

Effects of Moringa Oleifera Seeds Aqueous Extract on Type-II Diabetic Nephropathy in Adult Male Albino Rat

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Abstract

Background: Because of the potential antioxidant, anti-inflammatory, and immune-modulating properties of Moringa oleifera, there is an increased interest in its potential capacity in the prevention and control of diabetes mellitus and lipids peroxidation.

Aim of Study: Evaluating the potential antidiabetic efficacy and protective activities of aqueous extract of Moringa oleifera seeds in alloxan-induced diabetic nephropathy.

Material and Methods: This experimental study was started in September and ended at November, 2020. It was performed at Medical Physiology Department, Al-Azhar Faculty of Medicine, Cairo. Fifty adult male albino rats of a local strain were distributed randomly into 5 equal groups: Normal control group, control group received aqueous extract of Moringa seeds (500ml/kg body weight orally for 10 weeks), diabetic control group received a single dose of alloxan (140mg/kg body weight intraperitoneally), diabetic group received aqueous extract of Moringa seeds (500ml/kg body weight orally 10 weeks), and diabetic group received Moringa seeds aqueous extract before and after induction of diabetes in the same previous doses.

Results: Alloxan-induced diabetes mellitus was associated with significant higher levels of serum glucose, glycosylated hemoglobin (HbA1c), and lowered level of insulin as compared to the control normal group. It was also associated with histopathological changes of renal tissues and impaired renal function in the form of significant elevations of serum K⁺, Na⁺, creatinine, urea and uric acid levels as compared to the control normal group. Also, there were significant disturbance of lipid peroxidation represented by MDA Level and significant decrease in SOD level as compared to the control normal group. Moringa oleifera seeds aqueous extract showed significant lower levels of serum glucose, HbA1c and elevated level of insulin as compared with the diabetic rats. It was also associated with histological regeneration of damaged renal tissues and restoration of renal function in the form of significant decrease of serum K⁺, Na⁺, creatinine, urea and uric acid levels as compared to the diabetic group. Also, there were significant amelioration of lipid peroxidation represented by significant decrease of MDA level, and significant increase

of SOD level as compared to the control normal group. These results were prominent in pretreated group with Moringa seeds aqueous extract.

Conclusion: Moringa oleifera improved glycemic and lipidemic disturbances as well as have potent protective and regenerative effects for the kidney.

Key Words: *Moringa oleifera – Alloxan – Diabetes mellitus – Nephropathy.*

Introduction

DIABETES is a major public health problem. It is a clinical syndrome characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level [1]. A chronic increase in serum glucose levels can lead to macro- and micro-vascular complications, such as heart disease, hypertriglyceridemia, nephropathy, neuropathy, and retinopathy [2].

Diabetic nephropathy is a serious complication of diabetes mellitus. It is the most common cause of end stage renal failure [3].

Medicinal plants constitute an important source of potential therapeutic agents for diabetes mellitus [4]. Moringa oleifera (also known as drumstick) is a multi-purpose plant, owing to its ability to grow in both humid and hot dry lands [5]. It is rich in bioactive compounds as glycosides, antioxidants, vitamins A and C, beta-carotene, iron, potassium, minerals and other nutrients [6].

Dietary intake of antioxidants can inhibit or delay the oxidation of susceptible cellular substrates so prevent oxidative stress. Therefore, it is important to enrich our diet with antioxidants to protect against many chronic diseases [7].

The present work aimed to detect the possible effects of aqueous extract of Moringa oleifera on

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experimentally alloxan-induced type-II diabetic nephropathy in adult male albino rat.

Material and Methods

Plant material and preparation of extract: The fresh pods of *Moringa oleifera* were harvested from National Institute for Research farm in Cairo, and its identity was confirmed by expert taxonomist, Department of Pharmacognosy, Al-Azhar University, Cairo. Before crushing the *Moringa oleifera* seeds, the wings and brown coats were removed from each seed as well as any kernel that showed any signs of damage. Seeds were passed through a mesh sieve, and the powdered seed was used for the subsequent extractions. Aqueous extract was prepared by soaking exactly 100g of the powdered *Moringa oleifera* in 250ml distilled water at room temperature for 72 hours and filtered using Whatman filter paper No.1. The bulk filtrate obtained was reduced in rotavapor at 40 °C [8], and administered orally using oral cannula to rats 500mg/kg body weight (at 9.00-10.00 AM each day) for 10 weeks [9]. The treatments were initiated on the fifth day after alloxan exposure.

The doses of *Moringa oleifera* powder seeds tested revealed no adverse effects in experimental animals, including possible alterations such as food intake, unusual body growth, reduced activity, diarrhea, bleeding or death [10].

Isoflurine (Nile Pharmaceutical-Egypt) was used for anesthesia.

