Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **44** Issue **S-3 Year 2023** Page **117:124***

The Effect of Different *Moringa Oleifera* (*Moringaceae*) Leaves on Diabetic Rats

Khaled Abd El Ghafar El-Kady[1], Naeem Mohamed Rabeh[2], Nada Zaher Mohammed Alshehri[2*]

[1]*Department of General Botany Nutrition and Food Science, Faculty of Home Economics, Helwan University, Egypt.*

[2]*Department of Nutrition and Food Science, Faculty of Home Economics, Helwan University, Egypt.*

**Corresponding author's E-mail: randasokar@heco.helwan.edu.eg*

Article History

Received: 06 June 2023
Revised: 05 September 2023
Accepted: 21 September 2023

Abstract

Background: *Diabetes is a serious, long-term condition with a major impact on the lives. Type 2 diabetes is closely associated with insulin resistance and obesity and is characterized by impairments in physiological body processes and hyperglycemia and dyslipidemia. Moringa oleifera (MO) is a traditional herbal plant that has been used for a long time as a food ingredient or in traditional folk medicine.* ***Aim and objectives:*** *This study was conducted to investigate the effect of different Moringa oleifera leaves on diabetic rats.* ***Subjects and methods:*** *This study was carried out at the Postgraduate Lab of Home Economic Faculty, Helwan University. Thirty-five adult female Sprague-Dawley rats were fed on standard diet for one week for adaptation. Rats then was randomly divided into two main groups.* ***Result:*** *The results indicated that, STZ treated rats showed significant reduction ($P<0.05$) in serum insulin concentration and, increased glucose levels compared to normal rats. Supplementation with Moringa oleifera leaves in the diet caused significant ($P<0.05$) increase in the concentration of insulin while glucose level was significantly ($P<0.05$) decreased compared to the positive control one. It was also observed that, liver and kidney functions and lipid profile of the treated rats was improved compared to the positive control group.* ***Conclusion:*** *Administration of Moringa oleiferia to diabetic rats ameliorated all the adverse effects of diabetes via modulation of insulin, glucose, liver and kidney function, and lipid profile, therefore Moringa oleiferia leaves extract and dried could be used as a suitable supplementation therapy for diabetic patients.*

Keywords: *Moringa Oleifera (Moringaceae) Leaves, Extracts, Diabetes, Insulin, Rats*

1. Introduction

Chronic Diabetes is a serious, long-term condition with a major impact on the lives and well-being of individuals, families, and societies worldwide. It is among the top 10 causes of death in adults, and was estimated to have caused four million deaths globally in 2017. It was estimated that 285 million people had diabetes **(1)**. Nowadays, given the side effects due to man-made drugs, plants and traditional medicine are being increasingly used and the benefits of different plants are attracting more attention day-to-day **(2)**. Although various oral hypoglycemic drugs exist alongside insulin, there is still no promising therapy to cure diabetes. The plant kingdom represents a rich storehouse of organic compounds, many of which have been used for medicinal purposes, and could serve as leading substances for the development of novel agents, having good efficacy in various pathological disorders in the coming years **(3)**. More than 800 plants are used as traditional remedies for the treatment of diabetes due to their effectiveness, less side effects, and relatively low costs **(4)**. Diabetes

and related neurological complications are serious worldwide public health problems. The increasing number of affected individuals make it necessary to implement novel nutritional and therapeutic interventions. The tree Moringa oleifera has been used as a food source and for traditional medicine purposes due to possible antihyperglycemic, antioxidant, anti-inflammatory, and lipid regulating properties. These properties may be explained by the presence of numerous phytochemicals in the leaves, fruits,roots and, oil of the tree **(5)**.

Moringa oleifera (*Moringaceae*) is an important food commodity which has had enormous attention as the 'natural nutrition of the tropics. The leaves, fruit, flowers and immature pods of this tree are used as a highly nutritive vegetable in many countries, particularly in India, Pakistan, Philippines, Hawaii and many parts of Africa **(6)**. For centuries, M. oleifera has been cultivated for its nutritional values **(7,8)**. Moringa leaves have been reported to be a rich source of β-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants; and thus enhance the shelf-life of fat containing foods due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids **(9)**. The leaves of this plant are positioned as a tonic, strengthening and stimulating immune system for people living with HIV/AIDS **(10)**. The leaves have been used as antidiabetic, antibacterial, and anti-inflammatory herbal drugs **(11)**. The aim of the study was to investigate the effect of different Moringa oleifera leaves on diabetic rats.

