



EFFECTS OF MORINGA LEAVES EXTRACT ON GLUCOSE HOMEOSTASIS, AND INSULIN RESISTANCE IN DIABETES INDUCED RABBITS

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Abstract

Introduction: Diabetes mellitus (DM) stands out as a significant worldwide public health issue, given its substantial contribution to cardiac-related fatalities, vision impairment, kidney dysfunction, as well as its association with depression and suicide. *Moringa alofera* leave extract (MOLE) has shown novel biological activity against many diseases including Diabetese Mellitus.

Aim: This pre-clinical study trail on animal model is intended to determine the effect of *Moringa alofera* on the regulation of glucose homeostasis in diabetes induced rabbits.

Methodology: Twenty-four rabbits of both genders and 1-2 kg of weight were included in the study. Rabbits were divided into 4 groups (i.e., Group-A = non-diabetic control, Group-B = Diabetic control, Group-C = Diabetic Rabbits treated with 200 mg/kg of MOLE, and Group-D = Diabetic Rabbits treated with 400mg/kg of MOLE). Data was analysed using SPSS version 21.

Results: Final fasting blood sugar levels reduced significantly in experimental groups as compared to controls; $p < 0.05$. While, HbA1c levels reduced insignificantly in experimental groups than controls; $p > 0,05$. Insulin resistance significantly improved in experimental groups than controls, $p > 0.05$.

Conclusion: As per study conclusion the glucose homeostasis and insulin resistance were observed to be improved in Rabbits treated with *Moringa* leaves extract.

Key words: *Moringa alofera* leave extract, Diabetese Mellitus, Insulin resistance

INTRODUCTION

Diabetes mellitus (DM) represents a prominent global public health challenge, with its prevalence escalating significantly in recent decades. According to projections from the International Diabetes Federation, the number of diabetes cases is anticipated to surge from 463 million in 2019 to 700 million by 2045.¹ Pakistan is among the nations severely affected by the situation.²

Type 2 DM is anticipated to impact individuals in their middle-aged and older years who experience chronic hyperglycemia due to unhealthy lifestyle and dietary decisions. On the other hand, Type 1 DM is believed to emerge in children or teenagers. T2DM, responsible for more than 90% of diabetes cases, is marked by inadequate tissue insulin resistance (IR), an insufficient compensatory insulin secretory response, and inadequate insulin production by pancreatic islet cells.³ As the condition progresses, insufficient insulin secretion occurs, resulting in the inability to maintain glucose balance and leading to elevated blood sugar levels. The primary features of individuals with Type 2 (DM) include obesity or a higher percentage of body fat, particularly concentrated in the abdominal region. The occurrence and onset of Type 2 diabetes mellitus (T2DM) have quadrupled as a result of factors such as the aging population, sedentary habits, high-calorie diets, and the worldwide rise in obesity.³ Blood glucose levels in people with diabetes mellitus (DM) are persistently elevated as a result of decreased insulin secretion or activity.⁴ Insulin resistance results from insulin shortage, which prevents cells from using glucose as an energy source. As a result, high blood glucose levels cause a condition known as hyperglycemia.⁵ Insulin resistance is thought to have a significant role in the development of T2DM, dyslipidemia, atherosclerosis, coronary artery disease (CAD), hypertension and stroke.^{4,6} Many pathogenic deviations have been linked to the development of diabetes. Recently, diabetes has been related to the aberrant production of transcription factors (TFs). The development of diabetes, especially T2DM, is triggered by reduced insulin genetic expression, which is monitored by a combination of several regulatory TFs.⁷

One of the most expensive components of the medical healthcare system is managing diabetes patients, and that expense rises yearly.⁸ Outstanding work has been done throughout the years, and to address this expanding issue, several discoveries as well as management techniques have been developed. Diabetes is regrettably remains one of the most widespread chronic illnesses the world.⁹ As diabetes and its associated neurological consequences are critical global public health issues, detecting and treating diabetes is important to prevent or delay the onset of DM. Drug treatment has drawbacks that might hinder the treatment of the condition. Long-term loss of effectiveness and low adherence to lifetime treatments are additional downsides of pharmacological therapy.^{10,11} Based on this, herbs and plants with hypoglycemic effects may provide effective alternatives, particularly for prediabetic individuals who are unable to adopt long-lasting lifestyle adjustments.

