Oxidative stress is an imbalance condition between the number of free radical molecules and antioxidant compounds in the body which can cause damage to DNA, protein, and lipid. Damage to lipids is called lipid peroxides where oxidized lipids can trigger the production of toxic aldehydes such as Malondialdehyde and are associated with a state of insulin resistance.This study aims to determine the effect of the ethanol extract of Moringa leaves on constant insulin tolerance test (CITT) values and MDA levels in insulin resistance and insulin deficiency mice.Insulin resistance testing was carried out on a preventive basis for 28 days using a high-fat emulsion 0.5 mL//kgBW induction and a curative insulin deficiency test using 50 mg//kgBW of alloxan monohydrate induction.Each study was divided into 6 groups of mice test animals, namely negative control, positive control, control of the ethanol extract of Moringa leaves with a dose of 75 mg//kgBW, 150 mg//kgBW, and 300 mg//kgBW, a comparison control glibenclamide for insulin-deficient animals, and metformin control comparators for insulin resistance comparators. Parameters observed in the insulin resistance method KGD and CITT by giving insulin 0.0125 U//kgB Wintraperitoneal and in the insulin deficiency method, measuring KGD and MDA levels using a UV-Vis spectrometer. The results showed that the effective dose of the method of insulin deficiency, and insulin resistance was 75 mg//kgBW which could affect blood glucose levels, increase insulin

sensitivity and reduce malondialdehyde levels in mice. The conclusion of this study indicated that the ethanol extract of Moringa leaves affected the blood glucose levels of the tested animals by increasing the CITTvalue and reducing malondialdehyde levels.

Keywords: Antidiabetic, CITT, Malondialdehyde, Moringa *oleifera*

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders of fat, carbohydrate, and protein metabolism that results from defects in insulin secretion, insulin action (sensitivity), or both **[1]**. The prevalence of DM in the adult population worldwide in 2019 reached 10.7 million people, and this number is expected to increase to 13.7 million in 2030 **[2]**. According to epidemiology, diabetes mellitus is common in the Asia Pacific region. This is because the first, second, and fourth countries with the most populations in the world are in the Asia Pacific region, namely China, India, and Indonesia. International Diabetes Federation (IDF) data for 2017 found that the number of diabetics in Indonesia reached 10.3 million people and caused Indonesia to rank 6th with the highest number of adult diabetics in the world **[3]**. Data showed that almost 90% of people with diabetes suffer from diabetes mellitus type 2 (T2DM). T2DM is characterized by insulin resistance and insulin deficiency **[4]**.

Previous experimental and clinical studies report that oxidative stress plays a major role in the pathogenesis of T2DM and its complications **[5]**. Oxidative stress is an imbalance between the number of free radicals molecules and antioxidant compounds in the body **[6]**. Oxidative stress acts as a mediator of insulin resistance and its progression to glucose intolerance leading to microvascular and macrovascular complications **[7]**. Reactive oxygen species or free radicals can be produced by normal cellular metabolism and react with biomolecules like protein, lipid, and DNA to cause cellular damage and responsible for degenerative changes **[8]**. One of the resultsof excessive ROS (*Reactive oxygen species*) level is a modification of cellular structure and function proteins and lipids, leading to cellular dysfunction, including impaired energy metabolism, altered cell signaling and cell cycling control, impaired cell transport mechanisms, and immune activation **[9]**. Lipids are reported as one of the main targets of *Reactive oxygen species*(ROS) **[7]**. Besides, lipid-induced mitochondrial overload due to incomplete oxidation of fatty acids, a process associated with the formation of high ROS levels associated with the occurrence of

insulin resistance **[9]**. In clinical studies in T2DM patients, lipid peroxidation markers were seen according to disease severity, and typical markers include Malondialdehyde (MDA) **[9]**. Shodehindo et al. (2013) reported lipid peroxidation in diabetes-induced many secondary chronic complications, including atherosclerosis and neural disorders **[7]**. Therefore, to reduce damage caused by free radicals, the body needs antioxidants.

Plants are known as a source of natural antioxidant therapeutic potential for various diseases. One of the plants has been reported to possess strong antioxidant, and the antidiabetic effect is Moringa oleifera (*Moringa oleifera* L.) **[10]**. Moringa oleifera is one of the moringaceae families. Moringa oleifera is reputedly known as a "miracle tree" because many parts of this plant have been used for nutritional benefits, medicinal properties, etc. **[11]**. Empirically, Moringa leaves (*Moringa oleifera* L.) used by people as an antidiabetic. Based on the previous study, the ethanolic leaf extract*Moringa oleifera*dose 500 mg/kgBWshowed antidiabetic activity. It lowers blood glucose levels and improves insulin sensitivity in diabetic rats **[10]**. This research aimed to evaluate the activity of *M.oleifera* leaves as an antidiabetes using two animal model, that was insulin resistance and insulin deficiency animal model.