2. Materials And Methods

Chemicals: Casein, vitamins, minerals and cellulose was purchased from El-Gomhoria Company, Cairo, Egypt. Chemicals Streptozotocin (STZ) and all Chemical materials (analytical and HPLC grade) were obtained from Sigma Chemicals Co., St. Louis, USA.

Kits: for blood analysis was purchased from Alkan Company for Bio diagnostic Reagents, Dokki, and Cairo, Egypt.

Plant: Fresh leaves of Moringa were obtained from the Egyptian Scientific Society of Moringa at the National Research Centre

Rats: Adult female albino rats (Sprague- Dawley strain) (n=35 rat) weighing approximately (170 ±5 g.) was purchased from Helwan Experimental Animals Farm.

Sample collection: Fresh Moringa oleifera leaves were collected during March 2022 from the Model Plant Farm of Nub Arya, Egypt. The collected samples were purified, air- and sun- dried, and their leaves were manually ground in a mortar, then was passed through a 25-mm sieve, and was stored at 4-8 °C in a refrigerator for further analysis. Preparation of Moringa oleifera aqueous leaves extracts. And, Preparation of Moringa oleifera ethanolic leaves extracts.

Induction of diabetes: Streptozotocin was dissolved in a citrate buffer (pH 4.4) with a concentration of 15 mg/ml. All animals were fasted overnight and was injected interpretonial (ip) with a low dose STZ (60 mg/kg b.w.) to induce hyperglycemia, after 4 days, blood samples were obtained from medial canthus of eyes of each rat to estimate glucose levels. Animals with blood glucose level >200 mg/dl was considered as diabetic **(12)**.

Biological study: This study was carried out at the Postgraduate Lab of Home Economic Faculty, Helwan University. Thirty-five adult female Sprague-Dawley rats were fed on standard diet for one week for adaptation. The basal diet was formulated according to **Reeves et al., (13)**. Rats then was randomly divided into two main groups as follow: **The first main group** (n= 7) was be fed on basal diet only and served as control negative group. **The second main group** (n=28) was injected with STZ to induce diabetes. Then these diabetic rats were divided into four subgroups as follow: **The first subgroup:** was fed on basal diet and served as positive control group, **The second subgroup:** was fed on basal diet and given orally 1 ml/rat of Moringa water extract, **The third subgroup:** was fed on basal diet and given orally 1 ml/rat of Moringa ethanolic extract and **The fourth subgroup:** was fed on basal diet and supplemented with dried Moringa leaves at 10%.

At the end of the experimental period (6 weeks), rats were fasted overnight before sacrificing, two blood samples were collected, and the first sample was collected into a tube containing disodium salt of Ethylene Diamine Tetra Acetic Acid (EDTA) as anticoagulant and was used for assessment of

insulin hormone. The second blood sample was collected into a centrifuge tube without any anticoagulant and was centrifuged to obtain serum which was stored at- 20°C until used for subsequent analysis.

Biological evaluation: Feed intake, body weight was determined weekly. Body weight gain percent and feed efficiency ratio was calculated according to **(Chapman *et al*., (14)** using the following equation:

$$\text{BWG\%} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

FER= Body weight gain (g) / Feed intake (g)

Chemical analysis: Insulin activity was estimated using enzyme linked immunosorbent assay ELISA method as described by **Clark and Hales, (15)**. Glucose level was determined according to **Asatoor and King, (16).** Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to method of **Reitman and Frankel, (17)**. Serum creatinine, urea and uric acid level were determined by the method of **Tietz, (18**), **Wills and Savory, (19)** and **Patton and Crouch, (20)** respectively. Serum total cholesterol **Richmond, (21),** triglycerides **Wahlefeld, (22),** high density lipoprotein **(Albers et al., (23)** were determined. Meanwhile, low density lipoprotein and very low-density lipoprotein were calculated according to **Fridewald et al., (24).** LDL-c = TC- [HDL-c + (TG/5)] VLDL-c = TG/5.

Statistical Analysis: The obtained results were analyzed according to SPSS program. ANOVA test was used to compare results among groups and $P<0.05$ was considered to be significant **(25)**.

