ANTIDIABETIC AND ANTIOXIDANT POTENTIAL OF HYDROALCOHOLIC EXTRACT OF MORINGA OLEIFERA LEAVES IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

Ram Niwas Jangir and Gyan Chand Jain*

Center for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur-302004 (India).

Corresponding Author: Prof. Gyan Chand Jain
Center for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur-302004 (India).

ABSTRACT
The present study aimed to evaluate antidiabetic and antioxidant activities of 70% ethanolic extract of *Moringa oleifera* leaves (MOLEt) in streptozotocin-induced diabetic rats. Diabetes was induced in male Wistar rats by single intraperitoneal injection of streptozotocin (60 mg/kg b.wt.). The diabetic rats were administered orally with MOLEt at three different doses (100, 250 and 500 mg/kg b.wt./day) for 60 days. The results were compared with glibenclamide (5 mg/kg b.wt./day) treated rats. The streptozotocin treated control rats showed a significant increase in fasting blood glucose and glycosylated hemoglobin (HbA1c) with a concomitant decrease in serum insulin level and glycogen content in liver. Moreover, the levels of lipid peroxide (TBARS) in pancreatic tissue were significantly increased with a concurrent decline in antioxidant markers (SOD, CAT, GSH and ascorbic acid). The histopathological picture of the pancreatic islets showed degenerative and atrophic changes. Oral administration of MOLEt (100, 250 and 500 mg/kg b.wt./day) or glibenclamide for 60 days showed significant reduction in blood glucose and HbA1c levels and an elevation in serum insulin and hepatic glycogen levels. The extract treatment also reduced TBARS levels and improved the levels of antioxidant markers in the pancreas. The histomorphological picture of pancreas in MOLEt treated diabetic rats showed marked sign of amelioration. These results were comparable to reference drug, glibenclamide. The results of present study showed that MOLEt possesses significant antidiabetic and antioxidant activities.


INTRODUCTION
Diabetes mellitus (DM) is a chronic complicated metabolic disorder characterized by hyperglycemia, which often results from defects in insulin secretion, insulin action, or both. Moreover, DM is associated with severe disturbances of carbohydrate, fat, and protein metabolism. The incidence of DM is rising rapidly also including children and young persons of reproductive age. According to the diabetic atlas of the International Diabetic Federation, 382 million people were affected by diabetes worldwide in the year 2013 and diabetes prevalence is expected to reach 592 million by the year 2035. About 80% of them live in low- and middle-income countries.

To date there are different groups of oral hypoglycemic agents for clinical use, having characteristic profile of side effects. Insulin therapy affords effective glycemic control, yet its demerits are ineffectiveness through an oral administration, short half life and in the event of excess dosage, results fatal hypoglycemia. According to World Health Organization (WHO) 80% of the population in developing countries depends almost entirely on traditional medicine practices and herbal medicines for their primary health care needs. The use of plants in the management of diabetes is well documented and medicinal plants provide better alternatives as they are less toxic, easily available and affordable.

*Moringa oleifera* lam (family- Moringaceae) is a fast growing tree commonly known as Durmstick tree or Horse radish tree. It is also known as Miracle tree, Tree of life, Mother’s best friend, Savior of the poor and God’s gift to man. Nearby every part of this plant including root, bark, gum, leaf, fruit (pods), flowers, seeds and seed oil have been used for the treatment of various ailments in the indigenous system of medicine. *Moringa oleifera* has been reported to possess various pharmacological activities such as, analgesic, anti-asthmatics, anticalcancer, antifertility, antifungal, antihelminthic, antihyperlipidemic, anti-inflammatory, antioxidant, antipyretic, antimicrobial, antiulcer, antiurolithiatic.

Moringa oleifera leaves act as a good source of iron, calcium, phosphorus, copper, vitamins A, B and C, α-tocopherol, riboflavin, β-carotene, β-sitosterol, and essential amino acids. [33] Moreover, its leaves contain several phytochemicals such as two nitrile glycosides, niazirin and niazirinin and three mustard oil glycosides, 4-[(4′-O-acetyl-α-L-rhamnosyl)oxy] benzyl] isothiocyanate, niaziminin A and B, α-L-rhamnosides of 4-hydroxy-benzyl compounds with nitrile, carbamate and thiocarbamate groups, flavonoids, anthocyanins, proanthocyanidin and cinnamates, quercetin-3-O-glucoside and quercetin-3-O-(6′-malonyl-glucoside) and lower amounts of kaempferol-3-O-glucoside and kaempferol-3-O-(6′- malonyl-glucoside). The leaves also contained 3-cafeoylquinic (chlorogenic acid), 5-cafeoylquinic acid and benzyl amine (moringinine).[10, 34, 35]

Antihyperglycemic activity of different parts of Moringa oleifera has been reported in various diabetic animal models and in human patients. [36, 37, 38, 39, 40, 41] but except few, most of them are either acute or short duration studies.

