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ORIGINAL ARTICLE

Evaluation of antidiabetic and antioxidant activity of *Moringa oleifera* in experimental diabetes

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Abstract

Background: *Moringa* oleifera, a widely cultivated species in India, is an exceptionally nutritious vegetable with a variety of potential uses in treating rheumatism, venomous bites, and microbial infections. In the present study, we investigated the antidiabetic and antioxidant effects of methanol extracts of *M. oleifera* pods (MOMtE) in streptozotocin (STZ)-induced diabetic albino rats.

Methods: Diabetic rats were treated with 150 or 300 mg/kg MOMtE for 21 days and the antidiabetic effects of the extract were evaluated by measuring changes in biochemical parameters in the serum and pancreatic tissue. Two phytoconstituents, namely quercetin and kaempferol, were isolated from the MOMtE extract and their structures were determined using nuclear magnetic resonance and infrared spectroscopy.

Results: The progression of diabetes was significantly reduced after MOMtE treatment. In treated rats, both doses of MOMtE induced a significant reduction in serum glucose and nitric oxide, with concomitant increases in serum insulin and protein levels. Furthermore, MOMtE treatment increased antioxidant levels in pancreatic tissue, with concomitant decreases in levels of thiobarbituric acid-reactive substances. Histologic examination of the pancreas from diabetic rats showed degenerative changes in β-cells; MOMtE treatment significantly reversed the histoarchitectural damage to the islets cells.

Conclusion: In conclusion, *M. oleifera* exerts protective effects against STZ-induced diabetes. The MOMtE exhibited significant antidiabetic and antioxidant activity and active constituents may be isolated from the extract for evaluation in future clinical studies.

Keywords: antioxidant, histopathology, *Moringa oleifera*, oxidative stress.

Significant findings of the study: *Moringa oleifera* has promising antidiabetic and antioxidant effects. Phytochemical analysis revealed many active phytoconstituents that could be isolated and examined in future studies to develop potent antidiabetic agents.

What this study adds: To best of our knowledge there has been no previous comprehensive study to evaluate antidiabetic activity of pods from *M. oleifera* and present study adds the protective effect of *M. oleifera* pods as effective antidiabetic and antioxidant agent.

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Introduction

The incidence of diabetes is increasing in the developing world, with an increase in the number of diabetes patients in younger age groups.[1] The therapeutic management of diabetes without any side effects remains a challenge. In response, there is a growing interest in evaluating herbal remedies, which are seen to be less toxic and to have negligible side effects. *Moringa oleifera* is indigenous to the Indian subcontinent and is widely used in Ayurvedic medicine for the treatment of cardiac and circulation problems. Phytochemical investigations of *M. oleifera* have revealed the presence of 4-(4′-*o*-acetyl-α-L-rhamnopyranosyloxy) benzyl isothiocyanate, 4-(-L-rhamnopyranosyloxy)benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-(α-L-rhamnopyranosyloxy)benzyl glucosinolates.[2]

The mechanisms by which *M. oleifera* decreases hyperglycemic conditions remain uncertain. Therefore, the aim of present study was to evaluate the antidiabetic and antioxidant potential of a methanolic extract of *M. oleifera* (MOMtE) in diabetic rats with a view to providing information about active phytoconstituents for the clinical treatment of diabetes.

Methods

Plant material

Moringa oleifera pods were selectively collected fresh from a local market, washed with distilled water to remove any traces of dust, shade dried, and then powdered in an electric grinder. The plant at the fruiting stage was identified by Professor N.J. Sarna, Department of Botany, University of Rajasthan, and a voucher specimen has been deposited at the herbarium of the Department of Botany, University of Rajasthan, Jaipur, India (specimen no. RUBL-20393).

Extraction

Dried powdered pods (3 kg) were percolated with 100% methanol under reduced pressure for 72 h. The methanol was removed under vacuum at 35 ± 2°C for 48 h and a thick residue was obtained (yield 25.7 g w/w), which was stored at −4°C until use.

Phytochemical analysis

Pods were dried, powdered, and extracted (60–80°C) successively with petroleum ether, benzene, chloroform, alcohol, and water for 24–36 h. Extracts were filtered, dried *in vacuo*, and evaluated for the presence of carbohydrates, proteins, flavonoids, tannins, and alkaloids using standard procedures.[3] Two phytoconstituents were separated and identified through thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC). The nuclear magnetic resonance (NMR) and infrared (IR) spectra of these compounds were identical in every respect with a reference sample of quercetin.

