Ascorbic Acid-Rich *Moringa oleifera* Lam. Extract Inhibits Hepatorenal Toxicity and Enhances the Endogenous Antioxidant Levels in Streptozotocin-Induced Type II Diabetes

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Abstract:

**Background.** The cases of diabetes increase day by day due to unhealthy lifestyle, food habit, and less food intake. Novel drugs for the treatment of diabetes are urgently needed. Most researchers are looking for alternative drugs (plant-based drugs) for the treatment of diabetes.

**Objective.** The current experiment was designed to examine the hepatic and renal beneficial effect of *Moringa oleifera* Lam. (MO) extract in the streptozotocin (STZ)-induced diabetes.

**Methods.** Antidiabetic potential of the MO extract was estimated in terms of blood glucose levels, plasma insulin, hexokinase, and glucose-6-phosphate. Antihyperlipidemic effects of MO extract were evaluated through the estimation of low-density lipoprotein (LDL) cholesterol, total cholesterol (TC), triglyceride (TG), very LDL (VLDL) cholesterol, and high-density lipoprotein (HDL) level whereas the antioxidant effects were evaluated through estimation of catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione peroxidase (GPx) levels in diabetic rats.

**Results.** Dose-dependent treatment using MO extract significantly increased the body weight, hexokinase, plasma insulin, HDL, SOD, CAT, and GPx levels (*P* < 0.001) and significantly decreased the levels of fasting blood glucose, TC, TGs, LDL, VLDL, MDA, fructose-1,6-bisphosphate, glucose-6-phosphate, and glycated hemoglobin in STZ-induced diabetic rats (*P* < 0.001).

**Conclusion.** MO can be used as a therapeutic agent in the management of elevated blood glucose levels through the alterations in the blood glucose level, plasma level of insulin, and various biochemical parameters.

**Keywords:** Hyperglycemic, Hyperlipidemic, *Moringa oleifera*, Oral glucose tolerance test, Streptozotocin

1 Introduction

Hereditory and environmental factors play a significant role in diabetes. Diabetes is characterized by an increase in blood glucose levels and defects in insulin action and its secretion. Diabetes mellitus (DM) is a pathological disorder that results in severe non-physiological changes and metabolic imbalances in various tissues. DM exhibits the defect in insulin action and its secretion, which results in hyperglycemia with disturbances of protein, fat, and carbohydrates metabolism, gradually leading
to chronic complications such as neuropathy, cardiomyopathy, and retinopathy. According to a report, among the individuals aged 20 – 79 years from 219 countries, 382 millions of people have DM, and it is estimated that this number will increase up to 592 million in 2035 [1]. Previous studies suggested that oxidative stress plays an important role in the etiology of DM [2]. It is well documented that high oxidative stress level was observed in the DM patients and experimental rats due to persistent and induction of chronic hyperglycemia, which altered the endogenous antioxidant defense system and boost the generation of free radicals [2]. Streptozotocin (STZ) which is obtained from Streptomyces achromogenes is commonly used for the induction of experimental diabetes. The induction of DM using STZ starts the destruction of pancreatic β-cell, resulting in an increase in the blood glucose level.

*Moringa oleifera* Lam. (MO) (family: Moringaceae), normally referred to as horseradish or drumstick tree, is a plant that is widely distributed in sub-Himalayan regions and all over the Asian Nations. According to the traditional system of medicine, MO Lam. is the most useful tree and almost every part of the tree can be used for the treatment of numerous diseases [3]. MO is known for its medicinal values in hepatotoxicity [4], wound healing [5], inflammation (rheumatism) [6], and venous bites [7]. Besides, it also possesses antitumor [6] and antimicrobial activities [8-12]. A number of plant extracts, compounds, and formulations are known to have antihyperglycemic [13], antihyperlipidemic [14], and antioxidant activities [15]. To date, various antidiabetic drugs are available in the pharmaceutical market but still, many researchers search for new drugs that can effectively treat diabetes. At present, plant-based drug is the main focus in many studies due to lesser side effects, low cost, and specific actions. Furthermore, plant-based drug is easily available and easier to take in the form of syrup, tablet, capsules, and food. Various plant-based drugs are already scrutinized and proven to possess more beneficial effects on diabetes. A number of traditional medicines have been used for lowering blood glucose levels in diabetic patients. However, the researchers are looking for more specific drugs because they not only are effective in diabetes treatment but also exert hepatic and renal protection during diabetes. In the present study, we attempted to scrutinize the antidiabetic, antioxidant, and antihyperlipidemic effects of MO stem bark against STZ-induced diabetic rats.