Alloxan (Nile Pharmaceutical-Egypt) was dissolved in 0.9% NaCl and injected intraperitoneally at a dose of 140mg/kg body weight after overnight fasting.

Animals: This experimental study was started in September and ended at November, 2020. It was performed at Medical Physiology Department, Al-Azhar Faculty of Medicine, Cairo. A total number of seventy adult male albino rats of a local strain weighing between 80 and 120 grams (average weight was 100g) were used for this study. The animals were housed under similar standard environmental conditions in suitable cages (40 x 32 x 40cm for every 5 rats) with wide meshed raised floors to prevent coprophagia. The rats were kept ten days on ad libitum food and tap water at room temperature and natural light/dark cycle for acclimation. Animals were distributed randomly and equally into 5 groups:

Group I (Control Group) was provided standard animal pellet and water.

Group II (*Moringa* seeds aqueous extract-treated group): Rats received *Moringa* seeds aqueous extract (500ml/kg body weight per rat) for 10 weeks orally [10].

Group III (Diabetic control group): Rats were subjected to induction of diabetes by a single intraperitoneal injection of alloxan (140mg/kg body weight per rat) in normal saline, and fed on normal standard rat chow diet [11].

Group IV (Diabetic-*Moringa* seed aqueous extract-treated group): Diabetic rats were treated with *Moringa* seeds aqueous extract (500ml/body weight per rat) for 10 weeks orally.

Group V (Diabetic-*Moringa* seed extract-pretreated group): Rats were treated with *Moringa* seeds aqueous extract before and after induction of diabetes in the same previous dose.

Induction of experimental diabetes: Alloxan was slowly injected intraperitoneally in a dose of 140mg/kg BW. Just before alloxan injection, 2ml of glucose (5%) were given orally to prevent the initial hypoglycemic effect of alloxan [11].

The development of hyperglycemia in rats was confirmed by fasting serum glucose estimation after 2 days of alloxan injection using Accu-Chek glucometer (Roche, Germany). Animals with fasting blood glucose ≥ 200 mg/dL were considered hyperglycemic and were included in the study [12]. The serum glucose level was measured.

Rats with blood glucose over 400mg/dl were treated with 2-3 units of mixtard 30 insulin every other day in order to avoid development of ketoacidosis and coma. This dose of insulin did not correct hyperglycemia. Insulin treatment was discontinued 3 days prior to sample collection [13].

Blood sampling: Blood was collected (4ml of blood each) from the retro-orbital plexus using heparinized capillary tube (0.75-1.0mm internal diameter) inserted in the medial canthus. To obtain serum, the blood was collected into a dry clean graduated glass centrifuge tube. It was rapidly set to centrifuge at 3000 r.p.m. for 15 minutes. About half of the supernatant serum was sucked out into Eppendorf tubes and stored frozen at -20°C [14].

Biochemical estimation in renal homogenate: The isolated kidney tissue was cut into small pieces and washed with phosphate-buffered saline and then grinded in a homogenization buffer (Roche, Germany). The lysates were homogenized on ice using a Polytron homogenizer. The solution was sonicated in an ice bath to prevent overheating for 15 seconds followed by 5-minute centrifugation

at 12000 rpm and 4 °C. The resultant supernatant was used for measurement of antioxidant enzyme (SOD) and renal malondialdehyde (MDA) which were measured as thiobarbituric acid reactive substances (TBARS) by a colorimetric technique [15].

Histopathological studies: At the end of the 10th week, kidney was excised for histopathological studies. Specimens from the tissues were taken immediately and stained with Hematoxylin and Eosin (H and E) and examined microscopically according to William [16].

Statistical analysis: The data were detected by Mann-Whitney U test and reported as mean \pm SD. SPSS software version 25 was used for the statistical analysis to perform ANOVA followed by post hoc-test. $p < 0.05$ was considered to a statistically significant.

Results

The mean \pm standard deviation of serum glucose was 98.3 ± 5.9 , 95.3 ± 8.47 , 382.2 ± 20.9 , 296.9 ± 16.4 and 290.4 ± 9.58 mg/dl in groups I, II, III, IV and V respectively. Group II showed insignificant changes in respect to the group I (control group). Diabetes induced by alloxan resulted in a significant elevation in the levels of fasting serum glucose (FSG) in group III (diabetic group) in respect to control group I, while the treatment with Moringa seeds aqueous extract reduced the elevated fasting serum glucose in groups IV and V in respect to untreated alloxan-induced diabetic group, but still significantly higher than that of groups I and II. Also, there was no significant difference in fasting blood glucose levels between group IV and group V. Moringa seeds aqueous extract has no significant influence on plasma glucose level of normal rats. Also, these results were of prominent changes in pretreatment with Moringa seeds aqueous extract (Table 1).