MATERIALS AND METHODS

Animals

In this study, the animals were male Swiss Webster mice at an average bodyweight of 20-30 grams and 2-3 months of age obtained from breeders in Majalaya. All mice were adapted to the new environment for one week, including feeding. According to the Commission of the Ethics of Health Research Faculty of Medicine, animal experiments were conducted according to the University of Padjajaran Bandung (No. 227/UN6.KEP/EC/2020). The parameter measured in this experiment was blood glucose level and Malondialdehyde (MDA) level for insulin deficiency animal model and blood glucose level and the value of the constant of constant insulin tolerance test (CITT) for insulin resistance animal model.

Determination of Moringa oleifera leaves (*Moringa oleifera* L.)

Moringa oleifera leaves (*Moringa oleifera* L.) were obtained from Balitro Bogor, West Java. The determination of plants was then conducted in the Research Center for Biology, The Indonesian Institute of Science (LIPI), Bogor. (Plant Identification Certificate, Number:B-38954/ IPH.3/ KS/ XI/2019).

Characterization of Simplicia

Characterization was carried out on Moringa Simplicia powder, includingwater content, total ash content, water-soluble extract content, ethanol-soluble extract content, and drying shrinkage.

Phytochemical screening

Phytochemical screening was carried out on ethanol extract of Moringa leaf, which included Identifying alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids.

Ethanol Extract of Moringa oleifera Leaves

A total of 2000 grams of dried Simplicia was macerated with 12 L of 96% ethanol for 3 x 24 h at room temperature. The liquid extract was then evaporated using a rotary evaporator until it became a thick extract.

In Vivo Antidiabetic Acitivity Test:

Insulin Resistance

This method was conducted preventively. The insulin resistance animal model was induced in 30 mice divided into six groups: negative control group, positive control group, standard drug group (metformin 65 mg/kgBW), and extract group at dose 75, 150, and 300 mg/kgBW. Except for the negative control group, all groupswere induced using lipid emulsion with a modification at dose 0,5 mL/20 grams BW orally for 28 days. Before treatment,the mice fasted for 3 hours, and then the initial blood glucose levels were measured. After that, mice were given induction and treatment together for 28 days. Parameter measured were blood glucose on the 0, 7^{th}, 14^{th}, 21^{st}, and 28^{th} days. The data obtained were then analyzed, statistically using the ANOVA method. After 28 days, the insulin tolerance test was performed. An insulin tolerance test (ITT) was performed using insulin 0,0125 U/kgBW intraperitoneally. Blood glucose levels were measured using the Easy Touch® blood glucose meter at 0, 15, 30, 45, 60 minutes after insulin administration. The data was made into a graphical form, and the gradient was calculated to obtain the value of CITT.

Insulin Deficiency

This method was conducted curatively. Animal models of insulin deficiency were induced in 24 mice which were divided into six groups, namely the negative control group, the positive control group, the standard drug group (glibenclamide 0.65 mg/kgBW), and the extract group with doses of 75, 150, and 300 mg/kgBW. Except for the negative control group, all groups were induced using alloxan monohydrate 50 mg/kgBW **[12]**. After three days of alloxan administration,each test animal's blood glucose levels were measured to see an increase in blood sugar levels. Test animals are declared diabetic if

their fasting blood glucose levels are ≥ 200 mg/Dl **[13]**. They have then given therapeutic treatment for 15 days by measuring blood sugar levels on days 5th, 10th, and 15th using a glucometer and Easy Touch® striptest. The data obtained were then analyzed, statistically using the ANOVA method.

Measurement Of Malondialdehyde Conditions

After 15 days, measurements of malondialdehyde levels were taken from the mice's eye blood plasma Plexus Retroorbitalis **[14]**. The standard solution used was m 1,1,3,3-tetramethoxypropane (1: 80000) with a concentration of 25; 32.5; 50; 62.5; 75 µl, which was piped into the test tube, then added 125 µl of aquadest. Each test tube was added with 1.25 mL 20% TCA and 0.5 mL 0.67% TBA, then homogenized. The mixture was heated for 30 minutes in a boiling water bath and immediately cooled. A total of 0.25 mL of centrifuged blood supernatant was added to 1.25 mL of 20% TCA and 0.5 mL of 0.67% TBA and then homogenized. The mixture is heated for 30 minutes in a water bath then cooled. Standard TMP solutions and blood sample solutions were measured using a UV-Vis spectrophotometer at a wavelength of 532 nm. MDA levels were calculated using the standard curve regression equation 1,1,3,3-tetramethoxypropane **[15]**. The data obtained were then analyzed statistically using the ANOVA method.