### 2 Materials and methods

#### 2.1 Chemicals

STZ was purchased from Sigma Aldrich, USA. All the chemicals and reagents used in the experiment were purchased from the approved vendors.

#### 2.2 Collection of plant material

The stem bark of MO was collected and authenticated in March 2011 from Herbal Garden, Department of Pharmaceutical Sciences, Shalom Institute of Health and Allied Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Allahabad, Uttar Pradesh, India, and a specimen voucher (SIP/HD/054/17) has been submitted in the department for further reference.

#### 2.3 Preparation of MO extract

The collected stem bark of MO (3 kg) was blended and extracted with methanol (8 L) for 5 days. After extraction, the extract was filtered with Whatman filter paper to remove the particulate and debris substance from the extract. The rotatory evaporator was used to concentrate the extract [16-18]. A suspension of extracts in distilled water, prepared using 2% carboxymethyl cellulose, was used in the experiment.

#### 2.4 Animals

Albino rats (Wistar strain) weighed about 150 – 220 g were used in the experiment. The rats were kept under the standard conditions, i.e., temperature at 24 – 26°C, relative humidity at 55 – 65%, and coupled with a normal day/night cycle. To induce diabetes, the rats were fasted overnight and 60 mg/kg dose of STZ was used. After 5 days, the blood glucose level was estimated, and the rats having blood glucose level of more than 220 mg/dL were considered diabetic [19].

Briefly, the rats were divided into seven groups: Group I: Normal control (untreated); Group II: Normal control + MO (500 mg/kg); Group III: STZ-diabetic control; Group IV: STZ diabetes +
MO (100 mg/kg); Group V: STZ diabetes + MO (250 mg/kg); Group VI: STZ diabetes + MO (500 mg/kg); and Group VII: STZ diabetes + glibenclamide (10 mg/kg). Each group contained six animals.

All the work was done according to Principles of Laboratory Animal Care [20]. All animals received basal pellet diet and water ad libitum. The study was approved by the Institutional Animal Ethical Committee, Siddhartha Institute of Pharmacy (1435/PO/a/11/CPCSEA).

2.5 Acute oral toxicity study

For the estimation of acute oral toxicity study, the rats fasted overnight (12 h). Animals from every group received the graded dose of the MO extract up to 2000 mg/kg. The rats were kept under observation until 2 h for neurological, behavioral, and autonomic profiles and from 24 to 72 h for any signs of toxicity and lethality [21].

2.6 Oral glucose tolerance test (OGTT)

The Bonner-Weir method was used for the estimation of OGTT with minor modifications [22]. The rats were divided into five groups on the basis of treatments, i.e., glucose control, glucose control + MO extract (100 mg/kg), glucose control + MO extract (250 mg/kg), glucose control + MO extract (500 mg/kg), and glucose control + glibenclamide (10 mg/kg).

The rats in these OGTT groups received glucose (2 mg/kg) using the feeding tube [23]. Glucose oxidase-peroxidase kits were used for the estimation of glucose at regular time intervals (0, 30, 60, 90, 120, and 150 min).

