IJPSR (2022), Volume 12, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 21 March 2021; received in revised form, 27 September 2021; accepted, 05 December 2021; published 01 January 2022

HYPOGLYCEMIC AND HYPOLIPIDEMIC ACTIVITY OF *MORINGA OLEIFERA* LEAF POWDER IN ALLOXAN-INDUCED DIABETIC RABBITS

Krishnaveni Bommineni [*1, 2] and Madhusudhan Rao Yamsani [1]

Department of Pharmacognosy [1], Vaagdevi College of Pharmacy, Kishanpura, Kakatiya University, Hanamkonda - 506001, Telangana, India.
Mewar University [2], Chittorgarh - 312901, Rajasthan, India.

Keywords:

Moringa oleifera, Hypoglycaemic, Hypolipidemic, Glibenclamide, Diabetes, Rabbits

Correspondence to Author:
Krishnaveni Bommineni

Assistant professor,
Department of Pharmacognosy,
Vaagdevi College of Pharmacy,
Kishanpura, Kakatiya University,
Hanamkonda - 506001, Telangana,
India.

E-mail: bkvr22@gmail.com

ABSTRACT: The present study was carried out to investigate the hypoglycemic and hypolipidemic effect of *Moringa oleifera* leaf powder (MO) in alloxan-induced diabetic rabbits by administering oral doses (100, 200, and 400 mg/kg body weight). Diabetes was induced intravenously into rabbits with a single dose of alloxan (150 mg/kg). Diabetic rabbits were treated with MO (100, 200, and 400 mg/kg) and Glibenclamide (5 mg/kg/day). To determine fasting blood glucose, blood was drawn on days 1, 7, and 14, while it was drawn on day 14 for the determination of lipids, and relative body weight was measured at days 0 and 14. After 2 weeks of treatment, the fasting blood glucose levels of the MO-fed groups were significantly lower when compared with the diabetes mellitus (DM) group ($P < 0.05$), and the results were compared with standard Glibenclamide drug. Treatment with the MO also resulted in a significant decrease in serum total cholesterol, triglycerides, and low-density lipoprotein cholesterol ($P< 0.05$) accompanied by a significant increase in serum high-density lipoprotein cholesterol ($P< 0.05$). The overall results suggest that the MO possesses potential hypoglycemic and hypolipidemic activity in alloxan-induced diabetic rabbits.

INTRODUCTION: *Moringa oleifera* (Moringaceae) is a fast-growing perennial species native to North-western India, now cultivated in many parts of the world. This species has been used in folk medicine for the treatment of diabetes [1-3]. Scientists have shown Moringa cures type 1 diabetes and type 2. Type 1 diabetes is when patients do not produce insulin, which maintains a level of blood glucose at the necessary standard value.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(1).369-74

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(1).369-74

Type 2 diabetes is linked to insulin resistance. Type 2 diabetes can also be attributed to beta-cell dysfunction, which does not detect glucose levels and decreases insulin signaling, resulting in elevated blood glucose levels [4]. Several studies have shown that Moringa can act as an anti-diabetes agent. Several studies show that Moringa can act as an anti-diabetic agent.

Research has shown that aqueous extracts of *Moringa oleifera* can cure type 1 diabetes caused by streptozotocin and type 2 insulin-resistant diabetes in rats [5]. In another study, the researchers fed Moringa seed powder to STZ-induced diabetes rats and found that fasting blood glucose had decreased [6]. The antioxidant enzymes increased in the serum when the rats were treated with around 500 mg of Moringa seed powder/kg body weight.

This shows that antioxidants present in Moringa can reduce the ROS induced by STZ induction in beta cells[7]. STZ causes ATP dephosphorylation reactions and allows xanthine oxidase to form superoxides and reactive oxygen species (ROS) in beta cells [8]. In diabetic patients, the beta cells get impaired. As a result, high glucose reaches the mitochondria and releases reactive oxygen species. Since beta cells have a low number of antioxidants, this, in turn, causes beta-cell apoptosis. This decreases the insulin secretion contributing to hyperglycemia and, in turn, to type 2 diabetes mellitus [9, 10]. The antioxidants that induce a scavenging effect on ROS have been linked to flavonoids such as quercitin and phenolics. It can be presumed that flavonoids in Moringa scavenge the ROS released from mitochondria, thus protecting beta cells and, in turn, keeping hyperglycemia under control [4, 11].

