



## ***Moringa oleifera* Attenuates Oxidative Stress in STZ-Induced Diabetic Rats**

Sushma G<sup>\*1</sup>, Shivaprasad HN<sup>2</sup>, Nargund LVG<sup>1</sup>, Bhanumathy M<sup>1</sup>, Midhun T<sup>2</sup>

<sup>1</sup>Nargund College of Pharmacy, Dattatreya Nagar, Bangalore, Karnataka, India.

<sup>2</sup>Olive Lifesciences Pvt. Ltd., Bangalore, Karnataka, India.

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### **ABSTRACT**

Hyperglycemia in diabetes has been associated with increased formation of reactive oxygen species (ROS). Imbalance between formation and detoxification of ROS in biological systems exerts oxidative stress. Oxidative stress damages tissue compounds like DNA, protein and lipid. *Moringa oleifera* is a rich dietary source of natural antioxidants. The aim of this study is to evaluate the effect of leaves, stems and pods extracts of *M. oleifera* on lipid peroxidation, protein oxidation and antioxidant power in plasma as well as in liver in streptozotocin (STZ) induced diabetic rats. At the end of the treatment period, the levels of plasma glucose, HbA<sub>1C</sub> and Thiobarbituric acid reactive substances (TBARS) increased and free radical absorption power (FRAP) decreased in diabetic rats compared to normal rats. Administration of Moringa leaves extract (MLE), Moringa stems extract (MSE) and Moringa pods extract (MPE) for 4 weeks caused significant decrease in plasma glucose, HbA<sub>1C</sub>, plasma and liver TBARS, and an increase in levels of FRAP (both plasma and liver) in diabetic treated rats compared to untreated-diabetic rats. Phytochemical screening of the extracts revealed the presence of flavonoids, tannins, saponins, phenolic compounds and reducing sugar. Flavonoid and phenolics rich extracts MLE and MPE showed better attenuation of oxidative stress in diabetic rats. The trend was MLE>MPE>MSE. The present study confirms potential efficacy of *M. oleifera* in suppressing oxidative stress induced by hyperglycemia in rats.

### **KEYWORDS**

Oxidative stress; free radicals; Moringa; Diabetes; HbA<sub>1C</sub>; lipid peroxidation; TBARS.

### **INTRODUCTION**

Free radicals are generated as by-products of normal cellular metabolism; however, several conditions are known to disturb the balance between active oxygen species production and cellular antioxidant mechanisms. This imbalance can result in tissue injury. The level of intrinsic antioxidants critically influences the susceptibility of various tissues to oxidative stress. Beta islets are among the tissues that have lowest levels of intrinsic antioxidant defenses thus are at higher risk of tissue injury.<sup>1-4</sup>

Oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications. Enhanced oxidative stress and changes in antioxidant capacity, observed in both clinical and experimental diabetes mellitus, are thought to be the etiology of chronic diabetic complications.<sup>5</sup> Persistent hyperglycemia in diabetes causes increased production of active oxygen species, for all tissues from glucose auto-oxidation and protein glycosylation.<sup>6,7</sup>

In developing countries, metabolic disorders like diabetes and obesity are on the way to becoming major causes of morbidity and mortality as infectious diseases, due to the progressive transition in these countries to a sedentary and fast food lifestyle.<sup>8,9</sup> According to WHO projection, the prevalence of diabetes is

\*Address for Correspondence:

Sushma G

Nargund College of Pharmacy,

Banashankari III Stage,

Bangalore – 560085, Karnataka, India.

E-Mail Id: [sush.email@gmail.com](mailto:sush.email@gmail.com)

likely to increase by 35%. By 2025, world-wide diabetics are expected to cross 300 millions and India is suggested to become country with highest number of diabetics.<sup>10,11</sup> In recent years, there has been renewed interest in plant medicine for the treatment of diabetes. Most of the plants with antidiabetic activity are not edible and therefore, the studies on edible plants which have hypoglycemic effect would be of great value in dietary management of diabetes and its complications. For centuries, people in many countries have used *Moringa* as traditional medicine for common ailments. Further, scientific studies on *Moringa* have suggested its antidiabetic activity.<sup>12-16</sup> With such a great medicinal values being suggested for *Moringa* in traditional medicine, further scientific studies are very much needed.