2.7 Antidiabetic effect of MO extract

The rats in all experimental groups received oral treatment (one dose per day) using intragastric tube for 28 days continuously. On day 28, all animals were starved overnight (12 h), and their blood was collected through puncturing the retroorbital under mild anesthetic conditions. The blood samples were stored in anticoagulant-containing tubes and centrifuged at 5000 rpm for 15 min to separate the plasma. Other biochemical parameters such as total cholesterol (TC), triglycerides (TGs), and high-density lipoprotein (HDL) were estimated using commercial kits (Span Diagnostics, India) in adherence with the instructions provided by manufacturer. The levels of low-density lipoprotein (LDL) and very LDL (VLDL) cholesterols were calculated using the following formulae [25].

\[ \text{LDL (mg/dL)} = \frac{\text{TC}}{1.19} + \frac{\text{TG}}{1.9} - \frac{\text{HDL}}{1.1} - 38 \text{ (mg/dL)} \]

\[ \text{VLDL (mg/dL)} = \frac{\text{TGs (mg/dL)}}{5} \]

The coronary risk index and atherogenic index were estimated through using the following formulae:

\[ \text{Atherogenic index} = \frac{\text{Low density lipoprotein cholesterol}}{\text{High density lipoprotein cholesterol}} \]

\[ \text{Coronary risk index} = \frac{\text{Total cholesterol}}{\text{High density lipoprotein cholesterol}} \]

2.8 Effect of MO extract on biochemical parameters

At the end of the protocol, the blood samples of all rats were collected by puncturing the retroorbital under the mild anesthetic conditions. The blood samples were stored in anticoagulant-containing tubes and centrifuged at 5000 rpm for 15 min to separate the plasma. Other biochemical parameters such as total cholesterol (TC), triglycerides (TGs), and high-density lipoprotein (HDL) were estimated using commercial kits (Span Diagnostics, India) in adherence with the instructions provided by manufacturer. The levels of low-density lipoprotein (LDL) and very LDL (VLDL) cholesterols were calculated using the following formulae [25].

2.9 Effect of MO extract on antioxidant markers

For the estimation of antioxidant markers, the liver obtained from each animal was homogenized using Teflon homogenizer and prepared in ice-chilled 10% potassium chloride solution. The homogenate was then used for the estimation of antioxidant parameters. The evaluation of the antioxidant parameters such as superoxide dismutase (SOD) [26], catalase (CAT) [27], glutathione peroxidase (GPx), and malondialdehyde (MDA) [28-30] was carried out using the reported methods.

2.10 Effect of MO extract on pro-inflammatory mediators

The pro-inflammatory cytokines such as C-reactive protein (CPR), tumor necrosis factor (TNF-α), and interleukin-6 (IL-6) were estimated using the ELISA assays (RayBiotech, Georgia, USA) in accordance to the manufacturer’s instruction.
2.11 Statistical analysis

One-way ANOVA was used to analyze the data. All the data were expressed as mean ± S.E.M. Dunnett’s test was also carried out. The analyses in this study were performed using GraphPad Prism 7.0 (GraphPad Software, CA, USA). \( P \leq 0.05 \) was considered to be significant.

3 Results

3.1 Acute oral toxicity study

In the acute oral toxicity study, MO extract-treated rats did not show any effects in the behavioral, neurological, and/or autonomic pattern of rodents. MO extract-treated rats did not exhibit any changes in the water and food consumption but exhibited an increase in body weight. No animals in any group exhibited any signs and symptoms of toxic reaction. Thus, the dose of MO extract up to 2000 mg/kg was safe to use.

3.2 Effect of MO extract on OGTT

The rats in the glucose control group showed an increase in blood glucose levels at the end of the experiment. The rats in the MO extract-treated and glibenclamide-treated groups demonstrated reduced blood glucose levels during the OGTT study (Table 1 and Figure 1).

3.3 Effect of MO extract on fasting blood glucose levels

Table 2 shows the effect of the MO extract on the blood glucose level in the experimental rats. Normal control and normal control + MO extract did not show any significant changes in the blood glucose level. STZ-induced rats demonstrated an increase in blood glucose levels. The glucose control group showed a significant increase in blood glucose levels compared to the normal control groups.