Diabetes leads to many complications, such as retinopathy, atherosclerosis, nephropathy, etc. The therapeutic effects of Moringa will help prevent disease [12]. As hyperglycemia occurs, blood glucose interacts with proteins and causes advanced glycated end products (AGEs). These AGEs bind to RAGE, which is expressed on immune cell surfaces [13]. This interaction eventually contributes to the increase in cytokines such as interleukin-6 and interferons. At the same time, cell adhesion molecules are expressed on the surface of the artery endothelium. This promotes transendothelial migration, which induces inflammation of the arteries and contributes to atherosclerosis [14]. Moringa is used as an antiatherosclerotic agent [15]. The antiatherogenic nature of Moringa can be accounted for by its antioxidant properties.

Very few studies have studied the hypoglycemic and antihyperglycemic properties of *Moringa oleifera* leaves in rabbits. However, most findings have been obtained in leaf extract studies; therefore, this research investigates leaf powder, a popular method of ingestion, to investigate the effects on glucose, triglycerides, cholesterol, and body weight. Because of these aspects, the objective of this investigation was critically envisaged for the evaluation of the characteristics of *Moringa oleifera* leaf powder to continue to be used as a functional food ingredient in food and pharmaceutical products of concern also, ultimately, to enhance this significantly less explored leaf powder which is readily available in certain parts of the country in a safe, easily usable form.

MATERIALS AND METHODS: *Moringa oleifera* (MO) leaves were collected locally and identified by a botanist (Kakatiya University). The leaves were shade dried and pulverized to get powder; this powder was passed through sieve no.120 to get a fine powder. Alloxan monohydrate was purchased from Sigma –Aldrich co., USA. Serum glucose, total cholesterol, HDL (high-density lipoproteins), LDL (low-density lipoproteins), TG (triglycerides) were estimated using commercial kits purchased from span diagnostics, Surat, India.

Animals: Male albino rabbits (weighing 1.2 -1.6 kg) procured from Nishika labs, Hyderabad, India. Rabbits were maintained on a standard laboratory diet & potable water and housed at a temperature of 22±2°C with a 12 h light/dark cycle. They were acclimatized to laboratory conditions for one week before experimental work was carried out. The experimental protocol was accepted by the ethical committee of the institutional animal of Vaagdevi institute of pharmaceutical sciences, Warangal, India [16] (protocol No: VIPS/IAEC/02/2013/16). International guidelines issued by the International Council for Laboratory Animal Science were also followed [17]. These conditions were maintained throughout the experiment.

Induction of Experimental Diabetes Mellitus: Diabetes mellitus was induced by administering alloxan monohydrate (150mg/kg) dissolved in standard saline solution was injected intravenously into the overnight fasted rabbits through the marginal ear vein [18]. Food was provided 2 h after injection. After 72 h of injection, the animals were monitored for plasma glucose levels. Rabbits with above 250 mg/dL fasting blood glucose levels were selected for the study.

Experimental design: The diabetic rabbits were divided into five groups of six animals each (n=6). Group I are normal animals that were not given alloxan, served as a Normal control group, and received 1.5 ml of physiological NaCl solution (vehicle). Group II is an alloxan-induced diabetic

control group and also received 1.5 ml of physiological NaCl solution. Group III alloxan-induced diabetes animals treated with standard drug glibenclamide (5 mg/kg/day). Groups IV, V, and VI alloxan-induced diabetic animals were treated with MO at 100, 200 and 400 mg/kg. The treatment was continued for fourteen (14) consecutive days.

Estimation of Blood Glucose and Body Weight: Fasting blood glucose was measured after collecting blood samples from rabbit ear veins on 0, 7 and 14 days. Blood glucose levels were determined by the GOD-POD method (glucose oxidase method). Bodyweight changes were also recorded weekly. The procedure was performed according to the previous studies [19].

