EVALUATION OF ANTIDIABETIC AND ANTIHYPERLIPIDEMIC POTENTIAL OF AQUEOUS EXTRACT OF MORINGA OLEIFERA IN FRUCTOSE FED INSULIN RESISTANT AND STZ INDUCED DIABETIC WISTAR RATS: A COMPARATIVE STUDY

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ABSTRACT
The aim of the present study was to evaluate the effect of aqueous extract of Moringa Oleifera leaves on body weight, plasma glucose, insulin, lipid profile, HOMA and Oral Glucose Tolerance Test in Insulin resistant (IR) and type 1 diabetic rat models. IR was induced by high fructose diet and type 1 diabetes was induced by intraperitoneal injection of Streptozotocin (55 mg / kg body weight). The extract was administered at a dose of 200 mg / kg body weight by oral intubation for a period of 60 days. Fructose fed rats exhibited IR as reflected by an increase in body weight, hyperinsulinemia, hyperglycemia and increased HOMA. STZ induced diabetic rats showed hyperglycemia, hypoinsulinemia and failure to gain body weight. The severity of hyperglycemia was more in STZ diabetic rats. Both IR and STZ rats showed hyperlipidemia, which was more severe in IR rats. OGTT showed increased glucose intolerance in both IR and STZ diabetic rats, severity being more in IR rats. Administration of aqueous extract of Moringa oleifera for 60 days restored all the alterations to normal/ near normal. The study clearly reveals that aqueous extract of Moringa oleifera leaf possesses potent antihyperglycemic and antihyperlipidemic effect in both Insulin resistant and Insulin deficient rat models.

Key words: Moringa oleifera, Insulin resistance, Streptozotocin, glucose intolerance, lipids profile.

INTRODUCTION
Diabetes mellitus is now recognized as a metabolic disorder of multiple etiology which is characterized by chronic hyperglycemia resulting from absolute or relative deficiency in insulin secretion/insulin action or both. The number of diabetics was 171 millions in 2000, which might increase to 360 millions in the year 20301. As the number of people with Diabetes mellitus (DM) multiplies worldwide, national and international health care budget increases.

The vast majority of diabetic patients are classified into two broad categories: type-1 diabetes (IDDM), which is caused by an absolute deficiency of insulin, and type-2 diabetes (NIDDM), which is characterized by the presence of insulin resistance (IR) with an inadequate compensatory increase in insulin secretion. Lack of insulin at the metabolic level cause derangement in carbohydrate, fat and protein metabolism which eventually leads to a number of long term micro vascular (retinopathy, nephropathy, and neuropathy) and macro vascular (coronary artery disease, peripheral vascular disease and cerebro vascular disease) complications.

Despite considerable progress in therapies using expensive synthetic drugs, the search for herbal remedies is growing which can be accounted for the effectiveness, minimal side effects in clinical experience and relatively low cost of the herbal drugs. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown2.

Moringa oleifera Lam (syn Pteriogosperma Geartn), belongs to the monogenic family Moringaceae and it is one of the best known, most widely distributed and naturalized species3. It is popularly known as drumstick or horseradish in English. It has numerous medicinal uses, which have long been recognized in Ayurvedic and Unani systems of medicines4. Many parts of this plant i.e., leaves, immature pods, flowers and fruits are edible and are used as a highly nutritious vegetable in many countries5. This plant was well known to the ancient world, but only recently, it has been rediscovered as a multipurpose tree with a tremendous variety of potential uses. The leaves have been reported to be a rich source of β-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidant due to the presence of ascorbic acid, flavonoids, phenolics and carotenoids. M. oleifera contains nitrile mustard oil glycosides and thiocarbamate glycosides which are anti hypertensive and are very rare in nature7. The leaves exhibit strong antioxidant property expressed in terms of free radical scavenging activity and reducing power8. Niazimicin, a compound from the leaves has been proposed to be a potent chemoprotective agent in chemical carcinogenesis and Niazimicin (9+10), a thioribamate from the leaves of M. oleifera, exhibits inhibition of tumour promoter induced Epstein-Barr virus activation9. Leaves were found to contain lipid lowering activity in the serum of high fat diet fed rats which may be attributed to the presence of β-sitosterol 10, hepatoprotective activity11 and found to preserve and enhance the process of spermatogenesis in mice 12. The aqueous extract of leaves of M. oleifera has shown to lower the blood sugar in diabetic rats13. The fresh leaf juice was found to inhibit the growth of microorganisms, staphylococcus aureus and Pseudomonas aeruginosa, which are pathogenic to humans14.

Earlier studies on antihyperglycemic and anti hyperlipidemic activity of M. oleifera are fragmentary and no studies are available on the efficacy of M. oleifera in preventing IR. In the present study the efficacy of aqueous extract of M. oleifera leaf (AEMO) was evaluated for its antidiabetic and antihyperlipidemic potential in fructose fed insulin resistant (IR) and STZ induced diabetic rats (type-1).