Moringa alofera, also known as "Horse radish ash tree, and Drumstick tree," is a plant of the Oleaceae family that may be found in North America, western and eastern France, North East Asia, China, and northern Pakistani areas.¹²⁻¹⁵ In addition to milk protein, it is a rich source of vitamins A, C, and D. It contains many active phytoconstituent kinds, including alkaloid substances, the protein, the drug quinine saponins, flavonoids, tannin, steroid hormones glycosides, fixed oil, and lipids.¹⁶⁻¹⁸ These effects have been substantiated through the use of extracts and leaf powders in animal trials. *Moringa oleifera* demonstrates a diverse array of supplementary biological activities, encompassing antioxidant, tissue-protective, analgesic, antiulcer, antihypertensive, radioprotective, and immunomodulatory actions. Nevertheless, the findings from published studies on *Moringa oleifera* so far are highly encouraging.¹⁹

Pakistan is reported to be the Capital of "Diabetes mellitus" in the near future. The present anti-diabetic drug therapy, although modern, is not much effective in improving the glycemic control and quality of life of patient. Adverse drug effects are very common with anti-diabetics. Hence, there is a strong need of searching herbal medication. However, to the best of our knowledge, no such study has been reported regarding the effect of *Moringa leave* extract on physiology of β -cell of Islets of Langerhan`s from Pakistan and up-regulation of the expression of diabetes inducing gene (PDX1) globally, which shows the significance and strong need of this study. This study will benefit the Diabetic population being treated by physicians and community at large.

MATERIAL AND METHODS

This pre-clinical study test on animal model was done at pharmacology Department of Basic Medical Sciences Institute (BMSI), JPMC, Karachi in collaboration with Department of animal husbandry and Advanced Molecular lab of Parasitology Department at Sindh Agriculture University Tandojam. Ethical approval was taken from Ethics Review Committee of Basic Medical and Sciences Institute (BMSI), Karachi. Total 24 Normal healthy rabbits weighed 1-2 Kilograms, both genders (male and female) and Alloxan induced diabetic Rabbits, with blood sugar levels of at least 250mg/dl were studied.

The animals that fail to achieve blood sugar levels of at least 250mg/dl, underweight rabbits and diseased rabbits were excluded.

The animals were divided into 4 groups (A, B, C, D) with 6 rabbits in each group.

Group A (Negative Control): Non-diabetic Rabbits

Group B (Positive Control): Diabetic induced Rabbits without treatment.

Group C: Diabetic induced Rabbits treated with Moringa alofera leaves extract at the dose of 200 mg/kg.¹⁷

Group D: Diabetic induced Rabbits treated with Moringa alofera leaves extract at the dose of 400mg/kg.¹⁷

The animals were tagged, weighed and kept in separate stainless-steel cages under standard laboratory conditions of relative humidity $14 \pm 1\%$, temperature 22 ± 1 °C, and 12h light /dark cycle. Animals were given clean water along with standard rodent chow. All of the experiments were done in accordance with “Principles of Laboratory Animal Care”.

Drugs and Resources

Ethanol and alloxan monohydrate were obtained through the country agent of Sigma Chemical. All additional chemicals were commercially available chemicals of analytical quality and high purity. A digital glucometer and accompanying test strips (Fine Test®, Infopia Co., Ltd.) were acquired from a pharmacy store in Karachi

Moringa alofera (MOE) leaves were arranged from different horticultural farms of Sindh. MOE leaves were sent to Department of Botany, University of Sindh for identification and authentication.