The mean \pm standard deviation of serum insulin was 5.02 ± 1.1 , 5.1 ± 1.03 , 2.55 ± 0.4 , 3.6 ± 0.87 and 3.03 ± 0.7 mg/dl in groups I, II, III, IV and V respectively. Group II showed insignificant changes in respect to the control group I. Diabetes induced by alloxan resulted in a significantly decrease in the levels of serum insulin in group III (diabetic group) in respect to control group I, while the treatment with Moringa seeds aqueous extract reduced the elevated insulin in groups IV and V in respect to untreated alloxan-induced diabetic group, but still significantly higher than that of groups I and II. Also, there was no significant difference in serum insulin levels between group IV and group V (Table 1).

Moringa seeds aqueous extract has no significant influence on serum insulin level of normal rats. These results were of prominent changes in pretreatment with Moringa seeds aqueous extract (Table 1).

The mean \pm standard deviation of HbA1c was 3.06 ± 0.24 , 3.15 ± 0.26 , 5.56 ± 0.47 , 4.12 ± 0.41 and 4.68 ± 0.47 mg/dl in groups I, II, III, IV and V respectively. Group II showed insignificant changes in respect to the control group I. Diabetes induced by alloxan resulted in a significant elevation in the levels of HbA1c level in group III (diabetic group) in respect to control group I, while the treatment with Moringa seeds aqueous extract reduced the elevated HbA1c in groups IV and V in respect to untreated alloxan-induced diabetic group, but still significantly higher than that of groups I and II. Also, there was no significant difference in HbA1c levels between group IV and group V (Table 1).

It was noted that Moringa seeds aqueous extract has no significant influence on HbA1c level of normal rats. These results were of prominent changes in pretreatment with Moringa seeds aqueous extract (Table 1).

The mean \pm standard deviation of serum K⁺ was 4.79 ± 0.21 , 4.48 ± 0.33 , 5.9 ± 0.35 , 4.69 ± 0.23 and 4.36 ± 0.17 mg/dl in groups I, II, III, IV and V respectively. Group II showed insignificant changes in respect to the control group I. Diabetes induced by alloxan resulted in a significant elevation in the levels of serum K⁺ in group III (diabetic group) in respect to control group I. while the treatment with Moringa seeds aqueous extract reduced the elevated serum K⁺ in groups IV and V respectively in respect to untreated alloxan-induced diabetic group, but still significantly higher than that of groups I and II. Also, there was no significant difference in K⁺ level between group IV and group V (Table 2).

Moringa seeds aqueous extract has no significant influence on serum K⁺ level of normal rats. These results were of prominent changes in pretreatment with Moringa seeds aqueous extract (group V) (Table 2).

The mean \pm standard deviation of serum Na⁺ was 135.9 ± 4.07 , 138.5 ± 3.2 , 159.8 ± 4.74 , 144.05 ± 4.23 and 140.4 ± 2.46 mg/dl in groups I, II, III, IV and V respectively. Group II showed insignificant changes in respect to the control group I. Diabetes induced by alloxan resulted in a significant elevation in the levels of serum Na⁺ in group III (diabetic group) in respect to group I (control group), while the treatment with Moringa seeds aqueous extract

reduced the elevated serum Na^+ in groups IV and V in respect to untreated alloxan-induced diabetic group, but still significantly higher than that of groups I and II. Also, there was no significant difference in Na^+ level between group IV and group V. It was noted that Moringa seeds aqueous extract has no significant influence on serum Na^+ level of normal rats. These results were of prominent changes in pretreatment with Moringa seeds aqueous extract (Table 2).

The mean \pm standard deviation of serum creatinine was 0.94 ± 0.08 , 0.99 ± 0.18 , 1.98 ± 0.24 , 1.33 ± 0.42 and 1.25 ± 0.24 mg/dl in groups I, II, III, IV and V respectively. Group II showed insignificant changes in respect to the control group I. Diabetes induced by alloxan resulted in a significant elevation in the levels of serum creatinine in group III (diabetic group) in respect to control group I, while the treatment with Moringa seeds aqueous extract reduced the elevated serum creatinine in groups IV and V in respect to untreated alloxan-induced diabetic group, but still significantly higher than that of groups I and II. Also, there was no significant difference in serum creatinine level between group IV and group V. Moringa seeds aqueous extract has no significant influence on serum creatinine level of normal rats. These results were of prominent changes in pretreatment with Moringa seeds aqueous extract (Table 2).

The mean \pm standard deviation of blood urea nitrogen was 8.38 ± 2.40 , 8.53 ± 2.3 , 44.48 ± 2.2 , 20.48 ± 3.1 and 17.48 ± 1.1 mg/dl in groups I, II, III, IV and V respectively. Group II showed insignificant changes in respect to the group I (control group). Diabetes induced by alloxan resulted in a significant elevation in the levels of blood urea nitrogen in group III (diabetic group) in respect to control group I. While the treatment with Moringa seeds aqueous extract reduced the elevated blood urea nitrogen in groups IV and V respectively in respect to untreated alloxan-induced diabetic group, it still significantly higher than that of groups I and II. There was no significant difference in blood urea nitrogen level between group IV and group V. It was noted that Moringa seeds aqueous extract has no significant influence on blood urea nitrogen level of normal rats. Also, these results were prominent changes in pretreatment with Moringa seeds aqueous extract (Table 2).