Present study aimed to investigate the effects of 70% ethanolic extract of Moringa oleifera leaves (MOLEt) on fasting blood glucose, serum insulin, HbA1c and hepatic glycogen levels as well as lipid peroxidation (TBARS) and antioxidant defence marker parameters and histomorphological picture of pancreas in STZ-induced diabetic rats.

MATERIAL AND METHODS

Plant material and preparation of extract

Fresh young leaves of Moringa oleifera were collected from the adjacent areas of University of Rajasthan, Jaipur in the month of January –February, 2013. The plant was taxonomically identified by Prof. K.P. Sharma, Incharge, Herbarium, Department of Botany, University of Rajasthan, Jaipur, India where a voucher specimen (Specimen no. RUBL21056) was deposited. The fresh leaves were washed with distilled water, shade dried. The dried plant material was pulverized into coarse powder. The powder (250 g) was suspended in 70% ethanol and allowed to stand for 24 h. The mixture was subjected to Soxhlet apparatus for extraction at 60°C-70°C for 35 h. It was then filtered using a filter paper and the filtrate was evaporated to dryness in an oven at 40°C. A crystalline brownish residue weighing 37.5 g (15% of dried powder) was obtained. This was kept in an air tight bottle in a refrigerator until used. The extract was suspended in water before administering to experimental animals.

Animals

Colony bred, adult, healthy, male rats of Wistar strain (Rattus norvegicus) weighing 170-200 g were used in the present study. The animals were housed in polypropylene cages under standard husbandry conditions (12 hrs light / dark cycle; 25±3°C temperature). Rats were provided with water and nutritionally adequate pellet diet (Aashirwad Food Industries, Chandigarh, India) ad libitum. The animals were maintained as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study was approved by the Animal Ethical Committee of the Centre of Advanced studies, Department of Zoology, University of Rajasthan, Jaipur (India).

Chemicals

streptozotocin was obtained from Himedia Laboratory limited, Mumbai, India. Glibenclamide tablets (Daonil; Aventis Pharma, Ltd., India) were purchased from the medical store. All other chemicals and reagents used were of analytical grade.

Experimental induction of diabetes

Diabetes mellitus was induced by a single intraperitoneal injection of streptozotocin (STZ) dissolved in citrate buffer (pH 4.5) at a dose of 60 mg/kg body weight into overnight fasted rats. The STZ treated animals were given 2% glucose solution for 24 hours after 5 hours of STZ injection to prevent initial drug induced hypoglycemic mortality. Development of diabetes was verified after one week of STZ injection by measuring the blood glucose level in blood samples obtained from the tail vein of overnight fasted rats. The rats having blood glucose level above 250 mg/dl were considered to be diabetic and used in the study. This day was considered as the zero (0) day of the experiment.

Experimental design

The rats were divided into six different groups, each consisting of six animals and treated as follows.

- **Group I**: Control rats receiving vehicle (0.5 ml distilled water /rat/day) orally for 60 days.
- **Group II**: Diabetic rats receiving vehicle (0.5 ml distilled water /rat/day) orally for 60 days.
- **Group III**: Diabetic rats receiving M. oleifera extract (100 mg/kg bwt./day) orally for 60 days.
- **Group IV**: Diabetic rats receiving M. oleifera extract (250 mg/kg bwt./day) orally for 60 days.
- **Group V**: Diabetic rats receiving M. oleifera extract (500 mg/kg bwt./day) orally for 60 days.
- **Group VI**: Diabetic rats receiving glibenclamide standard drug (5mg/kg bwt./day) orally for 60 days.

After 24 hours of the last treatment, all the overnight fasted animals of different groups were weighed and autopsied under mild ether anesthesia. Blood was collected directly by cardiac puncture of which 2 ml. was added to an anticoagulant vial for the estimation of parameters in blood. Rest of the samples were allowed to
clot at 37°C and the serum was separated by centrifugation at 3000 rpm for 20 min and stored at -20°C until assayed.

**Body weight and blood glucose**

Body weights of all rats were recorded at 15 day interval for a period of 60 days and after overnight fasting blood samples were collected from the tail vein and blood glucose was determined at 0, 15, 30, 45 and 60 days using a glucometer (One Touch Ultra blood monitoring system from Life Scan, Johnson and Jonsson Company, California, USA).

**Oral glucose tolerance test (OGTT)**

At the 55 days of experimental period oral glucose tolerance test (OGTT) was performed in control, diabetic, *M. oleifera* high dose (500 mg/kg) and glibenclamide group animals. After overnight fasting blood glucose level was estimated (0 minutes). Without delay, a glucose solution at a dose of 2 gm/kg body weight was administered orally. Blood samples were taken at 30, 60, 90 and 120 minutes after glucose administration and blood glucose level was estimated.