Test animals

Experiments were performed using colony bred, sexually mature albino Wistar rats of both sexes (170–230 g). Rats were provided standard laboratory chow (Aashirwad Food Industries, Chandigarh, India) and water was available *ad libitum*. All animal procedures were approved by the Ethics Committee of the Centre for Advanced Studies, Department of Zoology (University of Rajasthan, Jaipur, India) and were in accordance with Indian National Science Academy (INSA), New Delhi guidelines for the maintenance and use of animals.

Toxicity study

The acute toxicity of MOMtE was evaluated using an established protocol that has been described in detail elsewhere.[4] Briefly, rats were administered different concentrations of extract and were examined for any signs of behavioral changes and/or mortality for the following 21 days. On the basis of these experiments, an LD_{50} 1300 mg/kg was established for MOMtE and two doses (150 and 300 mg/kg per day) were used in all further experiments.

Study design and doses

Streptozotocin (STZ) was injected at a dose of 50 mg/kg, i.p., to overnight-fasted rats.[5] Rats were considered diabetic when fasting glucose levels were >250 mg/dL. Control rats were injected with 0.1 mmol/L sodium citrate buffer alone. Diabetic rats were allowed to drink 2% glucose solution overnight to overcome the drug-induced hypoglycemia.

Rats were randomized into seven groups of seven rats each as follows: (i) an untreated control group; (ii) an untreated diabetic group; (iii) two treated control groups, in which normal rats were treated with either 150 or 300 mg/kg per day, p.o., MOMtE for 21 days; (iv) two treated diabetic groups, in which STZ-diabetic rats were treated with either 150 or 300 mg/kg per day, p.o., MOMtE for 21 days; and (v) a glibenclamide-treated group, in which diabetic rats were treated with 0.3 mg/kg per day, p.o.,

glibenclamide (dissolved in 0.5 mL distilled water) for 21 days.

Estimation of serum biochemical parameters
After 21 days, rats were killed by mild ether anesthesia and tissues were collected for the subsequent biochemical analysis of specific parameters in the pancreas and other vital organs. After autopsy under mild ether anesthesia, serum was separated and analyzed for insulin (RIA), total protein (Lowry et al.[6]) and albumin.[7]

Estimation of pancreatic biochemical parameters
After rats had been killed, the pancreas removed and stored at −4°C until biochemical analyses for protein,[6] lipid peroxidation,[8] superoxide dismutase (SOD),[9] reduced glutathione (GSH),[10] catalase,[11] and glycogen[12] using established methods.

A small portion of the pancreas from each rat was fixed in 10% formalin solution for sectioning (5 μm) and staining with hematoxylin and eosin to enable histological examination.

Statistical analysis

Data are given as the mean ± SEM and statistical analyses were performed using ANOVA. Results obtained at the end of experiment were compared with those of the control and diabetic groups using Student's *t*-test. Differences were considered significant at $P < 0.001$.

Results

Nuclear magnetic resonance of quercetin and kaempferol

The ^{1}H-NMR spectrum of the compound isolated from the extract revealed H-6′-H-6′ ortho-coupling (8.4 Hz) at 7.4 and 6.8 p.p.m., and H-6′-H-2′ meta-coupling (2 Hz) at 7.4 and 7.6 p.p.m. Another meta-coupling occurs between H-6 and H-8 (1.9 Hz) at 6.3 and 6.5 p.p.m. These results are identical in every respect with those obtained for the reference sample of quercetin.

The proton NMR spectrum (δ p.p.m.) revealed the following results: 6.08 (1H, *d*, J = 2.0 Hz, H-6), 6.44 (1H, *d*, J = 2.0 Hz, H-8), 8.10 (2H, *d*, J = 10 Hz, H-2′, 6′), 6.87 (2H, *d*, J = 10 Hz, H-3′, 5′); ^{13}C-NMR (δ p.p.m.): 145.5 (C-2), 136.2 (C-3), 177.1 (C-4), 157.1 (C-5), 98.6 (C-6), 164.2 (C-7), 93.8 (C-8), 162.0 (C-9), 103.7 (C-10), 120.4 (C-1′), 129.1 (C-2′, 6′), 115.4 (C-3′, 5′), 159.7 (C-4′). From the ^{1}H- and ^{13}C-NMR data, the compound was identified as kaempferol (Figs 1–4).

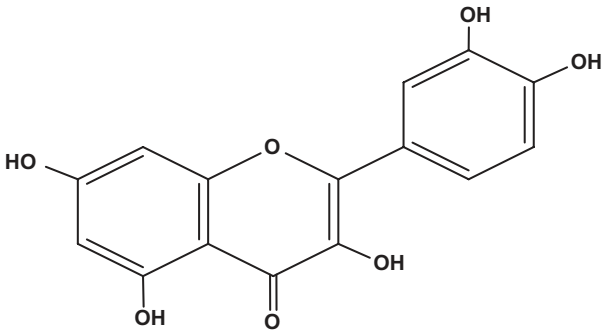

Figure 1 Chemical structure of quercetin.