The mean \pm standard deviation of serum uric acid was 12.05 ± 3.75 , 11.05 ± 2.75 , 22.90 ± 3.10 , 17.20 ± 4.11 and 13.30 ± 3.01 mg/dl in groups I, II, III, IV and V respectively. Group II showed insignificant changes in respect to the group I (control

group). Diabetes induced by alloxan resulted in a significant elevation in the levels of serum uric acid in group III (diabetic group) in respect to control group I, while the treatment with Moringa seeds aqueous extract reduced the elevated serum uric acid in groups IV and V in respect to untreated alloxan-induced diabetic group, it still significantly higher than that of groups I and II. Also, there was no significant difference in serum uric acid level between group IV and group V. It was noted that Moringa seeds aqueous extract has no significant influence on serum uric acid level of normal rats. These results were of prominent changes in pretreatment with Moringa seeds aqueous extract (Table 2).

The mean \pm standard deviation of MDA was 64.09 ± 6.43 , 59.05 ± 4.33 , 87.00 ± 5.22 , 59.06 ± 1.23 and 62.22 ± 1.44 mg/dl in groups I, II, III, IV and V respectively. Group II showed insignificant changes in respect to the group I (control group). Diabetes induced by alloxan resulted in an elevation in the levels of MDA in group III (diabetic group) in respect to control group I, while the treatment with Moringa seeds aqueous extract reduced the MDA in groups IV and V respectively in respect to untreated alloxan-induced diabetic group, but still significantly higher than that of groups I and II. Also, there was no significant difference in MDA level between group IV and group V. Moringa seeds aqueous extract has no significant influence on MDA levels of normal rats. These results were of prominent changes in pretreatment with Moringa seeds aqueous extract (Table 3).

The mean \pm standard deviation of SOD was 350.34 ± 8.14 , 360.14 ± 5.32 , 150.54 ± 5.13 , 267.11 ± 2.10 and 253.09 ± 7.16 mg/dl in groups I, II, III, IV and V respectively. Group II showed insignificant changes in respect to the control group I. Diabetes induced by alloxan resulted in a significant decrease in the levels of SOD in group III (diabetic group) in respect to control group I, while the treatment with Moringa seeds aqueous extract elevated the SOD levels in groups IV and V in respect to untreated alloxan-induced diabetic group, but still significantly higher than that of groups I and II. Also, there was no significant difference in SOD level between group IV and group V. It was noted that Moringa seeds aqueous extract has no significant influence on SOD level of normal rats. Also, these results were prominent changes in pretreatment with Moringa seeds aqueous extract (Table 3).

Histopathological examination of the renal tissues in groups I and II showed a normal renal

Table (1): Effects of Moringa oleifera seeds aqueous extract on serum glucose, insulin and HbA1c levels in different groups (Mean \pm SD).

Parameters	Control normal (Group I)	Moringa seeds aqueous extract-treated group (Group II)	Diabetic group (Group III)	Diabetic-Moringa seeds aqueous extract treated group (Group IV)	Diabetic -pretreated with Moringa seeds aqueous extract-treated group (Group V)
Fasting serum glucose (mg/dl)	98.3 \pm 5.9	95.3 \pm 8.47 $p>0.05^*$	382.2 \pm 20.9 $p<0.05^*$ $p<0.05^\circ$	296.9 \pm 16.4 $p<0.05^*$ $p>0.05^\#$ $p>0.05^\Omega$	290.4 \pm 9.58 $p<0.05^*$ $p>0.05^\P$ $p<0.05^\circ$
Insulin (ng/ml)	5.02 \pm 1.1	5.5 \pm 1.03 $p>0.05^*$	2.55 \pm 0.4 $p<0.05^*$ $p<0.05^\circ$	3.6 \pm 0.87 $p<0.05^*$ $p>0.05^\#$ $p>0.05^\Omega$	3.03 \pm 0.7 $p<0.05^*$ $p>0.05^\P$ $p<0.05^\circ$
HbA1c level	3.06 \pm 0.24	3.15 \pm 0.26 $p>0.05^*$	5.56 \pm 0.47 $p<0.05^*$ $p<0.05^\circ$	4.12 \pm 0.41 $p<0.05^*$ $p>0.05^\#$ $p>0.05^\Omega$	4.68 \pm 0.47 $p<0.05^*$ $p>0.05^\P$ $p<0.05^\circ$

N : Number of rats in each group = 10.

°: Groups V were compared to group III.