**Glycosylated hemoglobin, Hemoglobin and Insulin**

Glycosylated hemoglobin (HbA1c) was estimated by glycohemoglobin reagent set (Accurex Biomedical Pvt. Ltd. Mumbai, India) and total hemoglobin (Hb) concentration by using Sahli’s apparatus. Serum Insulin level was analysed through chemiluminescence in fully automatic Advia Centaur Immuno Assay System.

**Tissue Biochemistry**

Pancreatic tissue samples were carefully dissected out and stored at -70°C until biochemical analysis for total protein, lipid peroxidation assay (TBARS), superoxide dismutase (SOD) activity, catalase (CAT) activity, glutathione (GSH) and ascorbic acid levels. Quantitative estimations of glyco- and catalase activity was also made in the frozen liver samples.

**Histopathological study**

Pancreatic tissue was dissected out, washed in ice cold saline, fixed in Bouin’s fixative and processed through an ascending series of ethanol and cleared in xylene. The tissues were then embedded in paraffin wax and 5 μm thick sections were cut, stained with haematoxylin and eosin and observed under light microscope for histopathological changes.

**Statistical analysis**

All the data were calculated and statistically analyzed with SPSS 20.0 computer software package for windows (SPSS INC., Chicago, IL, USA). The data were expressed as mean ±SEM and tested for variance. All the data statistically analyzed with one way ANOVA followed by Tukey’s as a post hoc test. Differences in means were considered significant at p<0.05.

**RESULTS**

**Body weight**

Changes in body weight of control and experimental rats are depicted in Table 1. Mean body weight of rats of control group was significantly elevated by 7.22% (P≤0.05), 10.12% (P≤0.01), 16.65% (P≤0.001) and 23.34% (P≤0.001) respectively after the 15, 30, 45 and 60 days of treatment period when compared to their initial body weight. In contrast to this, the mean body weight of untreated diabetic rats (group II) significantly decreased by -5.57%(P≤0.05), -9.38%(P≤0.001), -13.47%(P≤0.001) and -15.61%(P≤0.001) respectively after the 15, 30, 45 and 60 days of experimental period compared with their initial body weight (0 day).

Diabetic rats treated with MOLEt at different doses (100, 250 and 500 mg/kg b.wt./day) showed duration dependent increase in the mean body weight. At lower dose treatment (group III) significant gain in body weight was observed (6.43%, P≤0.01) only after 60 days period. In medium dose group (group IV) a significant gain of 8.48% (P≤0.05) and 11.21% (P≤0.01) in body weight was observed after both 45 and 60 days of treatment. In highest dose group (500 mg/kg b.wt.), 7.49% (P≤0.01), 12.21% (P≤0.001) and 14.79% (P≤0.001) gain in body weight was observed respectively after 30, 45, and 60 days of treatment compared with their initial body weight (0 day). In diabetic rats treated with glibenclamide (group VI), the body weight gain recorded was 7.71% (P≤0.05), 10.25% (P≤0.01) and 14.29% (P≤0.001) respectively after 30, 45 and 60 days of treatment as compared with their initial body weight (0 day).

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Table 1: Effects of *M. oleifera* (MOLEt) on body weight in STZ-induced diabetic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>STZ Treatment Day</th>
<th>0th day</th>
<th>15th Day</th>
<th>30th Day</th>
<th>45th Day</th>
<th>60th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II</td>
<td>192.50±2.14</td>
<td>179.33±2.29</td>
<td>169.33±1.96*(+5.57)</td>
<td>162.50±1.78*(+9.38)</td>
<td>155.17±1.68*(-13.47)</td>
<td>151.33±3.23*(-15.61)</td>
</tr>
<tr>
<td>Group III</td>
<td>191.33±2.18</td>
<td>178.83±2.14</td>
<td>181.17±2.06*(+3.30)</td>
<td>183.00±1.90*(+2.23)</td>
<td>186.17±2.04*(+4.10)</td>
<td>190.33±2.29*(+6.43)</td>
</tr>
<tr>
<td>Group IV</td>
<td>189.17±3.10</td>
<td>176.83±2.91</td>
<td>180.67±2.98*(+2.17)</td>
<td>186.33±3.79*(+5.53)</td>
<td>191.83±3.85*(+8.48)</td>
<td>196.67±4.01*(+11.21)</td>
</tr>
<tr>
<td>Group V</td>
<td>194.83±2.69</td>
<td>180.17±2.23</td>
<td>185.83±2.36*(+3.14)</td>
<td>193.67±2.20*(+7.49)</td>
<td>202.17±2.81*(+12.21)</td>
<td>206.83±2.82*(+14.79)</td>
</tr>
<tr>
<td>Group VI</td>
<td>188.00±2.44</td>
<td>177.17±3.02</td>
<td>183.17±2.76*(+3.38)</td>
<td>190.83±2.61*(+7.71)</td>
<td>195.33±3.57*(+10.25)</td>
<td>202.50±4.25*(+14.29)</td>
</tr>
</tbody>
</table>

Level of significance: Value in parenthesis indicate % change Values represent mean ± SEM (n=6)  
ns = non significant; a = P ≤ 0.05; b = P ≤ 0.01; c = P ≤ 0.001, Normal, diabetic control, extract and glibenclamide treated rats compared with corresponding values on 0 day.