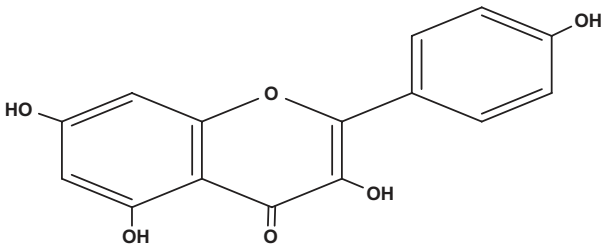

Figure 2 Chemical structure of kaempferol.

Effect of MOMtE on serum glucose and insulin

Serum glucose levels in control rats and in rats in the different experimental groups are summarized in Table 1. Serum glucose levels were significantly increased in untreated diabetic rats ($P < 0.001$). However, after treatment of diabetic rats with both 150 and 300 mg/kg MOMtE, glucose levels decreased significantly. Furthermore, serum insulin levels were significantly lower in untreated diabetic rats compared with control rats ($P < 0.001$). Treatment of diabetic rats with both 150 and 300 mg/kg MOMtE for 21 days significantly increased serum insulin levels compared with levels in untreated diabetic rats.

Effect of MOMtE on biochemical parameters

Serum protein and albumin levels in control and MOMtE-treated normal rats were unchanged throughout the experimental period. However, in untreated diabetic rats, serum protein and albumin levels decreased significantly compared with the control group ($P < 0.001$). Treatment of diabetic rats with MOMtE at both doses restored biochemical parameters to normal levels (Table 1).

Table 2 summarizes total protein, SOD, GSH, catalase, and lipid peroxidation levels in the different

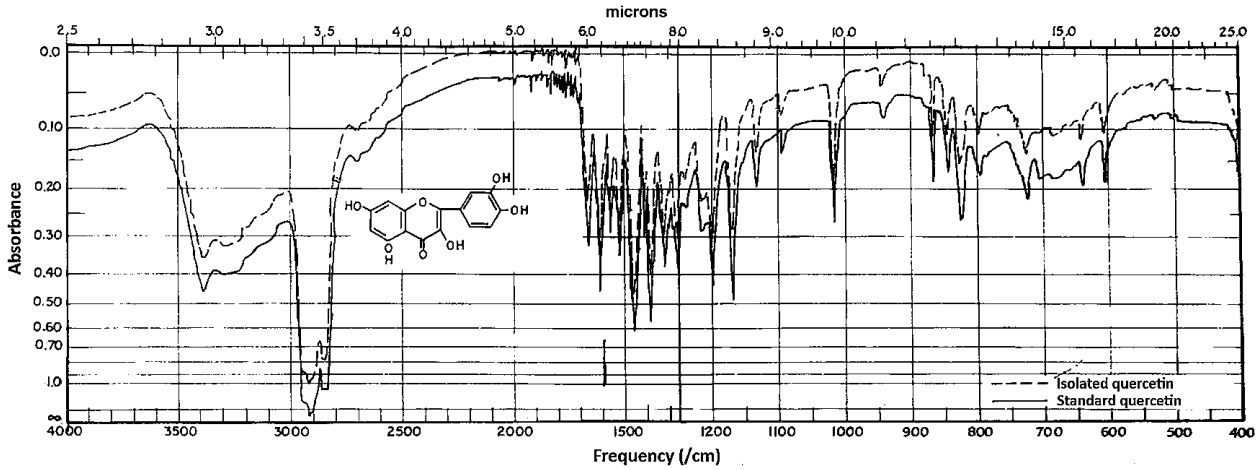

Figure 3 Infrared spectra of isolated and standard quercetin.