Lipid-Profile Changes: On day 14, rabbits fasted overnight, and blood was collected from the marginal ear vein. The blood was allowed to stand at room temperature for 2 hours in sterile EDTA test tubes and centrifuged at 1,500 rpm for 5 minutes.

The supernatant was immediately separated from the pellet to prepare serum samples to determine the level of triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), Low-density lipoprotein cholesterol LDL-c) and total cholesterol (TC) with the use of respective kits [20].

Statistical Analysis: The collected data were analyzed by GraphPad Prism 8 for Windows (version 8.01, Trial) and expressed as means ± standard error of the mean (SEM). Glycaemia data were analyzed using two-way ANOVA analysis of variance, followed by Dunnett's multiple comparison tests.

RESULTS: The blood glucose levels in all the groups were estimated on the 0th, 7th, and 14th days. In the normal control group, the glucose levels are maintained normal through all 14 days, and in the diabetic control group, the higher blood glucose levels are also continuously maintained. The standard drug-treated group has shown a progressive reduction in blood glucose levels on the 7th and 14th days. The test drug-treated groups IV, V, VI, given 100, 200 & 400 mg doses respectively, have shown considerable reduction in blood glucose levels. The 400mg dose level of the *Moringa oliefera* powder has shown an almost equal effect in the decrease in blood glucose levels as to that of the standard drug Glibenclamide. The results are shown in **Table 1.**

TABLE 1: THE EFFECT OF THE ORAL ADMINISTRATION OF *MORINGA OLEIFERA* POWDER AND GLIBENCLAMIDE ON BLOOD GLUCOSE IN NORMAL AND DIABETIC RABBITS FOR TWO WEEKS

Group	Dose (mg/kg)	Blood glucose (mg/dl)		
		0 day	7th day	14th day
Normal Control	-	91.2±1.64	90.4±1.2	90.8±1.42
Diabetic Control	-	264±6.4	279±4.8	318±7.8
Glibenclamide	5	268.4±7.2	198±4.4	130±5.4
MO	100	265±3.4	227.3±5.36	172.3±3.6
MO	200	270.2±1.2	215.6±4.4	158±2.68
MO	400	267.4±2.6	190±5.81***	135.4±7.24

The body weight changes were also recorded for the animals of all the groups. The normal control group animals have shown a gain of 10% in weight. The diabetic control group animals' body weight is less than that of the normal group. The standard drug-treated group and Test drug MO powder treated group animals have restored the bodyweight almost equal to the normal control group. The results are shown in **Table 2.**

TABLE 2: EFFECT OF ORAL ADMINISTRATION OF *MORINGA OLEIFERA* POWDER ON BODY WEIGHT IN NORMAL AND DIABETIC RABBITS FOR TWO WEEKS

Group	Dose (mg/kg)	Bodyweight (kg)		Changes in body weight (%)
		Initial	Final	
Normal	-	1.32±0.06	1.48±0.04	10.8% ↑
Diabetic Control	-	1.42±0.03	1.31±0.05	7.7% ↓
Glibenclamide	5	1.38±0.04	1.53±0.04	9.8% ↑
MO	100	1.48±0.05	1.54±0.04	3.8% ↑
MO	200	1.36±0.06	1.48±0.03	8.1% ↑
MO	400	1.50±0.04	1.65±0.04	9% ↑

The Total cholesterol, Triglycerides, HDLC & LDLC levels were on the 14th day. There is an increase in Total Cholesterol, Triglycerides, & LDLC levels and a decrease in HDLC levels in the diabetic control group. The Standard drug and Test drug-treated group animals have shown a significant reduction in Total Cholesterol, Triglycerides & LDLC levels and restored normal levels of HDLC. They were indicating beneficial effects of *Moringa oliefera* powder as anti-diabetic. The results are shown in **Table 3.**

TABLE 3: EFFECT OF ORAL ADMINISTRATION OF *MORINGA OLEIFERA* POWDER ON TC, TG, LDL AND HDL IN NORMAL AND DIABETIC RABBITS FOR TWO WEEKS