Blood glucose

Changes in fasting blood glucose level in normal control and experimental rats are depicted in Table 2. Normal control rats (group I) showed sustained blood glucose levels throughout the experimental period. In contrast to this, continues increase in fasting blood glucose levels was recorded in untreated diabetic control rats (group II) by 5.42%, 9.44% and 13.27% (P<0.05) and 16.40% (P<0.01) respectively after 15, 30, 45 and 60 days of treatment as compared with corresponding values on 0 day.

The blood glucose levels in diabetic rats treated with lower dose of MOLEt (group III) was significantly (P ≤ 0.01) reduced by -30.45%, -37.31% and -41.03% respectively after 30, 45 and 60 days of treatment. In medium dose treatment group (group IV), the fasting blood glucose level was significantly declined by -25.22%(P ≤ 0.01), -42.31%(P ≤ 0.001), -45.86%(P ≤ 0.001) and -49.15%(P ≤ 0.001) respectively after 15, 30, 45 and 60 days of treatment compared with corresponding values on 0 day.

After treatment of diabetic rats with glibenclamide (group VI), the fasting blood glucose level also reduced significantly (P ≤ 0.001) by -38.77%, -53.34%, -61.32 and -62.84% respectively after 15, 30, 45 and 60 days of treatment as compared with corresponding values on 0 day.

Table 2: Effects of *M. oleifera* (MOLEt) on fasting blood glucose in STZ-induced diabetic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>STZ treatment day</th>
<th>0th day</th>
<th>15th day</th>
<th>30th day</th>
<th>45th day</th>
<th>60th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>81.66±1.47</td>
<td>84.17±1.92</td>
<td>82.50±2.29*(-1.98)</td>
<td>85.33±1.84*(+1.38)</td>
<td>83.00±3.10*(-1.39)</td>
<td>81.50±1.67*(+3.17)</td>
</tr>
<tr>
<td>Group III</td>
<td>80.17±1.76</td>
<td>321.33±12.73</td>
<td>263.33±14.79*(-18.05)</td>
<td>223.50±17.67*(-30.45)</td>
<td>199.83±12.43*(-37.81)</td>
<td>189.50±12.16*(-41.03)</td>
</tr>
<tr>
<td>Group IV</td>
<td>83.00±2.41</td>
<td>323.83±13.01</td>
<td>242.17±17.99*(-25.22)</td>
<td>186.83±14.52*(-42.31)</td>
<td>175.33±9.50*(-48.86)</td>
<td>164.67±9.70*(-49.15)</td>
</tr>
<tr>
<td>Group V</td>
<td>82.17±1.64</td>
<td>338.17±13.60</td>
<td>220.50±10.43*(-34.80)</td>
<td>171.17±12.68*(-49.38)</td>
<td>158.33±14.50*(-53.18)</td>
<td>137.33±12.73*(-59.39)</td>
</tr>
<tr>
<td>Group VI</td>
<td>78.67±2.25</td>
<td>331.83±14.03</td>
<td>203.17±14.99*(-38.77)</td>
<td>154.83±13.83*(-53.34)</td>
<td>128.33±9.59*(-61.32)</td>
<td>119.33±10.39*(-62.84)</td>
</tr>
</tbody>
</table>

Level of significance: Value in parenthesis indicate % change Values represent mean ± SEM (n=6)  
ns = non significant; a = P ≤ 0.05; b = P ≤ 0.01; normal and diabetic control rats compared with corresponding values on 0 day, ns = non significant; b = P ≤ 0.01; c = P ≤ 0.001, various extracts and glibenclamide treated rats compared with corresponding values on 0 day.

Oral glucose tolerance test (OGTT)

Fig. 1 shows the changes in the levels of blood glucose in normal control and different experimental groups after oral administration of glucose (2g/kg b.w.t.). In all experimental groups oral feeding of glucose induced a significant hyperglycemia at 30 minutes as compared with corresponding values on 0 min. After 120 min the blood glucose level in normal control rats (group I) tend to return near normal level. Untreated diabetic rats (group II) showed maximum increase in blood glucose after 60 min and showed mild decline after 90 min. In
diabetic rats treated with 500 mg/kg b.wt. of MOLEt (group V) or glibenclamide (group VI), the blood glucose level showed continues decline after 60 min and after 120 min, the blood glucose levels tend to come down near to corresponding values at 0 min.