*: All groups were compared to control group I.

°: Groups IV was compared to group III.

°: Groups III was compared to group II.

°: Groups V was compared to group IV

Table (2): Effects of Moringa oleifera seeds aqueous extract on serum K⁺, Na⁺, creatinine, urea and uric acid levels in different groups (Mean \pm SD).

Parameters	Control normal (Group I)	Moringa seeds aqueous extract-treated group (Group II)	Diabetic group (Group III)	Diabetic-Moringa seeds aqueous extract treated group (Group IV)	Diabetic -pretreated with Moringa seeds aqueous extract-treated group (Group V)
Serum K ⁺ (mmol/L)	4.79 \pm 0.21	4.48 \pm 0.33 $p>0.05^*$	5.9 \pm 0.35 $p<0.05^*$ $p<0.05^\circ$	4.69 \pm 0.23 $p<0.05^*$ $p>0.05^\#$ $p>0.05^\Omega$	4.36 \pm 0.17 $p<0.05^*$ $p>0.05^\P$ $p<0.05^\circ$
Serum Na ⁺ (mmol/L)	135.9 \pm 4.07	138.5 \pm 3.2 $p>0.05^*$	159.8 \pm 4.74 $p<0.05^*$ $p<0.05^\circ$	144.05 \pm 4.23 $p<0.05^*$ $p>0.05^\#$ $p>0.05^\Omega$	140.4 \pm 2.46* $p<0.05^*$ $p>0.05^\P$ $p<0.05^\circ$
Creatinine (mg/dl)	0.94 \pm 0.08	0.99 \pm 0.18 $p>0.05^*$	1.98 \pm 0.24 $p<0.05^*$ $p<0.05^\circ$	1.33 \pm 0.42 $p<0.05^*$ $p>0.05^\#$ $p>0.05^\Omega$	1.25 \pm 0.24 $p<0.05^*$ $p>0.05^\P$ $p<0.05^\circ$
Blood urea nitrogen (BUN) (mg/dl)	8.38 \pm 2.40	8.53 \pm 2.3 $p>0.05^*$	44.48 \pm 2.2 $p<0.05^*$ $p<0.05^\circ$	20.48 \pm 3.1 $p<0.05^*$ $p>0.05^\#$ $p>0.05^\Omega$	17.48 \pm 1.1 $p<0.05^*$ $p>0.05^\P$ $p<0.05^\circ$
Serum uric acid (mg/dl)	12.05 \pm 3.75	11.05 \pm 2.75 $p>0.05^*$	22.90 \pm 3.10* $p<0.05^*$ $p<0.05^\circ$	17.20 \pm 4.11 $p<0.05^*$ $p>0.05^\#$ $p>0.05^\Omega$	13.30 \pm 3.01 $p<0.05^*$ $p>0.05^\P$ $p<0.05^\circ$

N : Number of rats in each group = 10.

°: Groups V were compared to group III.

*: All groups were compared to control group I.

°: Groups III was compared to group II.

°: Groups IV was compared to group III.

°: Groups V was compared to group IV

Table (3): Effects of *Moringa oleifera* seeds aqueous extract on oxidant and antioxidant renal homogenate levels in different groups (Mean \pm SD).

Parameters	Control normal (Group I)	Moringa seeds aqueous extract-treated group (Group II)	Diabetic group (Group III)	Diabetic-Moringa seeds aqueous extract treated group (Group IV)	Diabetic -pretreated with Moringa seeds aqueous extract-treated group (Group V)
MDA level (nmol/g wet renal tissue)	64.09 \pm 6.43	59.05 \pm 4.33 $p>0.05^*$	87.00 \pm 5.22 $p<0.05^*$ $p<0.05@$	59.06 \pm 1.23 $p<0.05^*$ $p>0.05\neq$ $p>0.05\Omega$	62.22 \pm 1.44 $p<0.05^*$ $p>0.05\P$ $p<0.05@$
SOD level (U/mg protein)	350.34 \pm 8.14	360.14 \pm 5.32 $p>0.05^*$	150.54 \pm 5.13 $p<0.05^*$ $p<0.05@$	267.11 \pm 2.10 $p<0.05^*$ $p<0.05\neq$ $p>0.05\Omega$	253.09 \pm 7.16 $p<0.05^*$ $p>0.05\P$ $p<0.05@$

N : Number of rats in each group = 10.

@: Groups V were compared to group III.

* : All groups were compared to control group I.

 Ω : Groups IV was compared to group III.

@: Groups III was compared to group II.

 \P : Groups V was compared to group IV

histological structure of renal parenchyma and glomeruli (Figs. 1,2). In diabetic group, there was collapsed glomerular tuft with marked tubular atrophy associated with interstitial inflammation and interstitial hemorrhage (Fig. 3). Meanwhile, the kidney sections of diabetic rats in group IV

and V that treated with the *Moringa* seeds aqueous extract before and after induction of diabetes seemed to be restoring the normal appearance of glomeruli and regenerated tubules with interstitial hemorrhage, and the kidney nearly restored the normal cortical tissue (Figs. 4,5).