*Moringa oleifera* Lam (syn. *M. pterygosperma*) commonly known as 'The Miracle Tree' is the best known and most widely distributed species of Moringaceae family. It is native to Western and sub-Himalayan tracts, India, Pakistan, Asia and Africa.<sup>17</sup> The antioxidant activity of *Moringa* has not been previously investigated for oxidative stress in diabetes. Thus, the purpose of the present study was to evaluate the effects of *Moringa* leaves, stems and pods extracts on oxidative stress in STZ-induced diabetic rats.

## MATERIAL AND METHODS

### Chemicals Used

All chemicals and drugs used were obtained commercially and of analytical grade.

### Preparation of Extract

The leaves, stems and pods of *Moringa oleifera* were purchased from local market and authenticated by IIHR, Bangalore. Plant material was washed properly and dried in shade. The dried samples were extracted with distilled water in round bottom flask under reflux condenser. The extracts were collected at least three times and were filtered and then concentrated on water bath followed by complete drying under vacuum for 25 minutes. Dried material was kept at 4°C until used for

study. The plant material and solvent mass ratio was 1:2.5 during extraction.

### Phytochemical Screening

A preliminary phytochemical screening of the leaves, stems and pods extracts of *Moringa* were done using standard methods of analysis.<sup>18</sup>

### Animals

The study was performed on matured normoglycemic male Wistar rats, weighing 180-200 g, which were separately housed in cages. Animals were maintained in a room at 23 ± 2°C, humidity 45-55% with a fixed 12 h artificial light and dark period and allowed to eat and drink *ad libitum*. Rats were fed with standard rodent diet until initiation of treatment. All animals received humane care, as outlined in the guide for the care and use of laboratory animals.

### Induction of Diabetes in Animals

Diabetes was induced by a single intraperitoneal administration of STZ (65mg/Kg b. w.) in 0.15 M NaCl with 100 mM sodium citrate buffer (pH 4.5). Control rats received the vehicle alone. After 5 days of development of diabetes, the rats with plasma glucose more than 200 mg/dl were considered as diabetic rats and used for experiment.

### Experimental Design and Treatment

Forty eight rats were divided into eight groups: (1) Non-diabetic control group: Rats (n=6) received normal saline containing 100 mM sodium citrate; (2) Diabetic group: Rats (n=6) received a single i.p. administration of STZ; (3) Treated-diabetic groups: rats (n=6) received a single i.p. administration of STZ and 250 mg and 500 mg of *Moringa* leaves extract (MLE), *Moringa* stems extract (MSE) or *Moringa* pods extract (MPE), 5 days after administration of STZ. The experiment was carried out for 4 weeks after the initiation of treatment. Body weight and food intake of all groups were measured at the end of experiment period. At the end of the treatment period overnight-fasted rats were anesthetized under light ether and blood samples were collected from tip of the tail vein. Blood sample was collected in EDTA

tubes for determination of HbA<sub>1C</sub> and preparation of plasma.

### Isolation of Liver Tissue

After blood collection, animals were sacrificed by anesthesia and livers were removed and rinsed of any adhering blood. Then, livers were quickly sliced, and fragments were homogenated in appropriate buffers.

### Liver Homogenization for Evaluation of Oxidative Stress

For TBARS and FRAP assay, a fraction of liver was homogenized (1:10, w/v) in cold 1.15% KCl and 0.05 M sodium phosphate buffer pH 7.4, respectively. Homogenates were centrifuged at 6000g for 20 min at 4°C. Evans et al method was followed for preparing tissue homogenates for protein carbonyl assay.<sup>19</sup> The resulting supernatants were used for biochemical assays.

### Assays

Determination of blood glucose levels was done by Glucose oxidase standard method.<sup>20</sup> HbA<sub>1C</sub> was estimated as per colorimetric method.<sup>21</sup> Spectroscopic method was used for the estimation of lipid peroxidation as per Uchiyama et al.<sup>22</sup> TBARS in plasma and liver was expressed in nmol/ml and nmol/mg of protein, respectively. FRAP was used to determine antioxidant power of plasma and liver. FRAP assay was performed according to the method as described by Benzie et al.<sup>23,24</sup> The method is based on the reduction of the Fe<sup>3+</sup>-TPTZ [2, 4, 6-tri-(2-pyridyl)-s-triazine] complex to the ferrous form at low pH. FRAP value in plasma and liver was expressed as μmoles/L and μmoles/mg of protein, respectively. Protein concentration was determined by Bradford's method using bovine serum albumin as standard.<sup>25</sup>

Dinitrophenylhydrazine (DNPH) reagent and spectrophotometric method was used for the measurement of protein carbonyls in plasma and liver homogenate.<sup>26</sup> The results were expressed as nanomoles of carbonyl groups per milligram of protein using a molar extinction coefficient of

22,000 M<sup>-1</sup> cm<sup>-1</sup>. Protein contents were determined on the HCl blank pellets using a bovine serum albumin standard curve in guanidine-HCl and reading the absorbance at 280 nm.