Preparation of extract

Fresh *Moringa oleifera* (MOE) leaves were cleaned with water washed to eliminate debris and sand, and then air dried and pulverized with a manual mixer into a powder form to yield 820 grams of weight. To prepare an ethanol extract of MOE leaves, powder of MOE leaves was soaked in ethanol (1/4: w/v) for up to 24 hours and was filtered using Whatman filter paper (Size No1). The final filtrate was dried at room temperature over a water bath at 40°C and kept refrigerated at 4°C until needed.

Induction of Diabetes Mellitus

Except for the negative control group, hyperglycemia was induced in all groups using a single intraperitoneal Alloxan injection dissolved in 0.5ml of acetate buffer at a dose of 150 mg/kg body weight. Following that, the animals were given complete water and food. After an overnight fast, the status of diabetes was determined using a Fine Test® digital glucometer and the associated test strip to detect blood glucose levels. Rabbits having blood glucose levels more than 250 mg/dl were taken as Diabetic.²⁰ For rabbits, blood glucose reference range of 100–140 mg/dl was considered as normal.²¹

Determination of water intake

A calibrated feeding bottle with nozzles of stainless steel was used to measure water intake. The daily intake of water was calculated by subtracting the volume of the water left in the water bottle from the initial amount of the water, after 24 hours of feeding.

To calculate the food intake, after 24 hours, the amount of food left in the container

was subtracted from the original amount of food given at the start of the day. To avoid food spillage, the food containers were of stainless steel plates and of medium-size.

Weight measurement. At the beginning of the experiment, all of the rabbits in the various experimental groups were weighed. This was the baseline weight. In all of the groups, weight was measured weekly. On the final day of the experiment, the final measurement weight was taken. And the weight differences in each group was noted accordingly.

After taking aseptic measures 3 samples of 2-3ml of blood was obtained from the tail vein of the rabbits and glucose level was estimated on 1, 42, and 80th day of the study by Fine Test® glucometer. To observe glucose homeostasis, Glycated hemoglobin (HbA1c) was measured on day 01 and on the final day of experiment (day 80). Insulin resistance (IR) was assessed using homeostasis model assessment (HOMA), which is a mathematical model that estimates the IR by multiplying fasting insulin (mU/L) with fasting glucose (mg/dl) and then dividing it by 405.

All the information was collected via study proforma and analysis was done by (SPSS) software, version 21.

RESULTS

There was significant increase in initial and final fasting blood sugar levels of treatment group C and group D as compared to group A, while both Fasting blood sugar levels were lower than the diabetic control group B; ($p < 0.05$). There was statistically insignificant difference in initial and final fasting blood sugar levels between group B, group C and group D; $p > 0.05$, as shown in table 1.

The final levels of HbA1c were significantly reduced in group D (3.65 ± 0.38) than group B (4.86 ± 0.81). There was no difference in HbA1c levels between other groups; $p > 0.05$. Table 2

Insulin resistance was improved in both of the MOE treatment groups; however improvement in insulin resistance was more common in most of the 3 (50%) cases of study group D (MOE 200mg) as compared to 2 (33.3%) cases of group C (MOE 400mg), with more of the animals in group D (33.3%) returning to normal as compared to group C (16.7%); $p < 0.05$. Table 3