RESULTS AND DISCUSSION

In this study, we tested insulin resistance and insulin deficiency by administering moringa leaves ethanol extract (*Moringa oleifera* L.). In terms of insulin resistance parameterswheredecreased in blood glucose levels on the 0, 7^{th}, 14^{th}, 21^{st}, and 28^{th} day and followed by measuring the values of constant insulin tolerance test (CITT). The parameters observed in the insulin deficiency method were decreased in blood glucose levels on the 5th, 10th, 15th day, andplasma MDA level was measured after the end of treatmentusing a UV-VIS spectrophotometric.

Characteristics of Moringa Leaves Simplicia

The simplicia characteristics were carried out on the simplicia ethanol of Moringa leaves (*Moringa oleifera* L.), and the following results were obtained (**Table 1**).

Simplicia characteristics were carried out to determine the quality of the simpliciaused for this study. The characteristics of Moringa leaves simplicia include drying shrinkage, water content, total ash content, ethanol-soluble extract content, and water-soluble extract content.

Phytochemical Screening of Ethanol Extract of Moringa Leaves

Phytochemical screening was carried out on simplicia and ethanol extract of Moringa leaves (*Moringa oleifera* L.*),* and the following results were obtained (**Table 1)**.

Phytochemical screening is carried out to determine the class of secondary metabolite compounds contained in the simplicia and ethanol extract of Moringa leaves to be used. **Table 2** shows that the simplicia and ethanol extract of Moringa leaves contain alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids, but do not contain quinone compounds. These metabolite compounds Moringa contains flavonoids that can regenerate pancreatic beta cells in diabetic rat test animals **[16]**.

Decreased Blood Glucose Levels in Insulin Resistance Animals

Blood glucose levels obtained in this study are shown in **Table 3**.

From **Table 3**, we could see a decrease in the value of blood glucose levels that are quite varied. From the statistical results using the *One Way ANOVA* method then continued using Post-Hoc Bonfferoni obtained on positive control day 21 to day 28 had a significant difference ($p < 0.05$) when compared with a negative control group, metformin comparison group at 65 mg/KgBw, and the leaves of moringa ethanol extract group at 75 mg/KgBw, 150 mg/KgBw and 300 mg/KgBw. This indicates that the metformin comparison group at 65 mg/KgBw and the moringa leaf ethanol extract test groups decreased blood glucose levels. However, these levels did not return to normal. Statistically, all doses of ethanol extract of Moringa leaves had a significant difference ($p < 0.05$) compared to the positive control group. But statistically, there were no significant differences in the three doses.

Constant Insulin Tolerance Test (CITT)

This test was carried out preventively on mice induced by high-fat emulsion with a dose of 0,5 mL/20 grams Bw. Testing of insulin tolerance test constants was performed to determine whether the animal tested exhibited insulin resistance. Insulin sensitivity was determined using the intraperitoneal insulin tolerance method. Blood glucose levels were measured every 15 min for one h following intraperitoneal administration of 0,0125 U/KgBw insulin. Lower C_{ITT} indicated lower insulin sensitivity **[17]**. The value of C_{ITT} was shown in **Table 4**.

Table 4 showed that high-fat emulsion administration with a dose of 0,5 mL/20 grams BW for 28 days could affect the C_{ITT} value. Using the *One Way ANOVA* method, the statistical results, then continued using Post-Hoc Bonfferoni obtained on a negative control group,have significantly different CITT values towards positive control. This shows a decrease in insulin sensitivity in the animals in the

positive control group. The metformin group and all test groups of ethanol extract of moringa leaves showed significant differences in the positive control ($p < 0.05$) and did not differ significantly in negative controls. This shows the effect of increasing insulin sensitivity by metformin and the ethanol extract of moringa leavesmaterial. The metformin group and the ethanol extract of moringa leaves groups had insulin sensitivity levelsthat were almost the same as the level of insulin sensitivity possessed by the negative control group or healthy animal group. Whereas in the ethanol extract of moringa leaves groups, based on the table above, it can be seen that the ethanol extract of moringa leaves groups did not have a significant difference to the metformin group used in this study as a comparison. This refers toindicates that the ethanol extract of moringa leaves material has the same effectiveness as metformin.