### STATISTICS

Results were expressed as mean ± SEM for six rats in each experimental group. Statistical analysis was performed using Graph Pad Prism 4 software. One way ANOVA followed by Tukey's post hoc test was used to compare differences between experimental groups. The criterion for statistical significance considered P<0.05.

### RESULTS

Results of the preliminary phytochemical screening of *M. oleifera* extracts revealed the presence of flavonoids, polyphenols, tannins, saponins and reducing sugars. Flavonoids were rich in leaves and pods extracts compared to stems extract. Saponins were high in pods (Table 1).

Table 1: Phytochemical screening of different *M. oleifera* extracts

Test	MLE	MSE	MPE
Reducing sugars	+	+	+
Saponins	++	+	+++
Tannins	++	+	++
Flavonoids	+++	++	+++
Glycosides	+	--	--
Alkaloids	--	--	--
Polyphenols	+++	++	+++

MLE: *M. oleifera* leaves extract; MSE: *M. oleifera* stems extract; MPE: *M. oleifera* pods extract.

Table 2: Effect of Moringa extracts on food intake, body weight, plasma glucose and HbA<sub>1C</sub> of rats in different experimental groups

	<b>Food intake (g/day)</b>	<b>Body weight (gm)</b>	<b>Plasma glucose (mg/dl)</b>	<b>HbA<sub>1C</sub> (%)</b>
Control	20.9±3.7	290±13.4	150±20.4	6.8±0.44
Diabetic	47.7±2.6 <sup>##</sup>	180±15.5 <sup>#</sup>	280±22.6 <sup>#</sup>	13.3±0.94 <sup>##</sup>
MLE (250 mg/kg)	24.7±3.2 <sup>***</sup>	277±17.5 <sup>**</sup>	168±17.4 <sup>**</sup>	7.5±1.2 <sup>***</sup>
MLE (500 mg/kg)	23.9±3.4 <sup>***</sup>	280±14.9 <sup>**</sup>	160±23.2 <sup>**</sup>	7.1±0.68 <sup>***</sup>
MSE (250 mg/kg)	34.2±3.5	260±13.1 <sup>*</sup>	186±19.5 <sup>*</sup>	8.6±0.94 <sup>*</sup>
MSE (500 mg/kg)	30.6±3.7 <sup>*</sup>	268±19.6 <sup>*</sup>	183±18.7 <sup>*</sup>	8.3±1.05 <sup>*</sup>
MPE (250 mg/kg)	28.5±2.9 <sup>**</sup>	276±19.6 <sup>**</sup>	177±19.9 <sup>*</sup>	8.0±0.72 <sup>**</sup>
MPE (500 mg/kg)	26.8±2.8 <sup>**</sup>	279±20.3 <sup>**</sup>	175±20.4 <sup>*</sup>	7.8±0.91 <sup>**</sup>

MLE: *M. oleifera* leaves extract; MSE: *M. oleifera* stems extract; MPE: *M. oleifera* pods extract. The values represent the mean ±SEM for six rats per group. Comparisons were made by one-way ANOVA test followed by Tukeys post hoc test. <sup>#</sup>P<0.01 compared to control group <sup>##</sup>P<0.001 compared to control group; <sup>\*</sup>P<0.05 compared to diabetic group. <sup>\*\*</sup> P<0.01 compared to diabetic group, <sup>\*\*\*</sup>P<0.001 compared to diabetic group.

Table 3: Effect of Moringa extracts on plasma and liver TBARS and PC in rats of different experimental groups