**Figure 1.** Oral glucose tolerance and area under curve of Moringa oleifera Lam. extracts in normal rats. Values are given as mean ± S.E.M. of six rats in each group. \(*P < 0.05, **P < 0.01, ***P < 0.001\) compared with normal control values.
| OGTT group                              | Pharmacokinetic parameters  |
|----------------------------------------|-----------------------------|
|                                        | $C_{\text{max}}$ (mg/dL) | $T_{\text{max}}$ (min) | AUC (mg.min/dL) |
| Glucose control                        | 150±2.864                  | 30                      | 19602         |
| Glucose control+MO (100 mg/kg)         | 141.2±1.068                | 30                      | 17919         |
| Glucose control+MO (250 mg/kg)         | 133±1.924                  | 30                      | 16692         |
| Glucose control+MO (500 mg/kg)         | 125.6±1.030                | 30                      | 15033         |
| Glucose control+glibenclamide (10 mg/kg)| 126.6±1.077               | 30                      | 15801         |

Each parameter represents the mean of six animals. AUC values. $t_{\text{max}}$: Time at maximum observed concentration, $C_{\text{max}}$: Maximum concentration, OGTT: Oral glucose tolerance test, AUC: Area under curve

increase in blood glucose level and concentration-dependent treatment with MO extract significantly decreased the blood glucose level ($P < 0.001$). Glibenclamide-treated groups also showed a similar result. MO extract showed the reduction of blood glucose level by 64.79%, 71.63%, and 76.69% at the dose level of 100, 250, and 500 mg/kg, respectively. Glibenclamide-treated rats demonstrated 75.03% reduction in blood glucose level.

### 3.4 Effect of MO extract on body weight

The rats in the group without receiving treatment showed an increase in body weight. STZ-induced diabetic rats had reduced body weight, and concentration-dependent treatment with MO extract increased body weight. Glibenclamide-treated rats showed an increase in body weight as compared to diabetes control group.

### 3.5 Effect of MO extract on plasma insulin

The plasma level of insulin was considerably reduced during diabetes, and similar result was observed in the experimental rats. STZ-treated group exhibited reduced plasma insulin and MO extract treatment significantly increased plasma insulin in a concentration-dependent manner ($P < 0.001$). Glibenclamide-treated rats showed similar results (Table 2).

### 3.6 Effect of MO extract on biochemical parameters

Table 2 shows the glycated hemoglobin level of normal and STZ-treated rats. STZ-induced diabetic rats demonstrated increased glycated hemoglobin levels, which was almost 4 times higher as compared to the control group. STZ-induced diabetic rats that received the MO extract showed a reduction in glycated hemoglobin levels in a dose-dependent manner.

STZ-induced diabetic rats had a reduced level of hexokinase as compared to the control group. MO extract significantly augmented the level as compared to STZ control in dose-dependent treatment ($P < 0.001$). Glibenclamide-treated rats also showed an increase in the level of hexokinase.

An opposite trend was observed in the level of glucose-6-phosphatase and fructose-1,6-bisphosphate, STZ-induced group rats exhibited increased level, and treatment with MO extract significantly reduced the level of glucose-6-phosphatase and fructose-1,6-bisphosphate ($P < 0.001$). Glibenclamide-treated rats showed a decline in the level of glucose-6-phosphatase and fructose-1,6-bisphosphate.

### 3.7 Effect of MO extract on lipid profile

STZ-induced diabetic rats exhibited increased levels of TC, TG, LDL, and VLDL and reduced level of HDL. STZ-induced diabetic rats treated with MO extract had significant reductions in the levels of TG, TC, LDL, and VLDL and increased the level of HDL in dose-dependent manner ($P < 0.001$). Glibenclamide-treated rats showed similar results (Table 2).