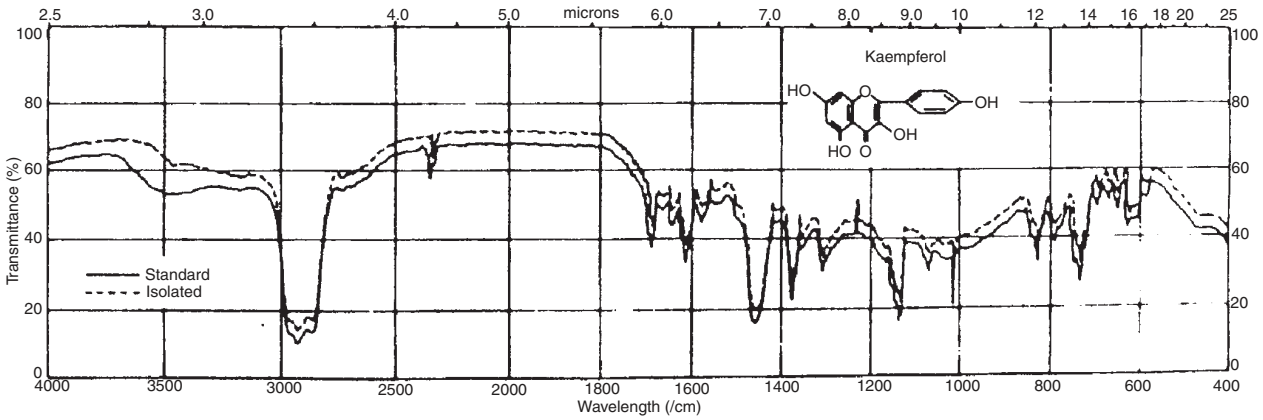

Figure 4 Infrared spectra of isolated and standard Kaempferol.

Table 1 Effect of the methanolic extract of *Moringa oleifera* on serum biochemical parameters in normal and streptozotocin-diabetic rats

Group	Serum glucose (mg/dL)	Total protein (mg/dL)	Albumin (mg/dL)	Serum insulin (μU/mL)	Serum NO (mg/dL)
Control rats					
Untreated	81.78 ± 12.84	6.84 ± 0.32	3.53 ± 0.19	19.07 ± 0.83	7.76 ± 0.87
150 mg/kg MOMtE	80.23 ± 11.71	7.12 ± 0.26	3.81 ± 0.22	19.31 ± 0.56	6.18 ± 0.13
300 mg/kg MOMtE	82.56 ± 21.71	7.47 ± 0.37	4.21 ± 0.09	20.43 ± 0.17	7.87 ± 0.25
Diabetic rats					
Untreated	261.28 ± 13.20**	3.77 ± 0.17**	2.11 ± 0.10**	9.14 ± 1.11**	4.89 ± 0.15**
150 mg/kg MOMtE	213.37 ± 28.36*†	4.64 ± 0.19*†	2.363 ± 0.07†	12.21 ± 0.35†	5.13 ± 0.16*†
300 mg/kg MOMtE	162.37 ± 22.78*†	5.21 ± 0.21*†	2.73 ± 0.08†	13.31 ± 0.78†	6.21 ± 0.22*†
0.3 mg/kg glibenclamide	141.54 ± 14.61†	5.43 ± 0.24*†	3.11 ± 0.24†	16.84 ± 0.56†	5.89 ± 0.21*†

Values are the mean ± SEM (n = 7 rats in each group). *P < 0.05; **P < 0.001 compared with the untreated control group; †P < 0. 001 compared with the untreated diabetic group.
MOMtE, methanol extract of *Moringa oleifera* pods; NO, nitric oxide.

groups. Treatment of normal rats with both doses of MOMtE resulted in non-significant changes in protein, SOD, GSH, catalase, and lipid peroxidation. In MOMtE-treated diabetic rats, there were increases in protein levels, SOD, GSH and catalase activity, but a significant decrease in lipid peroxidation (P < 0.01).

Table 2 Effect of the methanolic extract of *Moringa oleifera* on tissue biochemical parameters in normal and streptozotocin-diabetic rats

Group	Protein (mg/g)	Superoxide dismutase (μmol/mg protein)	Reduced glutathione (nmol/g tissue)	Catalase (μmol H_2O_2 consumed/min per mg protein)	Lipid peroxidation (nmol MDA/mg protein)
Control rats					
Untreated	53.52 ± 3.76	4.37 ± 0.23	2.68 ± 0.13	72.63 ± 6.14	2.51 ± 0.73
150 mg/kg MOMtE	59.37 ± 4.21	5.61 ± 0.13	2.92 ± 0.11	71.17 ± 4.15	2.18 ± 0.64
300 mg/kg MOMtE	48.71 ± 4.41	5.84 ± 0.17	2.73 ± 0.13	73.23 ± 5.31	2.83 ± 0.64
Diabetic rats					
Untreated	20.14 ± 1.11**	1.83 ± 0.31**	1.26 ± 0.09	32.45 ± 3.17**	9.18 ± 0.61**
150 mg/kg MOMtE	22.37 ± 3.18*†	2.02 ± 0.11†	1.37 ± 0.15†	41.35 ± 5.76†	6.31 ± 1.34†
300 mg/kg MOMtE	24.85 ± 1.09*††	2.17 ± 0.11††	1.78 ± 0.17†	48.84 ± 4.32††	5.13 ± 1.21†
0.3 mg/kg glibenclamide	32.14 ± 1.85*††	3.68 ± 0.02††	2.17 ± 0.15††	52.47 ± 3.14††	4.56 ± 0.75†

Values are the mean ± SEM (n = 7 rats in each group). *P < 0.05; **P < 0.001 compared with the untreated control group; †P < 0.05; †† P < 0.001 compared with the untreated diabetic group.
MOMtE, methanol extract of *Moringa oleifera* pods; MDA, malondialdehyde.