	<b>TBARS (nmol/ml)</b>	<b>TBARS in liver (nmol/mg protein)</b>	<b>Protein carbonyl (nmol/mg protein)</b>	<b>Protein carbonyl in liver (nmol/mg protein)</b>
Control	1.6±0.19	0.75±0.02	0.81±0.04	1.65±0.16
Diabetic	2.8±0.2 <sup>##</sup>	1.0±0.05 <sup>##</sup>	1.3±0.06 <sup>##</sup>	2.52±0.12 <sup>##</sup>
MLE (250 mg/kg)	2.0±0.15 <sup>**</sup>	0.80±0.03 <sup>**</sup>	0.89±0.05 <sup>***</sup>	1.82±0.11 <sup>**</sup>
MLE (500 mg/kg)	1.8±0.13 <sup>***</sup>	0.77±0.04 <sup>***</sup>	0.87±0.07 <sup>***</sup>	1.79±0.13 <sup>**</sup>
MSE (250 mg/kg)	2.1±0.10 <sup>*</sup>	0.85±0.01 <sup>*</sup>	0.95±0.08 <sup>**</sup>	2.02±0.14
MSE (500 mg/kg)	2.0±0.13 <sup>**</sup>	0.82±0.02 <sup>**</sup>	0.91±0.07 <sup>**</sup>	1.88±0.18 <sup>*</sup>
MPE (250 mg/kg)	2.1±0.11 <sup>*</sup>	0.81±0.04 <sup>**</sup>	0.91±0.06 <sup>**</sup>	1.87±0.13 <sup>*</sup>
MPE (500 mg/kg)	1.9±0.1 <sup>**</sup>	0.80±0.03 <sup>**</sup>	0.89±0.05 <sup>***</sup>	1.80±0.12 <sup>**</sup>

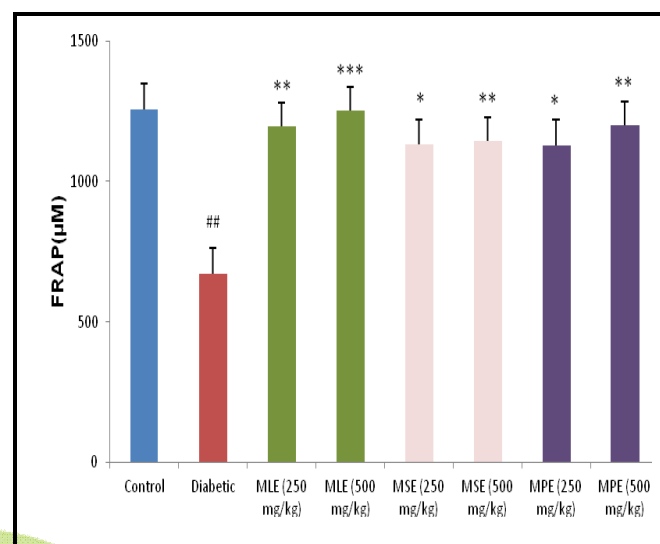
MLE: *M. oleifera* leaves extract; MSE: *M. oleifera* stems extract; MPE: *M. oleifera* pods extract. The values represent the mean ±SEM for six rats per group. Comparisons were made by one-way ANOVA test followed by Tukey's post hoc test. <sup>##</sup>P<0.001 compared to control group; <sup>\*</sup>P<0.05 compared to diabetic group. <sup>\*\*</sup> P<0.01 compared to diabetic group, <sup>\*\*\*</sup>P<0.001 compared to diabetic group.

Table 2 shows body weight, food intake, plasma glucose concentration and HbA<sub>1C</sub> in different experimental groups. Diabetic untreated rats showed significant weight loss (P<0.01) and polyphagia (P<0.001) compared to control group at the end of the treatment period. Administration of Moringa extracts (MLE, MSE and MPE) significantly reduced the body weight loss and polyphagia in treated-diabetic rats compared with untreated-diabetic rats. Both 250 mg and 500 mg of MLE and MPE showed significant (P<0.01) weight increase compared to untreated-diabetic rats. Whereas, MSE showed P<0.05 significant increase in weight compared to untreated-diabetic rats. Polyphagia was reduced significantly in all the treated groups compared to untreated group; MLE (P<0.001), MPE (P<0.01) and MSE (P<0.05). Significant reduction in plasma glucose concentration and HbA<sub>1C</sub> levels were observed after administration of Moringa extracts in diabetic rats (Table 2). Trend was MLE > MPE ≈ MSE effective on plasma glucose level. The efficacy was in the order MLE (P<0.001) > MPE (P<0.01) > MSE (P<0.05) on HbA<sub>1C</sub> level.

Table 3 shows concentration of TBARS in plasma and liver of control and diabetic animals. Diabetes caused a significant increase in TBARS concentration in plasma and liver compared to the control rats (P<0.001). Significant amelioration in lipid peroxidation in plasma and liver were noted in Moringa treated rats; MLE (P<0.001) being highly effective followed by MPE ≈ MSE. Protein carbonyl (PC) concentration in plasma and liver of all animal groups are also indicated in Table 3. The level of PC was increased in untreated-diabetic rats compared to control (P<0.001). MLE showed significant (P<0.001; P<0.01) inhibition of protein oxidation in plasma and liver proteins. Efficacy was in the order MLE > MPE > MSE. Dose-response effects on test parameters were observed.