3. Results and Discussion

Regarding to changes in body weight status, Table (1) illustrated the changes of body weight, feed intake and FER in the diabetic rats fed on diet supplemented with Moringa leaves. There were no significant differences in IBW among all groups. Diabetic rats had significant decrease ($P<0.05$) in the FBW compared to the negative control group. It was observed that STZ induced diabetic in rats caused significant decrease ($P<0.05$) in FBW compared to the healthy rats. This table showed that there was significant difference between studied groups as regards FBW, BWG%, FI and FER ($P<0.05$), in which Dried Moringa followed by 1 ml ethanolic extract had the best impact on body weight of diabetic rats. However, the supplementation with the tested materials caused a significant increase ($P<0.05$) in BWG% and FER compared to the positive control group. Dried Moringa caused the highest increase in BWG% and FER compared to other treatments.

Table (1): Effect of moringa leaves extract on body weight of diabetic rats.

Parameters / Groups	IBW (g)	FBW (g)	BWG%	FI (g/d/rat)	FER
Control –ve	166.00±1.48a	202.87±1.50a	22.24±1.56a	15.50	0.039±0.02a
Control +ve	168.62±0.85a	165.12±1.50d	-7.41±0.46c	11.00	-0.018±0.01d
1ml aqueous extract	169.50±1.25a	192.62±1.02c	13.64±0.27n	13.50	0.028±0.00c
1 ml ethanolic extract	169.75±2.39a	196.87±1.39b	16.04±1.79b	14.00	0.032±0.03bc
Dried Moringa (10%)	168.50±0.64a	202.32±0.90a	20.08±0.90a	15.00	0.037±0.01ab

Values were expressed as Means ± SE.
Values at the same column with different letters are significantly different at $P<0.05$.

Rats injected with STZ had significantly ($P<0.05$) higher glucose level and significantly ($P<0.05$) lower insulin concentration, compared to the control negative group Table (2). Feeding diabetic rats on diet supplemented with moringa leaves extract or dried caused a significant decrease ($P<0.05$) in the elevated serum glucose level, compared to the control positive group. This table shows that there was significant difference between studied groups as regards insulin and glucose ($P<0.05$), in which dried moringa followed by 1 ml ethanolic extract had the best impact on glucose and insulin levels of diabetic rats.

Table (2): Effect of moringa extract on glucose and insulin levels of diabetic rats.

Parameters / Groups	Insulin	Glucose	% of glucose reduction
Control –ve	1.23±0.02a	86.60±2.92e	-
Control +ve	0.373±0.03e	260.76±1.68a	-
1ml aqueous extract	0.650±0.02d	150.73±4.54b	42.19
1 ml ethanolic extract	0.830±0.03c	132.23±2.86c	49.29
Dried Moringa (10%)	1.01±0.04b	118.36±2.58d	54.60

Values were expressed as Means ± SE.
Values at the same column with different letters are significantly different at P<0.05.

The results in Table (3) revealed the effect of moringa leaves extract or dried on liver function of diabetic rats. The activities of serum ALT, AST and ALP significantly increased (P<0.05) in the diabetic group, compared with the corresponding value of normal control group. Supplementation with moringa leaves extract or dried significantly decreased (P<0.05) the elevated levels of both serum ALT AST and ALP compared to the negative control group. Moreover, there was significant difference between studied groups as regards AST and ALT (P <0.05), in which Dried Moringa followed by 1 ml ethanolic extract had the best impact on liver functions of diabetic rats. These findings suggest hepatic injury in type 2 DM and the hepatoprotective effect for moringa leaves extract or dried.

Table (3): Effect of moringa extract on liver functions of diabetic rats.

Parameters / Groups	AST (µ/dl)	ALT(µ/dl)	ALP (µ/dl)
Control –ve	88.10±2.58e	32.20±1.02d	64.93±2.87d
Control +ve	166.90±4.13a	62.83±4.47a	120.00±1.55a
1ml aqueous extract	137.30±2.13b	50.03±1.29b	105.14±3.16b
1 ml ethanolic extract	125.13±1.48c	42.60±1.70c	96.98±2.72b
Dried Moringa (10%)	110.03±1.29d	36.43±1.02cd	86.06±3.61c

Values were expressed as Means ± SE.
Values at the same column with different letters are significantly different at P<0.05.