Histology

Sections of the pancreas from control rats exhibited prominent cytoplasm with centroacinar cells containing serous acini. In contrast, pancreatic sections from diabetic rats showed degenerated islets with cellular debris. In addition, centroacinar cells with increased intercellular spaces were clearly visible. After 21 days treated of diabetic rats with MOMtE, there was a marked rejuvenation of pancreatic islets (Figs 5–9).

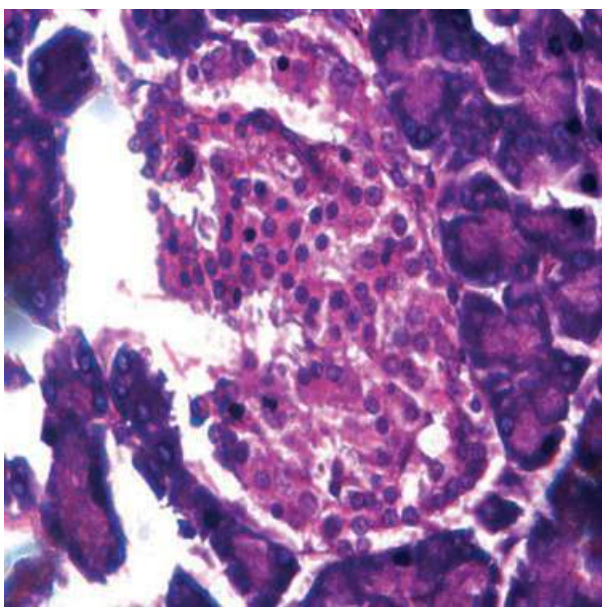

Figure 5 Pancreatic section from a control rat showing prominent cytoplasm. Islet cells with centroacinar cells containing serous acini are also visible. (Hematoxylin and eosin stain; original magnification ×400).

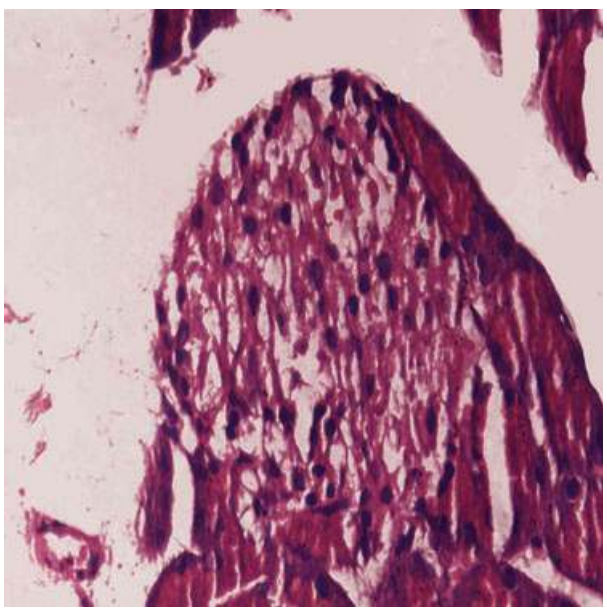

Figure 6 Microphotograph of a pancreatic section from a diabetic rat showing ruptured islets with degenerated cells. Damaged centroacinar cells with increased intercellular spaces and cellular debris are clearly visible. (Hematoxylin and eosin stain; original magnification ×400).

Discussion

In the present study 100% of rats treated with STZ developed hyperglycemia. Streptozotocin is a potent cytotoxin of the islets of Langerhans and causes severe diabetes.[13] In the present study, the diabetic group had significantly increased serum glucose levels, whereas regular administration of MOMtE to diabetic rats reduced serum glucose levels, in addition to increasing