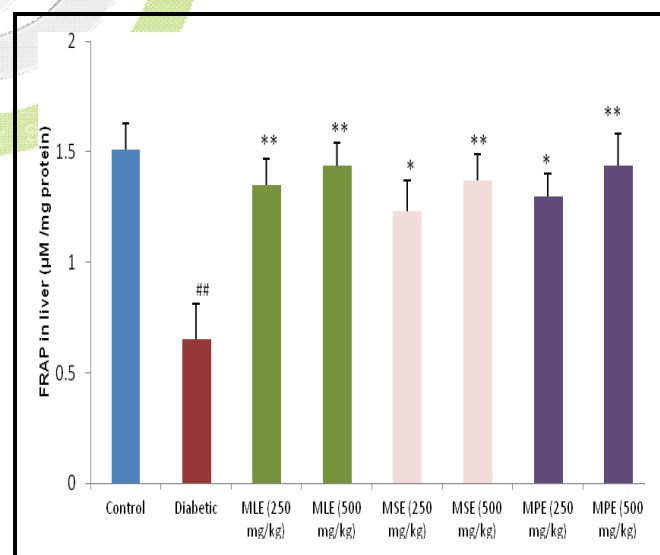
Effect of Moringa extracts (MLE, MSE and MPE) on FRAP antioxidant power in plasma and liver is shown in Figure 1 and Figure 2. Antioxidant power was significantly decreased in untreated-diabetic rats (P<0.001). FRAP of

the extracts was in the increasing trend with the concentrations in treated diabetic rats. The MLE (P<0.001) showed greater efficiency than MPE (P<0.01) ≈ MSE (P<0.01).



MLE; *M. oleifera* leaves extract; MSE; *M. oleifera* stems extract; MPE; *M. oleifera* pods extract. The values represent the mean ±SEM for six rats per group. Comparisons were made by one-way ANOVA test followed by Tukey's post hoc test. ##P<0.001 compared to control group; \*P<0.05 compared to diabetic group. \*\* P<0.01 compared to diabetic group, \*\*\*P<0.001 compared to diabetic group.

Figure 1: Effect of Moringa extracts on plasma FRAP in rats of different experimental groups



MLE; *M. oleifera* leaves extract; MSE; *M. oleifera* stems extract; MPE; *M. oleifera* pods extract. The values represent the mean ±SEM for six rats per group. Comparisons were made by one-way ANOVA test followed by Tukey's post hoc test. ##P<0.001 compared to control group; \*P<0.05 compared to diabetic group. \*\* P<0.01 compared to diabetic group, \*\*\*P<0.001 compared to diabetic group.

Figure 2: Effect of Moringa extracts on liver FRAP in rats of different experimental groups

## DISCUSSION

STZ induced diabetic model is a very commonly used model for diabetes induction in animals. STZ model represents most of the diabetic complication.<sup>27</sup> Advanced glycation end products commonly known as AGE are labile Schiff bases formed as a result of reaction of glucose with various proteins such as hemoglobin, albumin, collagen, low density lipoprotein, or crystalline proteins. It has been noted that patients with uncontrolled or poorly controlled diabetes have higher HbA<sub>1C</sub> level and the increase is proportional to the hyperglycemia status.<sup>28</sup> In diabetes it has been evident that glycation itself induces active oxygen species formation, and the level of HbA<sub>1C</sub> is a good indicator of oxidative stress in diabetes mellitus.<sup>29</sup> Thus, HbA<sub>1C</sub> level in plasma is a very sensitive index for glycemic control. Several studies have confirmed the anti-hyperglycemic activity of *Moringa* extracts (Jaiswal, Ndong, William, Kumari, Ghiridhari). Current study showed significant increase in HbA<sub>1C</sub> in diabetic rats compared to control rats. Treatment with *Moringa* significantly ( $P < 0.01$ ) reduced of HbA<sub>1C</sub> levels in treated-diabetic rats that could be due to an improvement in hyperglycemia. On the other hand, STZ induced diabetic rats were characterized by loss in body weight, and polyphagia. The decrease in body weight observed in uncontrolled diabetic might be the result of protein wasting due to unavailability of carbohydrate for utilization as an energy source. Body weight enhanced significantly ( $P < 0.01$ ) in *Moringa* leaves treated diabetic rats when compared with untreated-diabetic ones. Likewise, *Moringa* leaves decreased significantly ( $P < 0.001$ ) polyphagia in treated-diabetic rats when compared with untreated-diabetic group. *Moringa* stems and pods also showed increase in body weight and decrease polyphagia but the efficacy was high in *Moringa* leaves extract.