Group	Dose (mg/kg)	Serum Parameters			
		Total Cholesterol	Triglycerides	HDLC	LDLC
Normal Control	-	68.9±1.73	78±3.2	32±4.12	78.6±4.6
Diabetic Control	-	132±2.5	155±4.8	25±2.2	121±6.2
Glibenclamide	5	78.4±4.2	124±6.4	28±1.2	95±5.6
MO	100	91.2±3.64	148±7.2	25.8±1.25	111.2±4.6
MO	200	85.4±5.9	134±5.72	27.6 ±2.66	106.5±3.54
MO	400	80.4±4.3	126.6±6.04	29.4±1.15	100.5±2.3

DISCUSSION: In the present study, the anti-diabetic potential of *Moringa oleifera* leaf powder in alloxan-induced diabetic rabbits was estimated. Alloxan is one of the most commonly used substances to induce diabetes in rats. Alloxan induces diabetes through ROS, leading to rapid destruction of pancreatic beta cells, causing hyperglycemia [21]. Hyperglycemia, in turn, increases the generation of free radicals by glucose auto-oxidation [22]. Results revealed that the daily doses of *Moringa oleifera* leaf powder were able to cause significant reductions in mean blood glucose levels compared to diabetic control in a dose-dependent manner, with the 100, 200, and 400 mg/kg. *Moringa oleifera* leaf powder doses bring blood glucose levels down to normal within a few days of administration. One possible explanation for this could be the presence of various types of antioxidant compounds. M. oleifera is a source of antioxidants, vitamins, and a protease-resistant glycoprotein that functions as dietary fiber [23-25]. *Moringa oleifera* has antioxidant activity because it contains phenolic compounds and flavonoids, precisely three classes of phytochemicals: glucomoringin, flavonoids (quercetin and phenolic acids (chlorogenic acid) [25].

These bioactive compounds can exert antioxidant and anti-inflammatory effects to induce cellular protection [26, 27]. Flavonoids can regenerate damaged P-cells in alloxan-induced diabetic rabbits [28]. Polyphenols inhibit lipid peroxidation by acting as chain-breaking peroxyl radical scavengers and can protect LDL from oxidation [29]. Polyphenolic compounds also possess various other biological activities, such as reducing plasma lipids, which might be due to up-regulation of LDL receptor expression 30 and inhibition of hepatic lipid synthesis. Saponins, polyphenolic compounds, and gallic tannins possess potent inhibition of intestinal glucose transport by inhibiting sodium-glucose co-transporter-I (S-GLUT-1) intestines [31]. Diabetic *M. oleifera* treated rabbits increased in body weight. The increase in weight may be due to the content of leaf powder, specifically essential amino acids and vitamins A, B, C, and E. Antioxidants and antimicrobial compounds (phenols, tannins, alkaloids and coumarins) can also act as growth promoters[32]. On the other hand, Glibenclamide, a potent sulfonylurea used orally in small doses in the management of DM, causes stimulation of pancreatic beta cells leading to the release of insulin and causes hypoglycemia [33]. Glibenclamide metabolites, 4-trans-hydroxyglibenclamide (Ml) and 3-cis-hydroxyglibenclamide (M2) are hypoglycemic in humans [34]. These combined effects may have contributed to the regeneration of beta cells in the alloxan-induced diabetic rabbits under this study. Numerous epidemiological studies suggest that herbs/diets rich in phytochemicals and antioxidants execute a protective role in health and disease [35].

CONCLUSION: The various parts of the *Moringa olieffiera* plant were useful in diabetes. Therefore the present investigation has been taken up to prove its anti-diabetic effect scientifically compared to standard drugs. *i.e.*, blood glucose levels, Total cholesterol, Triglycerides, HDLC & LDLC levels in all the parameters evaluated. The Test drug

Moringa oliefiera has shown equal effect as that of the standard drug Glibenclamide. Therefore, the test drug Moringa olifiera powder can develop as a herbal anti-diabetic formulation.

CONFLICTS OF INTEREST: Authors have no conflict of interest regarding this article.