Insulin, total hemoglobin and glycosylated hemoglobin
Figures 2-4 shows the levels of serum insulin, total hemoglobin (Hb) and glycosylated hemoglobin (HbA1c) respectively, in control and experimental rats. The diabetic control rats (group II) showed a significant (P≤0.001) decrease in the levels of insulin and total Hb with a concomitant significant (P≤0.001) increase in percentage of HbA1c as compared to control rats (group I). Diabetic rats treated with different doses of MOLEt and glibenclamide showed significant increase in the levels of insulin (group III (P≤0.05), group IV (P≤0.01), group V (P≤0.001) and group VI (P≤0.001) and total Hb in group IV (P≤0.01), group V (P≤0.001) and group VI (P≤0.001) when compared to diabetic rats. In contrast to this, the percentage of HbA1c was significantly decreased in group III (P≤0.05), group IV (P≤0.001), group V (P≤0.001) and group VI (P≤0.001) as compared to diabetic rats.

Total protein and hepatic glycogen
The diabetic control rats (group II) showed a significant (P≤0.001) decrease in the concentration of both total protein in pancreas and glycogen in liver as compared to normal control rats (group I). Diabetic rats treated with different doses of MOLEt (100, 250 and 500 mg/kg b.wt./day) or glibenclamide showed significant increase in the levels of total protein in pancreas and glycogen in liver of group III (P≤0.05), group IV, V and VI (P≤0.001) as compared to diabetic rats. (Table 3; Fig. 5).
**Lipid peroxidation and antioxidant defence markers**

Table 3 depict the changes in lipid peroxidation (TBARS) and antioxidant defence markers in normal and experimental rats. Diabetic rats (group II) showed a significant (P≤0.001) elevation in TBARS concentration in pancreas as compared with normal control rats (group I). Diabetic rats treated with different doses of MOLEt or glibenclamide showed significantly reduced TBARS concentration in pancreas as compared to diabetic control rats.

There was significant (P≤0.001) reduction in the activity of SOD and CAT and concentrations of GSH and ascorbic acid in pancreas of diabetic rats (group II) as compared with normal control rats (group I). Diabetic rats treated with different doses of MOLEt or glibenclamide showed significantly increased activity of SOD and CAT [group III (P≤0.05), group IV (P≤0.01), group V and group VI (P≤0.001)], GSH [group IV (P≤0.01), group V and group VI (P≤0.001)] and ascorbic acid levels [group III (P≤0.05), group IV (P≤0.01), group V and group VI (P≤0.001)] as compared to diabetic rats.

**Table 3: Effect of M. oleifera (MOLEt) on lipid peroxidation and antioxidant parameters in pancreas of STZ -induced diabetic rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pancreas protein (mg/g)</th>
<th>LPO (nmole/mg tissue)</th>
<th>SOD (U/mg protein)</th>
<th>GSH (µg/mg tissue)</th>
<th>Ascorbic Acid (mg/g tissue)</th>
<th>Catalase (n mole H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>54.73±2.17</td>
<td>2.87±0.19</td>
<td>17.78±0.74</td>
<td>2.80±0.11</td>
<td>1.42±0.14</td>
<td>63.22±2.31</td>
</tr>
<tr>
<td>Group II</td>
<td>22.02±1.58***</td>
<td>8.34±0.76***</td>
<td>10.26±0.71***</td>
<td>1.13±0.10***</td>
<td>0.58±0.05***</td>
<td>27.25±1.57***</td>
</tr>
<tr>
<td>Group III</td>
<td>30.88±1.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.02±0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.76±0.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.75±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.10±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.05±3.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>36.31±2.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.29±0.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.13±0.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.01±0.18&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.19±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>47.20±4.46&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>39.03±2.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.21±0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.10±0.76&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.37±0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.39±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>54.6±2.63&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VI</td>
<td>42.33±2.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.35±0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.15±1.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.18±0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.18±0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.85±4.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Level of significance: Values represent mean ± SEM (n=6)**

*** = P ≤ 0.001, diabetic control rats compared with normal control rats.

a = P ≤ 0.05; b = P ≤ 0.01; c = P ≤ 0.001, various extracts and glibenclamide treated rats compared with diabetic control rats.

**Histopathological study**

Histomorphological picture of pancreas of normal control rats (group I) exhibited normal lobular structure of acini and islets of Langerhans. The islets of Langerhans are interspersed among the acini as compact spherical mass (Fig. 6). Histomorphological picture of pancreas of untreated diabetic control rat (group II) revealed degenerative and atrophic changes in islets of Langerhans. Significant reduction in the size, cellular density and granulation was observed in the islets (Fig. 7). Histomorphological picture of pancreas of diabetic rats treated with 100 mg/kg b.wt. of MOLEt (group III) exhibited mild prevention of degenerative changes. The islets showed reduced granulation with slight increase of size (Fig. 8). While diabetic rats treated with 250 mg/kg b.wt. of MOLEt (group IV) showed moderate amelioration of necrotic changes, reduction in vacuolization concomitantly with an increase in the size of islets (Fig. 9). Histomorphological picture of pancreas of diabetic rats treated with 500 mg/kg b.wt. MOLEt (group V) depicted significant improvement of histological alterations. The islets showed near normal morphology and size with cells showing mild vacuolization and degranulation (Figure: 10). Diabetic rats treated with glibenclamide (group VI) also showed restoration of the pancreatic histoarchitecture near to normal control rat. (Fig. 11).