Table (4) illustrates the effects of moringa leaves extract or dried in serum kidney functions on diabetic rats. Injection with STZ significantly increase (P<0.05) the level of urea, uric acid and creatinine, compared to the control normal group (control –ve). Feeding diabetic rats on diet supplemented with moringa leaves extract or dried at the tested level caused a significant decrease (P<0.05) in the mean values of uric acid, creatinine and urea as compared to the positive control group. There were no significant differences in serum uric acid between the groups fed either moringa leaves aqueous extract, and also between the rats fed on moringa leaves ethanolic extract. Moreover, there was no significant differences in serum uric acid among the two treated groups ethanolic extract and dried moringa, in which Dried Moringa followed by 1 ml ethanolic extract had the best impact on kidney functions of diabetic rats.

Table (4): Effect of moringa extract on kidney functions of diabetic rats.

Parameters / Groups	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Control –ve	44.15±2.07e	1.03±0.07d	0.410±0.020d
Control +ve	90.13±2.17a	2.01±0.10a	1.05±0.033a
1ml aqueous extract	77.60±1.28b	1.83±0.06ab	0.939±0.022b
1 ml ethanolic extract	65.23±2.43c	1.64±0.02bc	0.898±0.009b
Dried Moringa (10%)	53.67±1.96d	1.54±0.03c	0.539±0.031c

Values were expressed as Means ± SE.
Values at the same column with different letters are significantly different at P<0.05.

Results illustrated in Table (5) shows the effect of moringa leaves extract or dried on lipids profile of diabetic rats. STZ injection to rats caused a significant increase (P<0.05) in serum lipid profile, however, serum HDL-C was significantly lowered, compared to the healthy rats. Diet supplemented moringa leaves extract or dried significantly decrease (P<0.05) the mean value of serum TC, TG, VLDL-C and LDL-C, however, serum HDL-C level was increased significantly (P<0.05), compared to the positive control group. This table shows that there was significant difference between studied groups as regards TC, TG, HDL, LDL and VLDL (P <0.05), in which Dried Moringa followed by 1 ml ethanolic extract had the best impact on lipid profile of diabetic rats.

Table (5): Effect of moringa extract on of lipid profile of diabetic rats.

Parameters / Groups	TC	TG	HDL-C	LDL-C	VLDL-C
	(mg/dl)				
Control –ve	129.33±1.36d	79.12±4.03d	65.93±2.23a	47.57±3.55d	15.82±0.80a
Control +ve	180.26±1.67a	139.00±3.55a	24.86±0.98e	127.60±1.98a	27.80±0.71a
1ml aqueous extract	162.30±2.75b	126.83±2.93b	41.63±1.37d	95.30±3.78b	25.36±0.58b
1 ml ethanolic extract	148.56±2.25c	119.06±1.90b	50.06±2.41c	74.68±3.51c	23.81±0.38b
Dried Moringa (10%)	136.36±2.77d	108.44±1.89c	56.39±2.00b	58.28±4.66d	21.68±0.37c

Values were expressed as Means ± SE.
Values at the same column with different letters are significantly different at P<0.05.

In this study we found that there was significant difference between studied groups as regards FBW, BWG%, FI and FER (P <0.05), in which Dried Moringa followed by 1 ml ethanolic extract had the best impact on body weight of diabetic rats. In line with agreement with **Bamagous et al.** found that the body weight of diabetic rats (Group 2) showed significantly reduced weight gain as compared to normal rats of Group 1 (*P*<0.05). Diabetic rats treated with *M. oleifera* (Group 3) showed significantly better weight gain (*P*<0.05) in contrast to diabetic control rats. An average food efficiency ratio of diabetes control rats (Group 2) was significantly lower than Group 1 rats (*P*<0.05) whereas rats in Group 3 showed higher food efficiency ratio as compared to Group 2 rats *(P*<0.05) **(26)**. **Udeogu et al.** found that within the Moringa oleiferia treated groups (groups 3, 4 and 5), it would be noticed that there was an initial loss of weight amongst these groups of rats probably as a result of onset of diabetes (week two). However, as treatment was introduced, it could be noticed that they rats showed little weight gain. This suggests a possible short-term positive effect of the extracts on body weight of diabetic rats. At this point, it could be noticed that group 5 with the highest extract dose exerted the highest percentage weight gain (2.94%), while group 3 with the lowest extract does present the lowest percentage gain (0.15). This suggests a possible dose dependent weight gain. It seems that as the dose of Moringa increased, the weight gained increased **(27)**.