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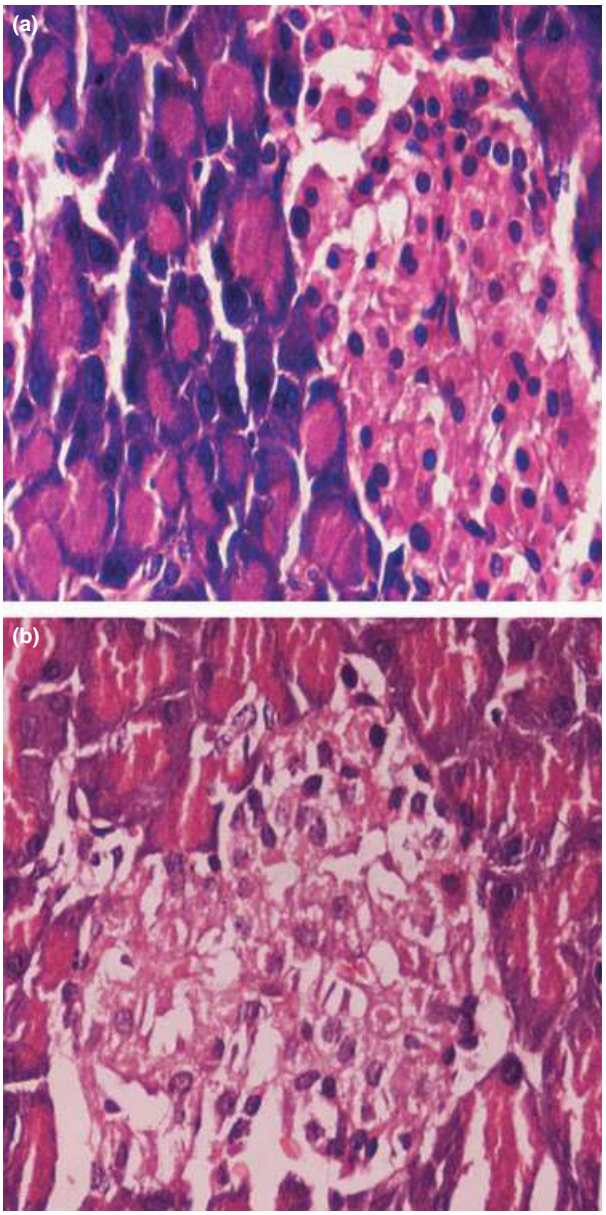

Figure 7 Photomicrographs of pancreatic sections from (a) a control rat and (b) a diabetic rat treated with 150 mg/kg per day methanol extract of *Moringa oleifera* pods (MOMtE) for 21 days. The section from the control rat shows normal pancreatic histoarchitecture (a). In the diabetic rat, MOMtE treatment has resulted in normal pancreatic islets (b). (Hematoxylin and eosin stain; original magnification ×400).

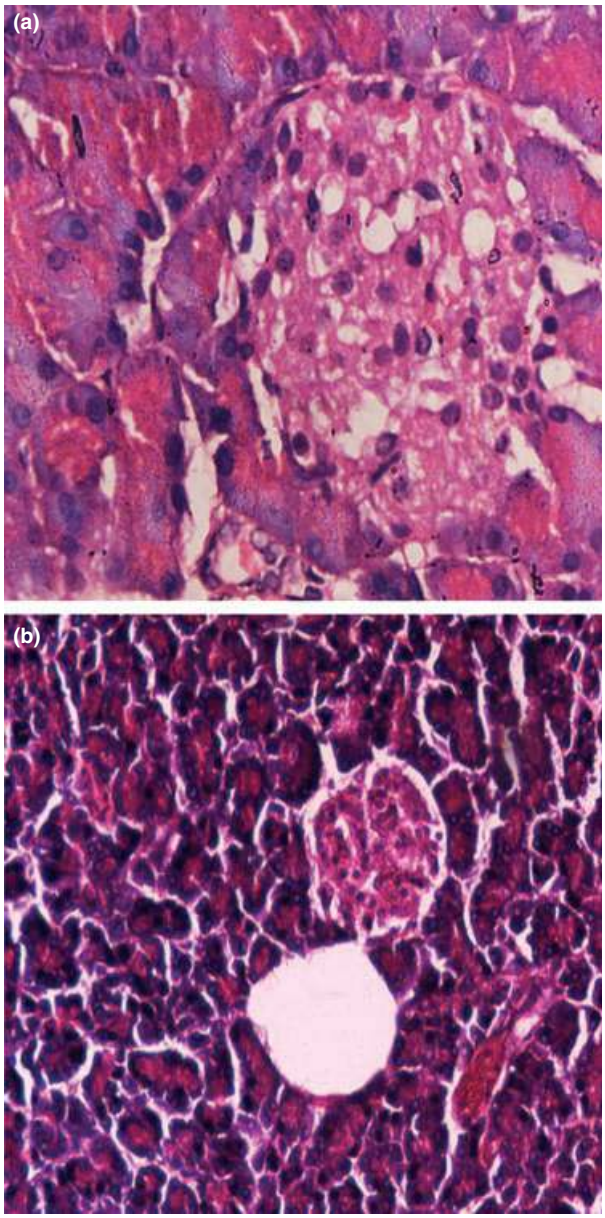

Figure 8 Photomicrographs of pancreatic sections from (a) a control rat and (b) a diabetic rat treated with 300 mg/kg per day methanol extract of *Moringa oleifera* pods (MOMtE) for 21 days. The section from the control rat shows normal pancreatic histoarchitecture (a). In the diabetic rat, MOMtE treatment has resulted in marked rejuvenation of the pancreatic islets (b). (Hematoxylin and eosin stain; original magnification ×400).