**Fig.6: Photomicrograph of pancreas of normal control rat (group I) showing normal islets of Langerhans interspersed among the acini as compact spherical mass (H & E x200).**

**Fig. 7: Photomicrograph of pancreas of diabetic control rat (group II) showing shrunken islets of Langerhans, displaying degenerative and necrotic changes (H & E x200).**
Fig. 8: Photomicrograph of pancreas of MOLEt (100mg/kg b.wt.) treated diabetic rat (group III) showing lesser degenerative changes, slight increase of size and cellular density of islets (H & E x200).

Fig. 9: Photomicrograph of pancreas of MOLEt (250mg/kg b. wt.) treated diabetic rat (group IV) showing improvement of histoarchitecture, reduction in vacuolization concomitantly with an increase in size of islets (H & E x200).

Fig. 10: Photomicrograph of pancreas of MOLEt (500mg/kg b.wt.) treated diabetic rat (group V) showing near normal morphology, size and cellular density of islets (H & E x200).

Fig. 11: Photomicrograph of pancreas of glibenlamide (5 mg/kg b. wt.) treated diabetic rat (group VI) showing restoration of pancreatic histology near to normal (H & E x200).

DISCUSSION

D.M. is one of the most important health problems worldwide that poses a major challenge to the scientist. Traditional antidiabetic plants might provide new oral antidiabetic phytochemicals which are considered to be less toxic and free from side effects than synthetic ones.[9]

Body weight is an indicator of good health and efficient metabolic homeostasis. The body weight of normal control rat showed a progressive increase throughout the experimental period. However, the body weight of untreated diabetic rats was found to be significantly decreased with duration of treatment. These results are in agreement with previous finding where similar decline in body weight was recorded in STZ-induced diabetic rats.[49, 50, 51] The observed weight loss in diabetic rats might be due to dehydration and excessive breakdown of tissue proteins and fats. Increased catabolism leading to muscle wasting might also be responsible for body weight loss.[52, 53, 54] Subchronic treatment of MOLEt at different doses (100, 250, 500 mg/kg) in diabetic rats prevented the loss of body weight both dose and duration dependently. These observations are parallel with the finding of other workers who also reported a similar prevention of body weight loss in diabetic rats after administration of M. oleifera extract.[39, 55, 56] This might be due to efficient, better glycemic and metabolic homeostasis induced by the plant extract.

Streptozotocin is a widely used chemical for the induction of experimental diabetes in animals. STZ selectively destroys the pancreatic beta cells involving uptake by glucose transporter -2 (GLUT-2). It also generates reactive oxygen species (ROS), which contribute to DNA fragmentation and evokes other deleterious changes in the beta cells of pancreas ultimately inducing beta cell necrosis and depletion of insulin biosynthesis and secretion.[57, 58, 59] This was evident from the marked decrease in serum insulin and increase in fasting glucose levels in STZ treated diabetic control rats observed in the present study. These results are parallel with other reports which have also observed similar depletion of insulin level and significant elevation in fasting blood glucose level in streptozocin treated diabetic rats.[39, 60, 61]

Administration of glibenclamide or MOLEt at different doses for 60 days in STZ-induced diabetic rats resulted in an elevation of serum insulin with significant improvement in fasting blood glucose levels. Our results are consistent with previous reports which have also mentioned that sub-chronic treatment with 70% ethanolic[39, 61, 62] or aqueous extract[29, 40, 51, 63] of M. oleifera leaves in diabetic rats significantly lowered fasting blood glucose level and increased plasma/serum insulin level. From these results it can be proposed that hoisted pancreatic insulin discharge might improve glucose uptake or else via hindering hepatic...
gluconeogenesis in addition to diminished blood glucose concentration.

The elevation of plasma insulin level with reduction of fasting blood glucose level by *M. oleifera* extract may be due to its many potential bioactive phytochemicals, especially quercetin 3-glycoside, kaempferol glycosides, chlorogenic acid and other polyphenols, moringine (alkaloid), beta carotene and other vitamin antioxidants (vita-A, C and E) present in the leaf extract,[10, 34] which might show ameliorative effect on glycemic index by virtue of their synergistic action resulting in an increased secretion of insulin by repair/ regeneration of beta cells of islets. Beside this, it might involve extra pancreatic action that include increased utilization of glucose by the body, inhibition of gluconeogenesis and inhibition of glucose absorption in the gut.[9, 64, 65]