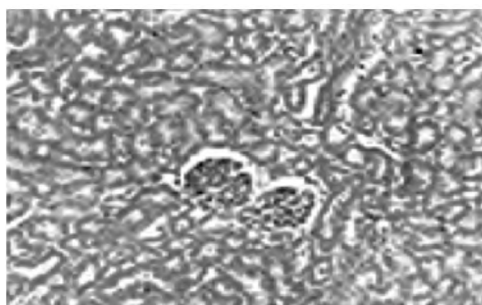


Fig. (1): Kidney of the normal control rats showed normal renal histological structure of renal parenchyma and glomeruli (Hx & E, 400X).

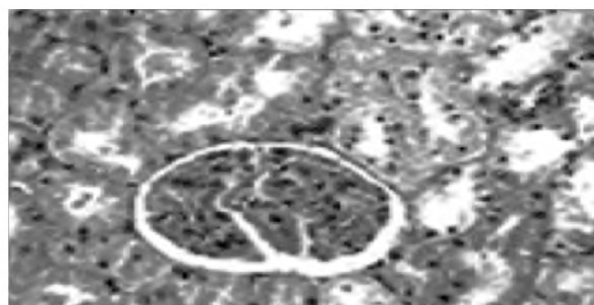


Fig. (2): Kidney of the normal control rats received Moringa seeds extract showed normal renal histological structure of renal parenchyma and glomeruli (Hx & E, 400X).

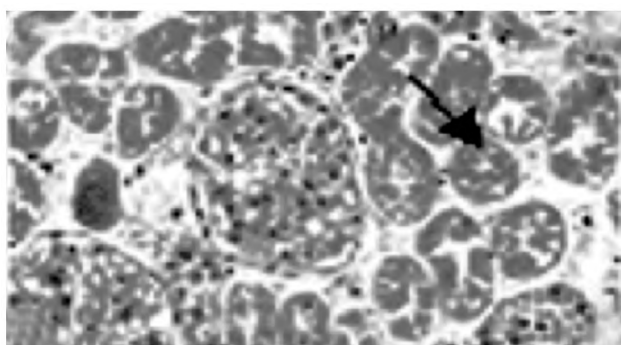


Fig. (3): Rats from the untreated diabetic group showed thickened glomerular basement membrane, vacuolated endothelial lining glomerular tuft, and vacuolated epithelial lining renal tubular cells (black arrow) (Hx & E, 400X).

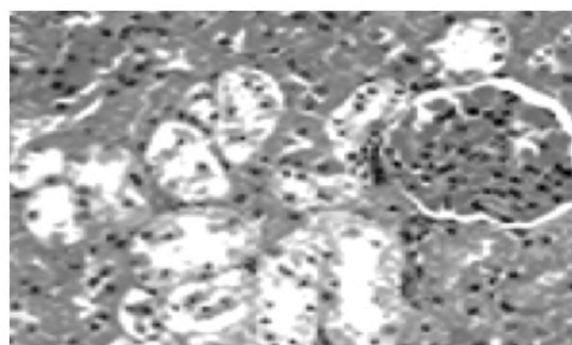


Fig. (4): Treating diabetic rats with Moringa seeds extract nearly restored the renal tissues to their normal histology with minimal histopathological changes (Hx & E, 400X).



Fig. (5): Pretreated diabetic rats with Moringa seeds extract showed the renal tissues nearly normal with minimal histopathological changes (Hx & E, 400).

Discussion

Results of the present study revealed that alloxan injection revealed a significant higher level of both serum glucose and HbA1C levels, and lower level of insulin when compared to control group. The toxic action of alloxan on pancreatic β -cells is the summation of several processes such as generation of free radicals, inhibition of glucokinase, selective destruction of pancreatic insulin secreting β -cells [17] and DNA damage [18]. Such damaged DNA activates nuclear polysynthetase which depletes the cellular pool of oxidized NAD⁺ resulting in β -cells damage and decreasing insulin level [19].

In the present study, treatment with Moringa seeds aqueous extract resulted in a significant reduction in fasting serum glucose (FSG) and HbA1c, and significant elevation of insulin level in comparison with diabetic untreated group. These findings could be attributed to improved hepatic insulin sensitivity resulting in decreased hepatic glucose production [20].

Jeong and his Co-Workers [21] mentioned that Moringa may improve muscle insulin sensitivity resulting in increased tissue glucose uptake. These results were also in consistent with that obtained in a study performed by Alessandro et al., [22] who found a significant reduction in serum glucose and HbA1c levels with Moringa treatment of diabetic patients.