### 3.8 Effect of MO extract on the antioxidant marker

STZ-induced diabetic rats had reduced levels of SOD, CAT, and GPx and increased level of MDA as compared to the control group. Treatment with MO extract significantly increased the level of SOD, CAT, and GPx and reduced level of MDA as compared to the STZ-induced rats ($P < 0.001$).
### Table 2. Effect of *Moringa oleifera* Lam. on biochemical parameters in STZ-induced diabetic rats

| Biochemical parameter                  | Normal control | Normal control+MO (500 mg/kg) | STZ-diabetic control | STZ-diabetic control+MO (100 mg/kg) | STZ-diabetic control+MO (250 mg/kg) | STZ-diabetic control+MO (500 mg/kg) | STZ-diabetic control+Glibenclamide (10 mg/kg) |
|---------------------------------------|----------------|-------------------------------|---------------------|-------------------------------------|-------------------------------------|-------------------------------------|---------------------------------------------|
| Fasting plasma glucose (mg/dL)        | 84.4±1.288     | 239.8±2.498***                | 394.8±2.498***      | 139.1±1.732**                      | 112.1±1.414**                      | 92.1±1.304***                       | 98.6±1.435***                              |
| Fasting plasma insulin (µU/mL)        | 11.4±0.509     | 11.4±0.245                    | 2.4±0.453***        | 4.2±0.373*                         | 6.2±0.374**                        | 10.6±0.438***                       | 9.6±0.123***                               |
| Glycated hemoglobin (A1c) (%)         | 1.24±0.092     | 1.24±0.0812                   | 4.8±0.178***        | 3.96±0.238*                        | 2.82±0.861**                       | 1.52±0.0431***                      | 1.86±0.0927***                            |
| Hexokinase (µg/mg of tissue)          | 151±3.24       | 150.6±3.108                   | 88.4±1.806***       | 115.2±1.497**                      | 131.1±1.643**                      | 147.2±1.281***                      | 141.6±2.088***                            |
| Glucose-6-phosphatase (unit/mg of tissue) | 8±0.707       | 8±0.717                       | 14.4±0.927***       | 13.4±0.509ns                        | 11.4±0.432                        | 8.4±0.436**                         | 8.4±0.374***                              |
| Fructose-1, 6-bisphosphatase (unit/mg of tissue) | 26.8±1.241 | 27±1.517                     | 54.8±1.281***       | 43±1.871*                          | 38.2±1.497**                       | 30.7±0.707**                        | 31.0±0.431***                             |
| Total cholesterol (mg/dL)             | 66.2±1.715     | 66.8±1.393                    | 141.2±2.634***      | 129.8±1.53*                        | 101.8±1.934**                      | 76.2±2.01***                       | 84.2±2.302***                             |
| Triglycerides (mg/dL)                 | 78±1.975       | 78.2±1.431                    | 144.8±3.04***       | 131.1±1.789*                       | 110.2±1.828**                      | 91±1.703***                        | 98.1±1.265***                             |
| Total HDL cholesterol (mg/dL)         | 57±1.14        | 57±0.124                      | 25.4±1.208***       | 39±1.225*                          | 44.4±1.03**                       | 51.6±0.927**                       | 53.4±0.927***                             |
| Total LDL cholesterol (mg/dL)         | 6.4±0.18       | 6.8±0.431                     | 86.8±0.818***       | 64.6±0.0528*                       | 35.4±0.538**                      | 8.4±1.121***                       | 11±0.742***                               |
| Total VLDL cholesterol (mg/dL)        | 15.6±0.295     | 15.72±0.132                   | 28.96±0.608***      | 26.2±0.375*                        | 22±0.365**                        | 18.2±0.253**                       | 10.6±0.341***                             |
| Weight variation (g)                  | 212.2±1.02     | 213.4±0.92                    | 175.6±3.011***      | 193.8±2.26**                       | 199.4±1.773**                     | 211.4±1.78***                      | 210.4±1.72***                             |
| HOMA-IR                               | 2.38           | 2.32                          | 2.34                | 1.44                                | 1.71                               | 2.41                               | 2.34                                      |
| HOMA-β                                | 191.78         | 212.64                        | 2.60                | 19.89                               | 45.55                             | 131.59                            | 97.08                                     |

All values are expressed in mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001. The comparisons were carried out using ANOVA, followed by Dunnett’s multiple comparison test. aCompared to normal control, bCompared to STZ-diabetic control. HOMA-IR: Homeostasis model assessment of insulin resistance, HOMA-β: Homeostasis model assessment of β-cell function, LDL: Low-density lipoprotein, VLDL: Very low-density lipoproteins, HDL: High-density lipoprotein, STZ: Streptozotocin.
A similar trend was observed in the glibenclamide-treated group rats (Table 3).