REFERENCES:

1. Mall TP and Tripathi SC: *Moringa oleifera*: a miracle multipurpose potential plant in health management and climate change mitigation from Bahraich (UP) India an overview. Int J Curr Res Bios Plant Biol 2017; 4(8): 52-66.
2. DeFilipps RA and Krupnick GA: The medicinal plants of Myanmar. Phyto Keys 2018; 102: 1.
3. Singh L, Yoti J and Singh J: Medicinal and Nutritional Values of Drumstick Tree (*Moringa oleifera*)–A Review. International Journal of Current Microbiology and Applied Sciences 2019; 8(5): 1965-74.
4. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, Ostolaza H and Martín C: Pathophysiology of type 2 diabetes mellitus. International Journal of Molecular Sciences 2020; 21(17): 6275.
5. Vargas-Sánchez K, Garay-Jaramillo E and González-Reyes RE: Effects of *Moringa oleifera* on glycaemia and insulin levels: A Review of animal and human studies. Nutrients 2019; 11(12): 2907.
6. Villarruel-López A, López-de la Mora DA, Vázquez-Paulino OD, Puebla-Mora AG, Torres-Vitela MR, Guerrero-Quiroz LA and Nuño K: Effect of *Moringa oleifera* consumption on diabetic rats. BMC Complementary and Alternative Medicin 2018; 18(1): 127.
7. Gopalakrishnan L, Doriya K and Kumar DS: Moringa oleifera: A review on nutritive importance and its medicinal application. Food Science and Human Wellness 2016; 5(2): 49-56.
8. Daiber A, Steven S, Vujacic-Mirski K, Kalinovic S, Oelze M, Lisa FD and Münzel T: Regulation of vascular function and inflammation *via* cross talk of reactive oxygen and nitrogen species from mitochondria or nadph oxidase implications for diabetes progression. International Journal of Molecular Sciences 2020; 21(10): 3405.
9. Forrester SJ, Kikuchi DS, Hernandes MS, Xu Q and Griendling KK: Reactive oxygen species in metabolic and inflammatory signaling. Circulation Research 2018; 122(6): 877-902.
10. Gerber PA and Rutter GA: The role of oxidative stress and hypoxia in pancreatic beta-cell dysfunction in diabetes mellitus. Antioxidants & Redox Signaling 2017; 26(10): 501-18.
11. Burgos-Morón E, Abad-Jiménez Z, Martinez de Maranon A, Iannantuoni F, Escribano-López I, López-Domènech S, Salom C, Jover A, Mora V, Roldan I and Solá E: Relationship between oxidative stress, ER stress, and inflammation in type 2 diabetes: the battle continues. Journal of Clinical Medicine 2019; 8(9): 1385.
12. Vargas-Sánchez K, Garay-Jaramillo E and González-Reyes RE: Effects of *Moringa oleifera* on glycaemia and insulin levels: A Review of animal and human studies. Nutrients 2019; 11(12): 2907.
13. Singh VP, Bali A, Singh N and Jaggi AS: Advanced glycation end products and diabetic complications. The Korean Journal of Physiology & Pharmacology: Official Journal of the Korean Physiological Society and the Korean Society of Pharmacology 2014; 18(1): 1.
14. Storch AS, Mattos JD, Alves R, Galdino ID and Rocha HN: Methods of endothelial function assessment: description and applications. International Journal of Cardiovascular Sciences 2017; 30(3): 262-73.
15. Rajbhar YP, Rajbhar G, Rawat PL, Shardulya S and Kumar M: Grow Moringa (*Moringa oleifera*), the miracle tree on the earth. Horticulture International Journal 2018; 2(4): 166-72.
16. CPCSEA guidelines for laboratory animal facility. Indian J Pharmacol 2003; 35: 257-74.
17. Demers G, Griffin G, De Vroey G, Haywood JR, Zurlo J and Bedard M: Harmonization of Animal Care and Use Guidance. Science 2006; 312: 700-701.
18. Lagisetty U, Mohammed H and Ramaiah S: Effect of Allicin on Pharmacodynamics and Pharmacokinetics of Gliclazide in Diabetic Animal Models.
19. Ahmad U and Ahmad RS: Anti diabetic property of aqueous extract of *Stevia rebaudiana* Bertoni leaves in Streptozotocin-induced diabetes in albino rats. BMC Complementary and Alternative Medicin 2018; 18(1): 1-1.
20. Jagroop IA, Persaud JW and Mikhailidis DP: A new rapid method to measure human platelet cholesterol: a pilot study. Clinical and Applied Thrombosis/Hemostasis 2011; 17(6): 578-84.
21. Macdonald Ighodaro O, Mohammed Adeosun A and Adeboye Akinloye O: Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. Medicina 2017; 53(6): 365-74.
22. Banda M, Nyirenda J, Muzandu K, Sijumbila G and Mudenda S: Antihyperglycemic and antihyperlipidemic effects of aqueous extracts of *Lannea edulis* in alloxan-induced diabetic rats. Frontiers in Pharmacology 2018; 9: 1099.
23. Vongsak B, Sithisarn P, Mangmool S, Thongpraditchote S, Wongkrajang Y and Gritsanapan W: Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. Ind Crop Prod 2013; 44: 566-71.
24. Mohsen M, Gholamreza A, Diana T, Mozhgan S and Salar N: Anti-inflammatory effect of *Moringa oleifera* lam. Seeds on acetic acid-induced acute colitis in rats. Av J Phytomed 2014; 4(2): 127-36.
25. Anudeep S, Prasanna VK, Adya SM and Radha C: Characterization of soluble dietary fiber from *Moringa oleifera* seeds and its immunomodulatory effects. International Journal of Biological Macromolecules 2016; 91: 656-62.
26. Alcántar-Díaz BE, Gómez-Meda BC, Zúñiga-González GM, Zamora-Perez AL, González-Cuevas J, Alvarez-Rodríguez BA, Sánchez-Parada MG, García- Bañuelos JJ and Armendáriz-Borunda J: Genotoxic evaluation of pirfenidone using erythrocyte rodent micronucleus assay. Food Chem Toxicol 2012; 50(8): 2760-5.
27. Olayemi AT, Olanrewaju MJ and Oloruntoba AC: Toxicological evaluation of *Moringa oleifera* Lam seeds and leaves in Wistar rats. Pharmacogn Comm 2016; 6(2): 100-11.
28. Al-Ishaq RK, Abotaleb M, Kubatka P, Kajo K and Büsselberg D: Flavonoids and their anti-diabetic effects: cellular mechanisms and effects to improve blood sugar levels. Biomolecules 2019; 9(9): 430.
29. Félix R, Valentão P, Andrade PB, Félix C, Novais SC and Lemos MF: Evaluating the *in-vitro* potential of natural