Olayaki and his Co-Workers [23] observed that oral administration of extract of Moringa significantly reduces serum glucose concentration and glycosylated-Hb in alloxan-induced diabetic rats. The possible mechanism by which Moringa oleifera brings its hypoglycemic action could be due to potentiating the insulin effect by increasing either the pancreatic secretion of insulin from cells of

islets of Langerhans, Mozo et al., [24], or its release from the bound form [25].

These results were in disagreement with Rutchaporn et al., [26] 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). In this study, the anti-diabetic activity of the extract in pretreated group (group V) was significantly higher than the non-treated group with the extract before induction of diabetes. This suggested that the extract had a protective effect against diabetes mellitus.

This study also showed a significant increase in the serum levels of Na⁺ and K⁺ in the diabetic control group compared with the control groups.

Al-Malki and El-Rabey [27] observed that treating diabetic rats with Moringa seeds powder significantly ameliorates both sodium and potassium levels compared with those of the control group.

Elevation in serum glucose concentration increase plasma tonicity, creating an osmotic driving force that favors the movement of water from the intracellular space to the extracellular space, thereby diluting the extracellular concentration of sodium [28].

Treatment with aqueous extract of Moringa oleifera seeds significantly increased Na⁺ concentration in all treated groups compared with the diabetic control group.

A significant elevation in serum creatinine and urea levels indicates an impaired renal function of diabetic animals [22]. An increase in creatinine, urea and uric acid levels occurred when there is damage to the kidney or when the kidney is not functioning properly. Increment of blood creatinine and urea levels with the increment of serum sugar level clearly indicates that the increase serum sugar level causes damage to the kidney [29].

Rawoof and his Co-Workers [30] mentioned that values of urea, creatinine, and uric acid in the serum of the diabetic group significantly increased indicates a progressive renal damage.

Diabetic nephropathy is the renal disease that occurs as a result of diabetes. Therefore the decreased levels of creatinine and urea in the treated groups shows that treatment of the disease with aqueous extracts of Moringa oleifera seed can guard against diabetic nephropathy, Al-Malki and El-Rabey [27].

Nabila and her Co-workers [31] mentioned that treatment with *Moringa* was effective which was represented by a decrease of serum urea and creatinine. In accordance, many studies reported that serum urea and creatinine increase in diabetic rats due to diabetic nephropathy.

Diabetes is characterized by disturbances in nephron function, which leads to impaired renal excretion of hydrogen and potassium, and result in hyperkalemia and a hyperchloremic normal-gap acidosis [22].

The renal tissues of diabetic rats showed also the symptoms of diabetic nephropathy illustrated in the hypertrophy of both glomerular and tubular elements, and increase in the thickness of glomerular basement membranes [28]. The histopathological alterations in the alloxan-induced diabetes together, with the other tests, achieved to diagnose diabetes which emphasized that diabetic nephropathy was grown as one of the diabetic complications [32].

Treating diabetic rats with *Moringa* seeds extract restored the normal renal function and histology of kidney with no pathological changes and controlled diabetic nephropathy [27].

Treatment with aqueous extract of *Moringa oleifera* seeds significantly decreased the level of potassium ion in all treated groups compared with the diabetic control group.

Oxidant stress markers which are critical factors in the progression of diabetic nephropathy increases by inflammatory mechanisms of injury in the kidney. Among antioxidative enzymes, SOD which catalyzes dismutation of the superoxide anion into hydrogen peroxide [33]. *Moringa oleifera* has antioxidant activity because it contains three classes of phytochemicals which are glucomoringin, flavonoides (quercetin and kaempferol) and phenolic acids [34].

Normal rats treated with aqueous extract of *Moringa oleifera* seeds showed insignificant changes in the renal tissue levels of both MDA and SOD.

Prasanna and his-Co-Workers [35] mentioned that treatment with *Moringa oleifera* decreases lipid peroxidation. This may be attributed to the ability of *Moringa oleifera* to increase the excretion of fecal cholesterol and hypolipidemic effects.

In this work, the reduced activities of SOD in kidney of alloxan-diabetic rats elevated significantly.

Serino and Salazar [36] mentioned that the untreated diabetic rats on their work showed a significant increase in the renal MDA, and the activity of SOD significantly diminished in renal tissue homogenate in relation to control group. It could reflect oxidative pressure which might be a mirror to diminished antioxidant defense potential as hyperglycemia and hyperlipidemia are associated with decreasing defense system body in warding off free radical [37].

These findings agreed with Karina and his Co-Workers [3] who reported a significant augment in serum SOD, and a significant decrease of MDA on *Moringa oleifera* ingestion to diabetic patients. The enhanced accumulation of these free radicals and dysfunction of antioxidant defense system resulted in oxidative stress [38]. These radicals can bind covalently to the macromolecules, and induce peroxidative degradation of the membrane lipids rich in polyunsaturated fatty acids, leading to the formation of lipid peroxides followed by multiple pathological changes Czerska et al., [39]. Illana et al., [40] reported that diabetes mellitus enhanced the free radical generation in various ways. Several studies suggested that disorders of lipid metabolism, hyperlipidemia and obesity are associated with overproduction of oxygen free radicals [41].