In this study we demonstrated that there was significant difference between studied groups as regards insulin and glucose (P <0.05), in which Dried Moringa followed by 1 ml ethanolic extract had the best impact on glucose and insulin levels of diabetic rats. **Yoon et al.** mentioned that Moringa may improve muscle insulin sensitivity resulting in increased tissue glucose uptake **(28)**. These results were also in consistent with that obtained in a study performed by **Alessandro et al.** who found a significant reduction in serum glucose and HbA1c levels with Moringa treatment of diabetic patients **(29)**. Similarly, **Oyedepo et al. and Soliman** postulated that the treatment of diabetic rats with moringa extract (400 mg/ kg) for 28 days significantly decrease blood glucose level. Moreover, many researchers recorded a decrease in insulin level in alloxan- diabetic rats (29,30,34). From this study, it is suggested that Moringa oleifera seed extract was able to reverse the inhibition of insulin secretion from the pancreatic beta cells and reduced the blood glucose level **(30, 31)**. These results were in disagreement with **Rutchaporn et al.** where they found insignificant reduction in serum glucose and HbA1c levels with Moringa treatment of diabetic patients. This could be due to the short duration of treatment (4 weeks) **(32)**.

In this study we illustrated that there was significant difference between studied groups as regards AST, ALT and ALP (P <0.05), in which Dried Moringa followed by 1 ml ethanolic extract had the

best impact on liver functions of diabetic rats. The obtained results are agreed with the results of **(33 and 34)** whom declared that M. oleifera extract has a hepato-nephroprotective therapeutic effect on obese rats; this was monitored from marked improvement in ALT, AST and ALP activities as well as urea and creatinine levels in serum; this result goes in parallel with that of **Mabrouki et al., (35)** who attributed that to the antioxidant potential of Moringa oleifera extract included phytochemicals. Similarly, M. oleifera displayed hepatoprotective effect on the liver of obese female rats, represented by a marked reduction in ALT and AST enzymes activity in serum **(36)**. **Fakurazi et al., (37)**, also reported the hepatoprotective activity of M. oleifera against hepatotoxicity induced by acetaminophen in rats.

In this study we cleared that there was significant difference between studied groups as regards Urea, Uric acid and Creatinine ($P < 0.05$), in which Dried Moringa followed by 1 ml ethanolic extract had the best impact on kidney functions of diabetic rats. Diabetic nephropathy is the renal disease that occurs as a result of diabetes. Therefore, the decreased levels of creatinine and urea in the treated group's shows that treatment of the disease with aqueous extracts of Moringa oleifera seed can guard against diabetic nephropathy **(38)**. These results were agreement with **Metwally et al., (39)** showed that M. oleifera treatment exhibited insignificant changes ($P > 0.05$) in serum creatinine level compared to the untreated obese group. **Saleh and Sarhat, (40)** observed that the effect of administration of the ethanolic extract of M. Oleifera significantly reduced urea and creatinine concentration of the diabetic rats to a level similar to the control and metformin-treated diabetic animals. Furthermore, M. oleifera extract seem to be safe for kidney and away from nephrotoxicity, which is indicated by the results of creatinine, which are very close to normal control values **(37)**.

In this study we found that there was significant difference between studied groups as regards TC, TG, HDL, LDL and VLDL ($P < 0.05$), in which Dried Moringa followed by 1 ml ethanolic extract had the best impact on lipid profile of diabetic rats. These results were agreement with **Metwally et al., (39)** revealed that administration of ethanolic extract of M. oleifera in obese females attenuated body weight gain, thus, it ameliorated obesity and atherogenic dyslipidemia. This was in accordance with **El-Gindy et al., (41)** indicated that supplementation of moringa leaves significantly stimulated and increased HDL cholesterol of rabbits under moderate heat stress. The decrease in the total cholesterol level, triglyceride, LDL and an increase in HDL cholesterol levels indicate that moringa leaves has a profound hypolipidemic activity because of their ability to control the mechanisms involved in lipids elimination from the body **(33 and 42).**

Flavonoids and saponins present in moringa led to increase HDL-C and reduce LDL-C and VLDL-c in hypercholesterolemic rats. In this concern, **Othman et al., (43); Negm, (33) and El-Bashshuti and Shanshan, (36) and El-Shehawi et al., (44)** revealed that M. oleifera reduced body weight gain, prevented the increase in cholesterol, LDL and TG and increased HDL compared to HFD-supplemented rats.

4. Conclusion

In conclusion, Administration of Moringa oleiferia to diabetic rats caused an improvement of the biochemical results from diabetes, therefore Moringa oleiferia leaves extract and dried could be used as a suitable supplementation therapy for diabetic patients.

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