Based on phytochemical screening, it was known that *M.oleifera* leaf extract contained flavonoid, saponin, tannins, steroids, and alkaloid. Flavonoids were thought to regenerate pancreatic beta cells by counteracting free radicals, increasing insulin release, and stimulating the absorption of $Ca2^{+}$ from cell tissue, which is very effective in not having enough insulin **[18]**.

Decreased Blood Glucose Levels in Insulin Deficiency Animals

Blood glucose levels obtained in this study are shown in **Table 5**.

From **Table 5**, it can be seen that the decrease in the value of blood glucose levels is quite varied. From the statistical results using the *One Way ANOVA* method, then continued using Tukey's posthoc. On testing from the first day to the 15th day, the negative control group had a significant difference ($p < 0.05$) to the positive group. Likewise, these data indicate a significant difference ($p < 0.05$) from the positive group to the comparison group given 0.65 mg/kgBW glibenclamide. In the three groups of test doses, namely the ethanol extract of Moringa leaves 75 mg/kgBW, 150 mg/kgBW, and 300 mg/kgBW, showed that the test group could significantly reduce blood glucose levels up to day 15 (t15) and statistically different significant ($p < 0.05$) to the positive control group.

Measurement Of Malondialdehyde Conditions

In determining MDA levels, the standard curve was first determined using 1,1,3,3 Tetramethoxypropane with PBS (Phosphate Buffer Saline) solvent **[18]** to measure the absorbance with five concentration series, namely 25; 32.5; 50; 62.5; 75 µl to obtain a linear regression equation **[15]**. This equation determines the amount of MDA

levels in mice. The value of Malondialdehyde was shown in **Table 6**.

Table 6 shows the effect of the ethanol extract of *Moringa oleifera* L. leaves on MDA levels in animal models of mice that have been induced by alloxan. In this **Table 6**, the positive control group had the highest MDA levels compared to the others. Then there was a significant difference ($p < 0.05$) in the positive control group against the other groups. In the diabetic model group treated with ethanol extract of Moringa leaves at a dose of 75, 150, 300 mg/kgBW showed a decrease in MDA levels when compared to the positive control group.

Table 1: Characteristics Of Moringa Leaves Simplicia

Parameters	%
Water content	0.90
Drying Shrinkage	9. 32
Total Ash Content	10. 17
Ethanol Soluble Extract Content	46.352
Water Soluble Extract Content	20.09

Table 2: Phytochemical Screening Of Simplicia And Ethanol Extract Of Moringa leaves (*Moringa oleifera* L.)

Compounds	Simplicia	Extract
Alkaloid	+	+
Tannin	+	+
Quinon	-	-
Steroid	+	+
Flavonoid	+	+
Saponin	+	+

+: Contains secondary metabolite compounds
-: Does not contain secondary metabolite compounds

Table 3: Average Blood Glucose Levels In Insulin Resistance Animals

Groups	Blood glucose levels (Mg/dL ± SD)				
	0	7	14	21	28
Negative control	83.2±4.15	93.40 ±18.45	91.40 ±19.09	91.00±3.08*	92.00 ±6.96*
Positive control	84.80 ±4.44	85.20 ±27.88	98.20 ±29.91	129.80 ±3.96	151.00 ±19.20
Metformin 65 mg/kg BW	84.80 ±9.26	76.40 ±10.88	85.40 ±16.91	78.20 ±9.34*	69.20 ±16.45*
Ethanol extract of moringa leaves 75 mg/kg BW	84.60 ±3.36	83.20 ±16.63	79.00 ±19.52	78.2±10.80*	76.80 ±22.96*
Ethanol extract of moringa leaves 150 mg/kg BW	83.20 ±4.32	80.80 ±15.40	90.80 ±23.94	94.80 ±9.12*	90.40 ±19.31*
Ethanol extract of moringa leaves 300 mg/kg BW	80.20 ±4.02	77.00 ±16.00	77.60 ±22.23	82.40 ±10.81*	77.40 ±12.14*

***: Significantly different compared to the positive control group ($p < 0.05$)**

Table 4: The value of C_{ITT}

Groups	C_{ITT} ± SD
Negative control	1.06 ± 1.03*
Positive control	0.27 ± 0.19
Metformin 65 mg/kg BW	1.35 ± 1.16*
Ethanol extract of moringa leaves 75 mg/kg BW	1.15 ± 1.48*
Ethanol extract of moringa leaves 150 mg/kg BW	0.47 ± 0.57*
Ethanol extract of moringa leaves 300 mg/kg BW	0.60 ± 0.67*