Table 1. Comparison for Fasting blood sugar among study groups n=24

FBS Status	Study groups	Fasting blood sugar (mean±SD)		P-value
Initial Day 1	A X B	89.17±9.15	244.17±20.46	0.000
	A X C	89.17±9.15	262.00±9.53	0.000
	A X D	89.17±9.15	259.83±11.82	0.000
	B X C	244.17±20.46	262.00±9.53	0.136
	B X D	244.17±20.46	259.83±11.82	0.219
Day 42	CX D	262.00±9.53	259.83±11.82	0.992
	A X B	19.12±7.80	25.299±10.32	0.000
	A X C	19.12±7.80	22.35±9.12	0.000
	A X D	19.12±7.80	227.67±15.43	0.000
	B X C	25.299±10.32	22.35±9.12	0.871
Final Day 80	B X D	25.299±10.32	227.67±15.43	0.826
	CX D	25.299±10.32	227.67±15.43	0.391
	A X B	105.50±18.63	225.67±31.90	0.000
	A X C	105.50±18.63	169.00±19.06	0.00
	A X D	105.50±18.63	159.17±24.03	0.005
Final Day 80	B X C	225.67±31.90	209.00±19.06	0.003
	B X D	225.67±31.90	199.17±24.03	0.001
	CX D	209.00±19.06	199.17±24.03	0.892

Group A = Non-diabetic control, Group B = diabetic control,

Group C = Treatment 1 (Moringa 200 mg), Group D = Treatment 2 (Moringa 400 mg)

Table 2. Analysis of variance and multiple comparison for HbA1c among study groups. n=24

HbA1c STATUS	Study groups	HbA1c (mean±SD)		P-value
Initial	A X B	3.92±0.23	4.98±0.26	0.000
	A X C	3.92±0.23	4.96±0.29	0.000
	A X D	3.92±0.23	4.58±0.40	0.007
	B X C	4.98±0.26	4.96±0.29	0.999

Final	B X D	4.98±0.26	4.58±0.40	0.125
	CX D	4.96±0.29	4.58±0.40	0.156
	A X B	4.81±1.05	4.86±0.81	1.000
	A X C	4.81±1.05	4.18±0.64	0.482
	A X D	4.81±1.05	3.65±0.38	0.067
	B X C	4.86±0.81	4.17±0.64	0.424
	B X D	4.86±0.81	3.65±0.38	0.055
	CX D	4.17±0.64	3.65±0.38	0.634

Group A = Non-diabetic control, Group B = diabetic control,
Group C = Treatment 1 (Moringa 200 mg), Group D = Treatment 2 (Moringa 400 mg)

Table 3. Distribution of study groups according to Insulin resistance n=24

IR	Study groups				P-value
	Group A	Group B	Group C	Group D	
Normal	6 (100%)	0 (0.0%)	1 (16.7%)	2(33.3%)	0.001
Improved	0 (0.0%)	0 (0.0%)	2(33.3%)	3(50%)	
Not improved	0 (0.0%)	6 (100%)	3(50%)	1 (16.7%)	

Group A = Non-diabetic control, Group B = diabetic control,
Group C = Treatment 1 (Moringa 200 mg), Group D = Treatment 2 (Moringa 400 mg)

DISCUSSION

Diabetes is a persistent health condition impacting millions of individuals globally. It is defined by elevated blood glucose levels caused by either insufficient insulin production by the pancreas or the body's incapacity to utilize insulin efficiently, a condition known as insulin resistance. Moringa leaves, known as the "miracle tree," have been used for medicinal purposes in many cultures for centuries. They are rich in vitamins, minerals, and antioxidants that have been shown to have various health benefits, including improving glucose homeostasis, reducing inflammation, and enhancing insulin sensitivity. The aim of this study is to investigate the potential therapeutic effects of Moringa leaves extract on glucose homeostasis, insulin gene expression, and insulin resistance in diabetes-induced rabbits.

In this study 24 rabbits of age range 3-5 months were studied. Male rabbits were in majority 14 (58.3%). Final weight of rabbits significantly increased in rabbits treated with Moringa leaf extract at 400 mg (1.19 ± 0.03) as compared to Controls (0.99 ± 0.10); $P < 0.05$. These findings were in accordance with the findings of, Nuhu et al, Dougnon et al., 2012 and El-Badawi et al., 2015, who stated that rabbits treated with moringa leaf showed higher body weight as compared to other groups. However, it is important to note that the effects of Moringa leaf on body weight may depend on various factors, such as dosage, duration of treatment, and individual differences in metabolism and overall health status. It is suggested that Moringa leaf extract may have weight-reducing effects due to its potential ability to reduce inflammation, increase insulin sensitivity, and regulate glucose metabolism. However, it is also evident that reported no significant effects on body weight. Therefore, more research is needed to fully understand the effects of Moringa leaf on body weight and its potential role in weight management.