extracts to protect lipids from oxidative damage. Antioxidants 2020; 9(3): 231.
30. Yang H: Advances in research on lipid-lowering mechanisms of eight medicinal plants. In AIP Conference Proceedings 2019; 020007).
31. Aba PE and Asuzu IU: Mechanisms of actions of some bioactive anti-diabetic principles from phytochemicals of medicinal plants: A review.
32. Akhtar N and Mirza B: Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. Arabian Journal of Chemistry 2018; 11(8): 1223-35.
33. Candasamy M, Murthy TE, Gubiyappa KS, Chellappan DK and Gupta G: Alteration of glucose lowering effect of Glibenclamide on single and multiple treatments with fenofibrate in experimental rats and rabbit models. J Basic Clin Pharm 2014; 5: 62-67.
34. Varma MV, Scialis RJ, Lin J, Bi YA, Rotter CJ, Goosen TC and Yang X: Mechanism-based pharmacokinetic modeling to evaluate transporter-enzyme interplay in drug interactions and pharmacogenetics of glyburide. The AAPS Journal 2014; 16(4): 736-48.
35. Arora J and Ramawat KG: Bioactive molecules, nutraceuticals, and functional foods in Indian vegetarian diet and during postpartum healthcare. Bioactive Molecules in Food. Reference Series in Phytochemistry Springer Cham 2018.

How to cite this article:
Bommineni K and Yamsani MR: Hypoglycemic and hypolipidemic activity of *Moringa oleifera* leaf powder in alloxan-induced diabetic rabbits. Int J Pharm Sci & Res 2022; 13(1): 369-74. doi: 10.13040/IJPSR.0975-8232.13(1).369-74.