Ghiridhari and his Co-Workers [42] mentioned that the administration of aqueous extract of *Moringa oleifera* seeds to diabetic rats for 10 weeks exhibited a significant amelioration of both MDA and SOD. SOD is a natural antioxidant produced by cells. The tissue SOD level is inversely proportional to plasma glucose.

Moringa oleifera has antioxidant activity because it contains three classes of phytochemicals which are glucomoringin, flavonoides (quercetin and kaempferol) and phenolic acids [43].

Swathi et al., (2017) reported that there was a significant decrease in MDA level and marked increase in SOD activities in rat receiving aqueous extract of *Moringa oleifera* seeds.

Conclusion:

Moringa extracts played a significant role as potent hypoglycemic agents which were capable of improving other clinical conditions associated with diabetes mellitus such as the kidney function. *Moringa oleifera* showed protective effect on blood glucose levels and kidney functions as well as lipid peroxidation.

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تأثير مستخلص حبوب نبات المورينجا المائي على قصور الكلى الناتج عن مرض البول السكري المستحدث تجريبياً بالألوكزان في ذكور الجرذان البيضاء البالغة

خلفية البحث: نبات المورينجا هو عشب يستخدم في الطب التقليدي لخواصه العلاجية.

الهدف من البحث: تقييم تأثير مستخلص حبوب المورينجا المائي على ذكور الجرذان البيضاء المصابة بمرض السكر على وظائف الكلى.

طرق ومواد البحث: أجريت هذه الدراسة في قسم الفسيولوجيا الطبية بكلية الأزهر بالقاهرة، بدأت الدراسة في شهر سبتمبر وانتهت في شهر نوفمبر من عام ٢٠٢٠، وتم تطبيق البحث على خمسين جرذاً ذكراً بالغاً أبيض من سلالة محلية كنموذج للدراسة. وقد قسمت الجرذان عشوائياً إلى خمس مجموعات متساوية مجموعة ضابطة، مجموعة ضابطة تم إعطاؤها مستخلص حبوب المورينجا المائي بجرعة ٥٠٠ مل على لكل جرذ يومياً لمدة ١٠ أسابيع، ومجموعة مصابة بالسكر تم إعطاؤها الألوكسان ١٤٠ مل بالحقن في التجويف البريتوني جرعة واحدة لكل جرذ، ومجموعة مصابة بالسكر تم إعطاؤها مستخلص حبوب المورينجا المائي بنفس الجرعة السابقة يومياً لمدة ١٠ أسابيع، ومجموعة تم إعطاؤها مستخلص حبوب المورينجا المائي قبل وبعد إحداث السكر يومياً لمدة ١٠ أسابيع.

وقد سحب عينات الدم في آخر التجربة (بعد عشرة أسابيع) لقياس نسب السكر، والإنسولين، والهيموجلوبين السكري السكر التراكمي، والصوديوم، والبوتاسيوم، والكرياتينين، واليوريا، وحمض اليوريك، وإنزيم المألون داي د هيد والسوبر أوكسيد ديس ميوتاز. كما تم أيضاً أخذ عينات من أنسجة الكلى وعمل فحص مجهري لها.

نتائج البحث: أدى مستخلص حبوب المورينجا المائي عن طريق الفم للجرذان المصابة بالسكر إلى نقص ذا دلالة إحصائية في مستويات السكر، والهيموجلوبين السكري التراكمي، والصوديوم، والبوتاسيوم، والكرياتينين، واليوريا، وحمض اليوريك، وإنزيم المألون داي د هيد. كما أدى أيضاً إلى ارتفاع ذا دلالة إحصائية في مستوى الإنسولين والسوبر أوكسيد ديس ميوتاز. وقد أظهر فحص أنسجة الكلى وجود تحسن واضحاً في التغيرات الهستوباثولوجية التي يسببها السكر المرتفع بهذه الأنسجة.

الاستنتاج: استخدام مستخلص حبوب المورينجا المائي يمكن أن يكون ذا فائدة تطبيقية وعاملاً مساعداً في علاج مرض السكر وفي قصور الكلى الناتج عنه. وقد يرجع ذلك إلى قدرته على زيادة إفراز هرمون الإنسولين من البنكرياس وزيادة حساسية الجسم له وتحسن وظائف الكلى وزيادة مضادات الأكسدة، مع الأخذ في الاعتبار أن مستخلص حبوب المورينجا المائي قبل الإصابة بالسكر قد يقلل من الإصابة به ويحمي الكلى من اعتلالها.