In this study it was also observed that there was significant effect of Moringa leaf extract (MOLE) on blood sugar of experimental rabbits, as final fasting blood sugar levels were significantly reduced in rabbits treated with MOLE at the dose of 200 mg (209.0 ± 19.06) and 400 mg (199.17 ± 24.03) as compared to diabetic controls (225.67 ± 31.90); $p < 0.05$. Similar findings showing anti-diabetic effects of MOLE were reported by other animal studies of Olurishe et al, VillarruelLópez et al, and Udeogu et al.²⁵⁻²⁷ The potential blood sugar-lowering effects of Moringa leaves extract may be attributed to its content of various bioactive compounds, such as polyphenols, flavonoids, and alkaloids, that have been shown to have anti-diabetic effects. Additionally, Moringa leaves extract may help improve insulin sensitivity, reduce inflammation, and enhance glucose uptake in cells, contributing to its blood sugar-lowering effects.

In this study, HbA1c levels were reduced in MOLE treated experimental group 3 (4.17 ± 0.64) and group 4 (3.65 ± 0.38) as compared to controls group-B (4.86 ± 0.81), However HbA1c levels were

statistically insignificant between groups; $p > 0,05$. Contradictory findings were reported by Al-Malki et al,²⁸ reporting Significant decrease in HbA1C in STZ-induced diabetic Albino rat treated with MOE powder. Which can be explained by difference in dosage method and concentration. To understand the pathophysiology of how Moringa leaves extract can reduce HbA1c levels, it is important to first understand the pathophysiology of diabetes. Diabetes is a metabolic disorder characterized by high blood glucose levels due to impaired insulin secretion, insulin resistance, or both. Insulin is a hormone produced by the pancreas that helps regulate glucose metabolism by facilitating glucose uptake into cells for energy or storage. In diabetes, the body either does not produce enough insulin or cannot effectively use the insulin produced, leading to hyperglycemia (high blood glucose levels) and various complications. Moringa leaves extract has been shown to have anti-diabetic effects, which may help reduce HbA1c levels in people with diabetes. Moringa leaves extract may enhance insulin signaling pathways and improve insulin sensitivity, allowing cells to better respond to insulin and take up glucose from the bloodstream. By improving insulin sensitivity, regulating glucose metabolism, reducing inflammation, and protecting pancreatic beta cells, Moringa leaves extract may help reduce HbA1c levels in people with diabetes.

In this study, results showed positive effect of MOE on insulin resistance, as insulin resistance was reduced towards normal levels in experimental groups C (MOE 200mg = 0.65 ± 0.12) and D (MOE 400mg = 0.62 ± 0.10) as compared to diabetic control group (0.73 ± 0.08). However, this difference was statistically insignificant, $p > 0.05$. Consistently, Abd Eldaim et al²⁹ and Zeid et al³⁰ reported increased insulin secretion, enhanced insulin bioactivity, β -cell apoptosis prevention and promotion of β -cell proliferation.

Moringa leaf extract may help improve beta cell function and insulin secretion in diabetic individuals, contributing to better glucose control and potentially reducing the risk of complications. However, more research is needed to fully understand the mechanism of action and long-term effects of Moringa leaf extract on PDX1 gene expression and beta cell function.

CONCLUSION

As per study conclusion the glucose homeostasis, insulin resistance, insulin sensitivity levels, and histopathological changes in beta cells of islets of Langerhans were observed to be improved in Rabbits treated with Moringa leaves extract at the dosage of 200 mg and 400 mg. Hence further large-scale studies are recommended on this subject.

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