serum insulin levels. Treatment of normal rats with MOMtE had no adverse effects (Table 1). The presence of active principle(s) in *M. oleifera* may potentiate glucose-induced insulin secretion from existing β-cells or cause its release from the bound form, thus decreasing serum glucose levels in treated diabetic rats. In this context, a number of other plants have been screened for their hypoglycemic effects.[5]

Flavonoids act as insulin secretagogues or insulin mimetics, probably by influencing the pleiotropic mechanisms to attenuate diabetic complications. The present phytochemical investigation of *M. oleifera*

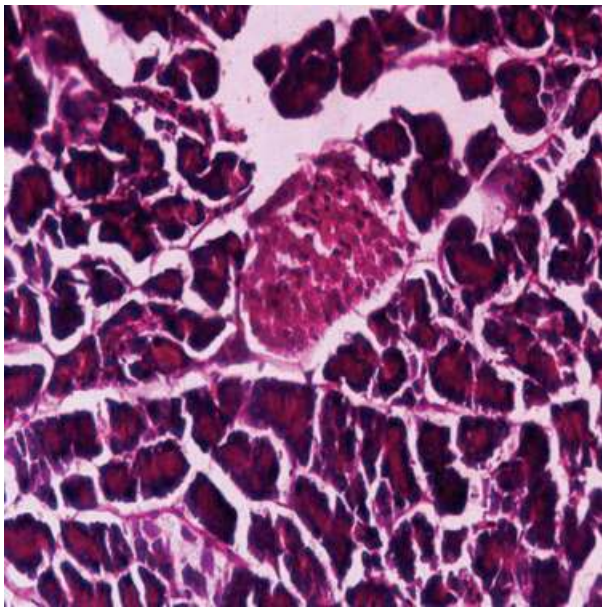

Figure 9 Photomicrograph of a pancreatic section from a glibenclamide-treated diabetic rat showing normal histoarchitecture of the islets with an increased number of islet cells. Centroacinar cells in the serous acini are completely normal. (Hematoxylin and eosin stain; original magnification ×400).

revealed the presence of bioflavonoids that may be responsible for the stimulation of glucose uptake in peripheral tissues and regulation of the activity and/or expression of the rate-limiting enzymes involved in carbohydrate metabolism.[14]

Albumin is necessary for appropriate distribution of body fluids between body tissues and intravascular compartments and functions as a plasma carrier by binding several hydrophobic hormones.[15] Albumin also keeps the blood from leaking out of blood capillaries.[16] Administration of STZ decreases serum albumin levels due to increased non-enzymatic glycosylation of protein.[17] In the present study, MOMtE treatment significantly elevated serum albumin concentrations, which is possibly associated with a decreased affinity of albumin towards glucose.

Oxygen free radicals have been suggested to contribute to the development of complications of diabetes leading to β-cell cytotoxicity. Indeed, many studies have been performed to evaluate changes in parameters of oxidative stress in diabetes.[5] In present study, an increase in lipid peroxidation, with concomitant decreases in SOD, CAT and GSH, was observed in the pancreas of diabetic rats. Lipid peroxidation is an index of malondialdehyde production.[18] The production of free radicals increases the peroxidation of lipid molecules. The decrease in SOD activity during the progression of diabetes could be due to enzyme glycosylation that occurs in the diabetic state. Decreased SOD activity could also be due to accumulation of H_2O_2 in affected tissues.[19] Reduced glutathione is a main factor in detoxification and antioxidant systems, providing a defense against free radicals and cytotoxins. An increase in GSH levels in MOMtE-treated diabetic rats may be due to decreased production of reactive oxygen species (ROS). The maintenance of GSH levels depends on various enzyme activities, such as γ-glutamyl transferase, glutathione *S*-transferase, glutathione peroxidase, glutathione reductase, glucose 6-phosphate dehydrogenase etc. Although these enzymes were not investigated in the present study, there is the possibility that the extract affected these enzymes, thus increasing the glutathione pool in diabetic rats.