### 3.9 Effect of MO extract on the atherogenic index and coronary risk index

Table 4 shows the atherogenic and coronary risk indices. STZ-induced diabetic rats exhibited an augmented level of atherogenic and coronary risk indices, and dose-dependent treatment with MO extract significantly reduced the atherogenic and coronary risk indices ($P < 0.001$). Glibenclamide significantly reduced the atherogenic and coronary risk indices as compared to STZ-induced rats ($P < 0.001$) (Table 4).

### 3.10 Effect of MO extract on TNF-α, CPR, and IL-6

Table 5 showed the effect of MO extract on TNF-α, CPR, and IL-6 in normal and experimental rats. Normal control and normal control receiving MO (500 mg/kg) showed almost similar levels of TNF-α, CPR, and IL-6. On the contrary, STZ-induced rats showed increased levels of TNF-α, CPR, and IL-6 and treatment with MO extract significantly reduced the level in a concentration-dependent manner ($P < 0.001$) (Table 5).

### 4 Discussion

The incidence of diabetes increased day-by-day due to a change in lifestyle and food habits. This rapid upsurge in the number of diabetic patients has encouraged scientists to find new therapeutics for the treatment of diabetes. Allopathic system of medicine is not sufficient to cure diabetes due to their limitations and side effects of those medicines. An alternative system of medicine is the best choice of medicine to successfully cure the diabetes [31]. In the current study, we scrutinized the antihyperglycemic and antihyperlipidemic effects of MO extract against STZ-induced diabetic rats. MO is a very popular herbal drug which is employed in the treatment of inflammation, but it also has the potential to be used as a new antidiabetic and antihyperlipidemic drug in the treatment of diabetes.

The evaluation of the acute oral toxicity study of the MO extract was to provide guiding ideas related to neurological, behavioral, and economical effects related to STZ-diabetic rats.
Table 4. Effect of *Moringa oleifera* Lam. extract on the atherogenic index and coronary risk index in STZ-induced diabetic rats

| Group No. | Description                        | Atherogenic index | Coronary risk index |
|----------|------------------------------------|-------------------|---------------------|
| I        | Normal control                     | 0.115             | 1.226               |
| II       | Normal control+MO (500 mg/kg)      | 0.115             | 1.242               |
| III      | STZ-diabetic control<sup>a</sup>   | 3.496***          | 5.739***            |
| IV       | STZ diabetes+MO (100 mg/kg)<sup>b</sup> | 1.641**        | 3.328**             |
| V        | STZ diabetes+MO (250 mg/kg)<sup>b</sup> | 0.775***       | 2.293***            |
| VI       | STZ diabetes+MO (500 mg/kg)<sup>b</sup> | 0.131***       | 1.426***            |
| VII      | STZ diabetes+Glibenclamide (10 mg/kg)<sup>b</sup> | 0.225***       | 1.628***            |

All values are expressed in mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001. The comparisons were carried out using ANOVA, followed by Dunnett’s multiple comparison test. <sup>a</sup>Compared to normal control, <sup>b</sup>Compared to STZ-diabetic control. STZ: Streptozotocin