In *M. oleifera* leaves the flavonol quercetin is found in high concentration and is a potent antioxidant[66] having multiple therapeutic properties.[67] It has been shown hypoglycemic properties in diabetic rats.[68, 69, 70] Vessal et al., (2003) reported that quercetin significantly increased heptic glucokinase activity, showing insulin like effect.[71] Kaempferol has been shown to have hypoglycemic activities.[38] and the mechanisms of action could be either by increasing the tissue utilization of blood glucose[72] by inhibiting hepatic gluconeogenesis or favoring absorption of glucose into the muscles and adipose tissue.[10, 38, 73] Furthermore, they also improve chronic hyperglycemia impaired pancreatic beta cells viability and insulin secretion in-vitro.[74] There are many reports about hypoglycemic activities of kaempferol derivatives present in many medicinal plants.[75, 76, 77] Ali et al., (2015) studied the effect of moringine, quercetin and chlorogenic acid isolated from ethanolic extract of *Moringa oleifera* leaves on alloxan induced diabetic rats. They reported that all these three phytoconstituents individually showed glucose lowering effect in diabetic rats, but the quercetin was most potent followed by chlorogenic acid.[65] Chlorogenic acid has an effect on glucose metabolism through inhibition of glucose 6-phosphate translocase in rat liver, reducing hepatic gluconeogenesis and glycogenolysis.[78] Beside this, immune modulatory activities of *M. oleifera* leaf extract may also be responsible for blood glucose lowering effect.[29] Insulinotropic action of *M. oleifera* leaves involves the induction of membrane depolarization and enhancement of intracellular calcium concentration mediated via the KATP dependent pathways of insulin release.[79] The anti-hyperglycemic effect of the *M. oleifera* extract is possibly linked to the strong antioxidant properties of the phytoconstituents which could counteract the toxic and pro-oxidant effects of streptozotocin.

Oral glucose tolerance test is well accepted and frequently used assay to screen antihyperglycemic activity of any drug,[66] and also to identity the altered carbohydrate metabolism during post glucose administration.[60] The results of oral glucose tolerance test in the present study suggest that rats treated with different doses of MOLEt have better glucose utilization capacity. This action could be due to enhanced insulin secretion from the beta cells, improved glucose utilization and transport.[81] These results are consistent with earlier finding which have also reported significant improvement in oral glucose tolerance in *M. oleifera* leaf extract treated diabetic or Goto-Kakizaki (GK) rats[36, 51, 61, 82, 83] According to Ndong et al., (2007), *Moringa* leaves extract might decrease gastric emptying which can improve glycemic control by prolonging the postprandial absorption.[86]

Glycosylated hemoglobin is a considered as the most reliable marker of glycemic control in the body.[84] In streptozocin treated diabetic control group HbA1c was significantly increased but in MOLEt / glibenclamide treated group it was significantly decreased. Glycation is a post translational non-enzymatic addition of sugar residues to N-terminal end of the hemoglobin beta chain. The rate of synthesis of HbA1c is directly related to exposure of RBC to glucose.[85] HbA1c is formed progressively and irreversibly over a period of time and it stable till the life of RBC. Our results are supported by the finding of Soliman et al. (2013) who also reported significant fall in HbA1c level in STZ diabetic rats after treatment with 70% ethanol extract of *M. oleifera* leaves.[36] Similarly, Girdhari et al. (2011) also reported ameliorative effect of *M. oleifera* leaves tablets on HbA1c in type-2 diabetic patients.[86]

Glycogen is chief intracellular storage form of carbohydrates mainly stored in the liver and muscles and serves as tissue reserve for the body’s glucose needs. The transformation of glucose to glycogen relies on the existence of insulin. Insulin favors glycogen synthesis by means of stimulation of glycogen synthase and hindering glycogen phosphorylase. Glycogen synthase and glycogen phosphorylase are the two key regulatory enzymes that catalyze the rate limiting steps of glycogen synthesis (glycogenesis) and degradation (glycogenolysis) respectively.[87, 88] In the present study there was significant depletion of glycogen content in the liver of STZ treated diabetic control rats which is in agreement with previous studies.[39, 60] Since STZ causes destruction of pancreatic beta cells resulting in marked decline of insulin level in blood. It is rational that glycogen level in liver and muscles of diabetic rat decreases as it depends on insulin mediated influx of glucose.[89] Diabetic rats treated with MOLEt at different doses showed dose dependent increase in hepatic glycogen reserve indicating enhanced rate of glycogenesis which may be resulted from the glucose uptake in liver cells by sensitization of insulin receptors or insulin release by beta cells of islets or by forcing the activities of enzymes which are involved in glycogen synthesis. These results are also corroborated by the finding of Soliman et al. (2013) and Suganabai et al. (2014) who also reported significant increase in glycogen
content in liver of STZ-induced diabetic rats treated with 70% ethanolic or aqueous extract of *M. oleifera* leaves respectively.\[^{39, 90}\]

The level of lipid peroxidation was measured indirectly by estimating thiobarbituric acid reactive substances (TBARS). Proxidants and antioxidants balance is vital for normal biological functions of the cell. Any disturbances that change this balance can provoke excessive production of reactive oxygen species (ROS), which creates a condition frequently known as oxidative stress. Oxidative stress is suggested as mechanism underlying diabetes and diabetic complications.\[^{91, 92, 93}\]