Free radicals and ROS are known to abnormally alter proteins and to generate protein carbonyl products, which are currently considered markers of oxidative injury to proteins.[20] Structural changes are considered to be among the molecular mechanism leading to the progression and development of diabetes and its complications.[21] Advanced oxidation protein products are also considered markers of oxygen-mediated protein damage.[22] In present study, we observed a decrease in the protein content of the pancreas from diabetic rats. However, MOMtE treatment of diabetic rats significantly increased protein levels. This clearly indicates that MOMtE, by decreasing oxidative stress, may be effective in preventing oxidative protein damage, which is thought to be involved in β-cell damage in the diabetic condition.

Conclusions

In conclusion, the methanolic extract of pods from *M. oleifera* protects β-cells against ROS-mediated damage by enhancing cellular antioxidant defenses and minimizing hyperglycemia in STZ-induced diabetes. The experimental findings clearly indicate an exciting opportunity to develop a potent antidiabetic drug from this plant.

Acknowledgments

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Disclosure

None declared.

References

1. World Health Organization (WHO). *Diet, Nutrition and the Prevention of Chronic Diseases: Report of a Joint WHO/FAO Expert Consultation*. WHO Technical Report Series 916. WHO, Geneva, 2003.
2. Fahey JW. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. *J Trees Life*. 2005; **1**: 5.
3. Subramanian SS, Nagarajan S. Flavonoids of the seeds of *Crotlaria retusa* and *C. striata*. *Curr Sci*. 1969; **38**: 65–8.
4. Lorke D. A new approach to practical acute toxicity. *Arch Toxicol*. 1983; **53**: 275–89.
5. Gupta R, Gupta RS. Hypolipidemic activity of *Pterocarpus marsupium* in streptozotocin induced diabetes. *J Complementary Integrative Med*. 2009; **6**: 1–28.
6. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem*. 1951; **193**: 265–75.
7. Corcoran RM, Durnan SM. Albumin determination by a modified bromcresol green method. *Clin Chem*. 1977; **23**: 765–6.
8. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979; **95**: 351–8.
9. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem*. 1974; **47**: 469–74.
10. Moron MJ, Depierre JW, Mannivik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochim Biophys Acta*. 1979; **582**: 67–8.
11. Aebi H. Catalase *in vitro*. *Methods Enzymol*. 1984; **105**: 121–6.
12. Hassid WZ, Abraham S. Chemical procedures for analysis of polysaccharides. *Methods Enzymol*. 1957; **3**: 34–7.
13. Brenna O, Qvigstad G, Brenna E, Waldu HL. Cytotoxicity of streptozotocin on neuroendocrine cells of the pancreas and the gut. *Dig Dis Sci*. 2003; **48**: 906–10.
14. Gupta R, Sharma AK, Sharma MC, Dobhal MP, Gupta RS. Antidiabetic and antioxidant potential of β-sitosterol in streptozotocin-induced experimental hyperglycemia. *J Diabetes*. 2011; **3**: 29–37.
15. Zunszain PA, Jamie GJ, Komatsu T, Tsuchida E, Curry S. Crystal structural analysis of human serum albumin complexed with hemin and fatty acid. *BMC Struct Biol*. 2003; **3**: 6.
16. Forker LL, Chaikoff IL. Turnover of serum proteins in diabetes as studied with S^{35} labeled proteins. *J Biol Chem*. 1951; **188**: 829–40.
17. Guthrow CE, Morris MA, Day JF, Thorpe SR, Baynes JW. Enhanced nonenzymatic glucosylation of human serum albumin in diabetes mellitus. *Proc Natl Acad Sci USA*. 1979; **76**: 4258–61.
18. Kakkar R, Mantha SV, Radhi J, Prasad K, Kalra J. Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes. *Clin Sci*. 1998; **94**: 623–32.
19. Karasu C. Increased activity of H_2O_2 in aorta isolated from chronically streptozotocin-diabetic rats: Effects of antioxidant enzymes and enzyme inhibitors. *Free Radic Biol Med*. 1999; **27**: 16–27.
20. Reznick AZ, Packer L. Oxidative damage to proteins: Spectrophotometric method for carbonyl assay. *Methods Enzymol*. 1994; **233**: 357–63.
21. Altomare E, Grattagliano I, Vendemaile G, Micelli FT, Signorile A, Cardia L. Oxidative protein damage in human diabetic eye: Evidence of a retinal participation. *Eur J Clin Invest*. 1997; **27**: 141–7.
22. Locckie LZ, Meerman JHN, Commander JNM, Vermeulen NPE. Biomarkers of free radical damage: Application in experimental animals and in humans. *Free Radic Biol Med*. 1999; **26**: 202–6.