***: Significantly different compared to the positive control group (p <0.05)**

Table 5: Average Blood Glucose Levels In Insulin Resistance Animals

Groups	Blood Glucose Levels (mg/dL ± SD)			
	0	5	10	15
Negative Control	97.00 ± 9.27*#	110.75 ± 26.66*	117.00 ± 13.19*	113.50 ± 32.18*
Positive Control	369.50 ± 135.12@	362.5 ± 169.86#@	395.75± 187.61#@	360.50 ± 168.09#@
Glibenclamid 0.65 mg/kgBW	300.25 ± 108.74@	172.75 ± 64.01*	130.25 ± 28.54*@	153.00 ± 18.88*@
Ethanol extract of moringa leaves 75 mg/kg BW	363.75 ± 114.57@	185.00 ± 72.23*	172.25 ± 19.17*	174.00 ± 29.95*
Ethanol extract of moringa leaves 150 mg/kg BW	352.25 ± 143.59@	262.25 ± 148.258@	149.50 ± 34.51*	162.50 ± 38.44*
Ethanol extract of moringa leaves 300 mg/kg BW	393.50 ± 109.082@	217.25 ± 55.22*	154.75 ± 26.76*	123.75 ± 22.29*

***: Significantly different compared to the positive control group (p <0.05)**
@: Significantly different compared to the negative control group (p <0.05)
#: Significantly different compared to the glibenclamid group (p <0.05)

Table 6: Average levels of malondialdehyde in mice

Groups	MDA (nmol/ml ± SD)
Negative Control	1.0302 ± 0.37*
Positive Control	3.4293 ± 1.76
Glibenclamid 0.65 mg/kgBW	1.3261 ±1.30*
Ethanol extract of moringa leaves 75 mg/kg BW	0.7134 ±0.20*
Ethanol extract of moringa leaves 150 mg/kg BW	0.6241 ±0.34*
Ethanol extract of moringa leaves 300 mg/kg BW	0.3790 ±0.44*

***: Significantly different compared to the positive control group (p <0.05)**

It is known that diabetes mellitus is a disease that can disrupt antioxidant defense and the occurrence of oxidative stress, which can worsen and develop complications. The source of oxidative stress that occurs comes from the increased production of free radicals due to glucose autoxidation, disruption of enzymatic antioxidant defense activity, and decreased concentrations of low molecular weight antioxidants in tissues. Sources of oxidative stress in diabetes include a shift in the balance of redox reactions due to changes in carbohydrate and lipid metabolism, increasingthe formation of ROS from glycation and lipid oxidation reactions reducing the antioxidant defense system **[19]**. Markers of lipid damage occur when the production of reactive oxygen species is excessive, for example, hydrogen peroxide and oxygen molecules, which modulate the biological function of all biomolecules that become lipid targets to oxidize to produce Malondialdehyde **[20]**. In diabetes, hyperglycemia will exacerbate and exacerbate the formation of ROS by several mechanisms. ROS will increase the formation of Tumor necrosis factor-α (TNF-α) expression and exacerbate oxidative stress. TNF-α can lead to fatty acid circulation, change β cell function, increase triglyceride levels, and reduce HDL levels. Oxidative stress in diabetics will increase the formation of ROS in the mitochondria which will result in various oxidative damage in the form of diabetes complications and will worsen the condition of diabetics. Therefore it is necessary to normalize ROS levels in the mitochondria to prevent oxidative damage **[20]**. In connection with this study, alloxan can significantlyincrease in Malondialdehyde levels compared to the negative control group.

The test therapy group with ethanol extract of Moringa leaves showed a very good effect, and there was a very significant difference ($p < 0.05$) compared to the positive control group. The most effective dose of Moringa leaf ethanol extract based on the data obtained is 300 mg/kgBW. This shows that the treatment of alloxan-induced diabetic mice with ethanol extract of Moringa leaves can significantly affect malondialdehyde levels. Moringa leaves have strong in vitro antioxidant activity by collaborating with the content of its secondary metabolite compounds, namely flavonoids. One of the mechanisms of action of flavonoids is an agent that can bind free radicals to reduce Reactive Oxygen Species (ROS) **[21]**.

CONCLUSION

Ethanol extract of Moringa leaves affectson increasing insulin sensitivity and reduces

blood sugar levels, which improve malondialdehyde levels.

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