Table 5. Effect of *Moringa oleifera* Lam. extract on inflammatory mediators in STZ-induced diabetic rats

| Group No. | Description                        | IL-6 (pg/ml) | TNF-α (pg/ml) | CRP (ng/ml) |
|----------|------------------------------------|--------------|---------------|-------------|
| I        | Normal control                     | 33.8±3.25    | 164.6±7.90    | 6027±423.6  |
| II       | Normal control+MO (500 mg/kg)      | 34.2±2.89    | 166±6.64      | 6007±310.2  |
| III      | STZ-diabetic control<sup>a</sup>   | 57±3.52***   | 227.4±9.86*** | 12738±870.6*** |
| IV       | STZ diabetes+MO (100 mg/kg)<sup>b</sup> | 48.6±2.51*   | 215.6±5.73*   | 10721±1086* |
| V        | STZ diabetes+MO (250 mg/kg)<sup>b</sup> | 41.8±1.28**  | 188.4±8.41*   | 8314±487.2** |
| VI       | STZ diabetes+MO (500 mg/kg)<sup>b</sup> | 31.2±2.85*** | 160.8±6.59*** | 5979±302.6*** |
| VII      | STZ diabetes+Glibenclamide (10 mg/kg)<sup>b</sup> | 31.4±2.23*** | 165.4±7.57*** | 6229±336.10*** |

All values are expressed in mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001. The comparisons were carried out using ANOVA, followed by Dunnett’s multiple comparison test. <sup>a</sup>Compared to normal control, <sup>b</sup>Compared to STZ-diabetic control. IL-6: Interleukin-6, TNF-α: Tumor necrosis factor-alpha, CRP: C-reactive protein

of the drug on the rats. Till the end of the acute toxicity study, MO extract, up to the dose of 2000 mg/kg, did not show any signs and symptoms of the toxicity. This suggests that the dose of 2000 mg/kg is safe for experimental use.

STZ (2-deoxy-2-(N-methyl-N-nitrosourea)-1-Dglucopyranose) is a cytotoxic agent obtained from the *Streptomyces achromogenes* (soil microbe) that can affect the pancreatic β-cells through glucose transporter 2, causing the induction and damage to the DNA due to the presence of the nitrourea moiety. It causes damage to the DNA, activates the poly (adenosine diphosphate-ribose) polymerase, and causes the nicotinamide adenine dinucleotide<sup>+</sup> diminution in the pancreatic β-cells. The breakage of the DNA strand leads to changes in the plasma insulin and blood glucose concentration [32]. During STZ-induced diabetes, hyperglycemia commonly occurred due to the destruction or reductions of the β-cells, resulting in a decrease in the secretion of insulin. STZ induces abnormality in the β-cells function which in turn suppresses the secretion of insulin [33]. Oral administration of the MO extract significantly decreases the blood glucose level in STZ treated rats. The possible mode of action of MO extract may stimulate the secretion of insulin from β-cells and improves the β-cells. This hypothesis was confirmed by pancreatic histopathology of the MO-treated rats which showed the protection against the pancreatic toxicity induced by the STZ. Oral glycemic drug such as glibenclamide acts on the pancreatic β-cells and increases the secretion of insulin from the pancreatic β-cells. The induction of diabetes starts with the destruction of acinar tissue, β-cells, islet of Langerhans, and exocrine function, which then induces pancreatic atrophy. Pancreatic hypertrophy is accompanied by the changes in mitochondria, Golgi bodies, and ribosomes. The histopathology of STZ-induced control group rats displayed the destruction of β-cells, and MO extract increased the insulin level, suggesting an improvement to the β-cells.

Hyperlipidemia is the common problem in diabetes. Diabetes directly or indirectly affects the pancreatic β-cells, which further reduces insulin secretion from
the β-cells and induces insulin deficiency. Insulin deficiency starts with the deposition of lipids such as TGs and cholesterol (TC) in the serum, which deranges the regulatory and metabolic processes [34]. Hypertriglyceridemia and hypercholesteremia are the major diseases which occur in DM. The alteration to the lipid profile induces the heart disease. STZ-induced rats significantly altered the lipid parameters ($P < 0.001$), suggesting the protective effect of MO against heart disease. The mechanism of action associated with this protective effect is possibly an improvement in the insulin level and the secretion of lipid from the bound form.