The increase free radicals may react with polyunsaturated fatty acids in the cell membranes leading to lipid peroxidation. Lipid peroxidation is highly destructive process that effects cellular organelles, enzymes and other molecules and cause them to lose biochemical functions and/or structural integrity leading to cell death.\[^{94, 95}\]

In the present study, it was observed that TBARS levels in pancreas of STZ-induced diabetic rats was significantly increased with concomitant decrease in the activities of SOD, CAT, GSH and ascorbic acid levels when compared with normal control rats. The decreased activity of antioxidant molecules along with elevated TBARS levels in diabetic rats could probably be associated with oxidative stress and decreased antioxidant defense potential.\[^{92}\]

The increased levels of TBARS in STZ diabetic rats could be due to increased production of ROS as a consequence of persistent hyperglycemia. It is well established that increased level of serum glucose may result in increased formation of ROS through autoxidation of glucose, through non enzymatic protein glycation, increased sorbitol pathway and depletion of some non enzymatic or enzymatic scavengers.\[^{96}\]

Secondarily, the oxidative stress may also be induced by diabetogenic agent streptozotocin.\[^{58, 59}\] Similar elevation of TBARS level and significant decline of antioxidant defense parameters (SOD, CAT, GSH, ascorbic acid etc) has been reported in STZ-induced diabetic rats by various workers.\[^{97, 98, 99}\] It was suggested that decreased antioxidant enzymes activity in diabetic state could be due to overutilization of these in scavenging excessive free radicals generated due to hyperglycemia, glucose autoxidation and glycation of these enzymes.\[^{100}\]

Treatment of diabetic rats with MOLEt showed significant dose dependent decline of TBARS level with concomitant increase in antioxidant markers (SOD, CAT, GSH and ascorbic acid) in pancreas. These results are parallel with earlier reports which have also reported decreased TBARS level with a concomitant increase in free radical scavenging antioxidant molecules in pancreas of diabetic rats receiving extract of *M. oleifera* leaves.\[^{40, 62}\]

It has been reported that *M. oleifera* leaves are potent source of polyphenols especially quecertin and kaemferol and vitamins which are capable of scavenging peroxyl and superoxyl radicals.\[^{101, 102}\] Flavonoids can exert their antioxidant capacity by various mechanisms viz. by scavenging or quenching free radicals, by chelating metal ions or by inhibiting enzymatic systems responsible for free radical generation.\[^{64, 69, 103}\]

Robertson et al. (2005) demonstrated that antioxidants may prevent the worsening of diabetes by improving beta cells function in animal models and suggested that enhancing antioxidant defence mechanism in pancreatic islets may be a valuable pharmacologic approach for managing diabetes.\[^{104}\]

The biochemical finding observed in the present study are further supported by histopathological study of pancreas. The histopathological examination of islets of Langerhans in pancreas of STZ diabetic rats showed severe degenerative and atrophic changes. The islets were shrunken showing decreased cellular density and granulation and vacuolization. These results are in agreement with previous reports which have also shown similar type of histopathological lesions in pancreas of STZ diabetic rats.\[^{40, 60, 99}\] Such pathological changes could be attributed to glucotoxicity, which arise from excessive uptake of glucose by beta cells in diabetes.\[^{57, 58, 59}\] The excessive sugar glycation reactions and mitochondrial electron transport chain produces reactive oxygen species (ROS) at the level beyond the antioxidant capacities of the cell. The ensuing oxidative stress impairs insulin synthesis, secretion and initiates a cascade of cellular events that ultimately lead to beta cells cytotoxicity and death.\[^{105}\]

The histopathological study of the pancreas in *M. oleifera* extract treated diabetic rats showed dose dependent restoration of histoarchitecture. The observed ameliorative effects of MOLEt may be due to presence of secondary metabolites like phenols and flavonoids which exerts antioxidant like effects consequently alleviating oxidative stress and enhancing insulin secretion possibly by virtue of regeneration of beta cells. This is probably because the pancreas contains stable (quiescent) cells which have the capacity of regeneration \[^{106}\] or the self duplication/ self proliferation of cells increase the number of beta cells in islets.\[^{107, 108, 109}\] Our results reinforced the previous reports which also observed significant restoration of pancreatic histoarchitecture in 70% ethanolic \[^{62}\] or aqueous extract of *M. oleifera* leaves treated diabetic rats.\[^{40, 63}\] Diabetic rats treated with MOLEt showed ameliorative effects on glycemic markers, pancreas histology and antioxidant defense parameters comparable to reference drug glibenclamide.

**CONCLUSION**

Results of present study suggest that the 70% ethanolic extract of *M. oleifera* leaves has potential antidiabetic and antioxidant action in STZ-induced diabetic rats. It may
provide a lead for the development of antidiabetic drug of plant origin.

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