Increased lipid peroxidation has been associated with chronic hyperglycemia due to increased carbonyl stress.<sup>30</sup> Increased lipid peroxidation causes increased peroxy radical and hydroxyl radical formation. Thus, lipid peroxidation

induced oxidative stress is one of the characteristics features of chronic uncontrolled diabetes. TBARS is the most common indicator for lipid peroxidation.<sup>31</sup> Significant ( $P < 0.001$ ) increase in TBARS level in plasma and liver homogenate were observed in diabetic rats in the present study. The increase in TBARS levels was may be due to the increased lipid peroxidation in diabetic rats. Administration of *Moringa* extracts decreased TBARS significantly in treated diabetic rats when compared with untreated-diabetic rats. Inhibition of lipid peroxidation was high in MLE followed by MPE and MSE, respectively. Oxidative stress in diabetes coexists with a reduction in the antioxidant power.<sup>32</sup> Cakatay et al also indicated that total antioxidant capacity (FRAP) levels in plasma of chronic diabetic animals were decreased significantly as compared to those of control animals.<sup>33</sup> The present work, indicated the similar results and showed significant ( $P < 0.001$ ) reduction in plasma and liver homogenate FRAP of diabetic rats when compared with control animals. Treatment with *Moringa* extract (MLE>MPE>MSE) improved significantly antioxidant power in treated-diabetic rats when compared with untreated-diabetic group. Elevated protein carbonyl (PC) levels have been detected in diabetes.<sup>34,35</sup> High plasma PC levels in diabetic children and adolescents without complications compared with control subjects indicate that oxidative protein damage occurs at the onset of disease and tends to increase in the later stages. Furthermore, decreased antioxidant defenses might increase the susceptibility of diabetic patients to oxidative injury.<sup>36</sup> The results of the present study also showed that tissue and plasma PC levels were increased in untreated diabetic rats compared to controls ( $P < 0.001$ ) and *Moringa* extracts showed improvement in protein oxidation.

Polyphenols are important secondary metabolites present in plants and are also responsible for their antioxidant action and various beneficial effects in a multitude of diseases. Based on C-skeleton, polyphenols are classified as flavonoids and phenolic acids.

Flavonoids are secondary metabolites characterized by flavan nucleus and C<sub>6</sub>-C<sub>8</sub>-C<sub>6</sub> carbon-skeleton. Flavonoids have been reported to exert wide range of biological activities. These includes: anti-inflammatory, antibacterial, antiviral, antiallergic, cytotoxic antitumor, treatment of neurodegenerative diseases, vasodilatory action. In addition flavonoids are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyclo-oxygenase and lipoxygenase enzyme activities. They exert these effects as antioxidants, free radical scavengers, chelators of divalent cation. These are also reported to inhibit variety of enzymes like hydrolases, hyaluronidase, alkaline phosphatase, arylsulphatase, cAMP phosphodiesterase, lipase,  $\alpha$ -glucosidase, kinase.<sup>37</sup> In the present study water extracts of *Moringa* (MLE, MSE and MPE) were found to contain phytochemicals like flavonoids, phenolics, saponins, tannins and reducing sugars. MLE and MPE were rich in flavonoids and polyphenols. Saponins were present in higher concentration in MPE. The result show MLE to be more effective in all the parameters tested compared to other two extracts of *Moringa*. The higher efficacy of MLE may be due to higher concentration of antioxidants like flavonoids and polyphenolic compounds compared to MPE and MSE. Scientific studies on herbs containing flavonoids, tannins and phenolic compounds have demonstrated antioxidant property and attenuation of cell death induced by oxidative stress which supports our present findings. Results of the present study indicate that the beneficial activity of *Moringa* extracts in diabetes and its complication may be due to its antioxidant potential. Further studies are required to isolate, identify and structure elucidation of constituents responsible for its pharmacological activities.

## CONCLUSION

The present study showed that water extract of *Moringa oleifera* leaves, stems and pods attenuated oxidative stress in STZ-induced diabetic rats, which suggest the presence of biologically active antioxidant components

which may be worth further investigation and elucidation. The effective dose was found to be 250 mg/kg b.w. for leaves and pods where as 500 mg/kg b.w. for stems.

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