Diabetes is strongly correlated with oxidative stress generation. In diabetes, there is an overproduction of free radicals, which results in the development of reactive oxygen species (ROS), thus starting the reaction of oxidative stress. ROS reacts with all biological substances, such as polyunsaturated fatty acids, thus attenuating the endogenous antioxidant defense. Diabetic rats showed decreased levels of GPx, CAT, and enhanced levels of MDA. Free radicals result in an increase in the levels of MDA (a lipid peroxidation indicator) by reacting with polyunsaturated fatty acids [35]. During diabetes, the level of MDA level was significantly boosted which increases the oxidation reaction. STZ-induced diabetic rats showed the increased MDA level, and MO extract significantly decreased the MDA level, suggesting its antioxidant role. SOD and CAT are considered as the first-line endogenous antioxidant enzymes that protect the cells from oxidants. Both SOD and CAT are capable of scavenging hydrogen peroxide and superoxide radicals. During STZ-induced diabetes, the levels of first-line endogenous antioxidants such as SOD and CAT were reduced, and the treatment with MO improved the first-line antioxidant enzymes level. The mechanism of action associated with MO treatment is probably an improvement in the level of endogenous antioxidants.

STZ-induced diabetes showed an effect on various types of hepatic enzymes, including glucose-6-phosphatase, hexokinase, and fructose-1,6-bisphosphatase [36]. During diabetes, the serum enzymes directly or indirectly alter the metabolic function of hexokinase, fructose-1,6-bisphosphate, and glucose-6-phosphatase [37]. Research suggests that hepatic tissue is a vital part of the body, which plays a significant role in the regulation of various glucose-metabolizing enzymes. In diabetes, fats start depositing on the hepatic tissue and reduce the activity of hepatic enzymes. Hepatic enzymes such as hexokinase are an important enzyme that converts the glucose into the energy and glucose-6-phosphate [38]. During diabetes, the level of hexokinase reduced, and glucose converts into energy and glucose-6-phosphate. MO extract significantly increased the activity of hexokinase ($P < 0.001$), suggesting better utilization of glucose by the rodent. Glucose-6-phosphatase, another hepatic enzyme, plays an important role in the glycolysis reaction and utilization of fats and carbohydrates. Several researchers suggested that during diabetes, the level of glucose-6-phosphatase enzymes was increased, resulting in the deposition of carbohydrates and fats in the hepatic tissue and increased gluconeogenesis enzymes reaction in the hepatic tissue [39,40]. Fructose-1,6-bisphosphatase which is also a hepatic enzyme plays a significant role in the initiation of metabolic pathways and the production of glucose from non-carbohydrate sources [41,42]. The level of fructose-1,6-bisphosphatase increased considerably in diabetes due to activation of hepatic enzymes, and increased synthesis of the hepatic enzymes contributes to the enhanced glucose production. Elevated levels of hexokinase and reduced levels of fructose-1,6-bisphosphatase and glucose-6-phosphatase result in the inhibition of gluconeogenesis, glycolysis, or regulation of cyclic AMP.

5 Conclusion

In the present study, two general methods were used to determine whether the plant extract has an antidiabetic activity or not. Normoglycemic rats were used to analyze the hypoglycemic effect of the MO extract while STZ-induced diabetic rats were used to evaluate the antihyperglycemic effect of the extract. Different doses of MO extract caused a significant decline in the blood glucose levels and enhanced the level of plasma insulin. The result of this study indicates that MO extract possessed antihyperlipidemic, antioxidant, and antidiabetic activities. The present study could be beneficial for the development of the pharmaceutical drug for the treatment of type II diabetes in the future.
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Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

V.K. and M.R. conceived, designed, and performed the experiments. V.K. verified the analytical methods. V.K. and A.S.B. analyzed the data. A.S. and P.K. contributed reagents/materials/analysis tools. V.K. and M.R. wrote the paper. V.K., A.S.B., and M.R. reviewed drafts of the paper. All authors read and approved the final manuscript.

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