Chemicals
Streptozotocin was procured from Sigma chemical Co St. Louis, MO, USA. Olympus system packs (Japan) were used for assays of plasma glucose, triacylglycerols, cholesterol. Direct HDL-C and LDL-C kits were procured from ACCUREX. All other chemicals and solvents were of analytical grade and procured from SISCO research Laboratories Private Ltd., Mumbai, Maharashtra, India.

Plant Material
Aqueous extract of Moringa oleifera leaf (AEMO) dry powder (product code P/DSM/MOOL-01 and batch number (P8060947) was purchased from Chemiloids (Manufacturers and exporters of herbal extracts, Vijayawada, Andhra Pradesh, India). Herb to product ratio was 1:1. The extract was dissolved in distilled water prior to use.

Animals
Male albino Wistar rats were procured from Sri Venkateswara Enterprises, Bangalore, India, and were acclimatized for 7 days to animal house (Regd. No. 470/01/a CPCSEA) and maintained at a temperature of 22 ± 2º C. The animal room was regulated by a 12 h light; 12 h dark schedule. All the procedures were performed in accordance with the Institutional Animal Ethics Committee.
Diet

The standard pellet diet was procured from Sri Venkateswara Enterprizes, Bangalore, India and the high fructose diet was obtained from National Centre for Laboratory Animal Science, National Institute of Nutrition (Hyderabad, India).

Experimental design

In the present study, fifty-four male albino Wistar rats aged about 4-5 weeks with average body weight of 150-160 g were acclimatized to our animal house before induction of IR/type-1 diabetes. IR was induced in 16 rats by feeding fructose enriched diet throughout the experimental period. The fructose diet contained 66% fructose, 18% protein, 8% fat, 4% cellulose, 3% mineral and 1% vitamin mix. About twenty two rats were made diabetic by a single intraperitoneal injection of freshly prepared Streptozotocin (STZ) in 0.05M citrate buffer pH 4.5, at a dose of 55 mg/kg bodyweight. After a window period of 72 hours, rats with fasting plasma glucose levels above 300 mg% were considered diabetic. The remaining 16 rats served as controls. Both STZ induced diabetic and control rats were maintained on standard pellet diet. Each set of animals (Control, IR and type 1 DM) was further subdivided into two groups thus comprising a total of six groups: control (C), control rats administered with AEMO (C+MO), fructose fed rats (F), fructose fed rats administered with AEMO (F+MO), STZ diabetic (D) and STZ diabetic rats administered with AEMO (D+MO). Rats in the groups C+MO, F+MO and D+MO were administered with the AEMO at a dose of 200 mg/kg body weight in ~2 ml of distilled water and the remaining groups were administered with 2 ml of water once a day through gastric intubation for a period of 60 days.

Preliminary analysis of AEMO

Qualitative screening for phytochemicals i.e., alkaloids, anthracone glycosides, flavonoids, gallic tannins, catecholic compounds, phenols, saponins, steroidal and triterpenes was performed by following the standard methodology. 

Biochemical analysis

Plasma glucose, plasma insulin and body weight were measured at 15 day interval for a period of 60 days. Lipid profile were measured at the end of experimental period. Blood was collected in heparinised Eppendorf tubes by means of heparinised capillary tube through retro-orbital plexus. Plasma was separated immediately by centrifugation at 4º C using REMI-24 model centrifuge, aliquoted and frozen for insulin and other biochemical assays.

Oral Glucose Tolerance Test (OGTT)

At the end of the experimental period (60days), OGTT was performed in all groups of rats. The 12 hr fasted animals were challenged with a glucose solution at a dose of 2 g / kg body weight by oral intubation and blood samples were collected at 0 min (before glucose administration) and 30, 60 and 120 min after glucose administration.

After the experimental period of 60 days, rats from all six groups were sacrificed by cervical dislocation following 12 h fasting.

Plasma glucose, triacylglycerols (TAG), total cholesterol (TC), HDL and LDL cholesterol were assayed on fully automated chemistry analyser, Olympus AU 400. VLDL-C was calculated using the Friedewald formula as follows:

\[ \text{VLDL-C} = \text{TAG/5} \]

The values were expressed as mg/dl.

The antithrombogenic index (AAI) was calculated according to the method of Guido and Joseph as follows:

\[ \text{AAI} = \text{HDL-C X 100} / \text{TC} \cdot \text{HDL-C} \]

The values were expressed as percentage.

Effect of AEMO on fasting Plasma glucose

The plasma glucose levels of F group and D group increased in body weight where as STZ diabetic rats as a model of type -1 diabetes, showed loss of body weight when compared to C group (Fig 1).

Fig 1: Effect of AEMO on body weight of fructose fed IR and STZ induced diabetic rats.

At the end of 60 days, F group showed 9.7% increase and D group showed 31.4% decrease in body weight compared to C group. AEMO gave total protection against abnormal weight gain in F+MO which is evident from the un deviated bodyweight compared to C group. Though D+MO group showed a significantly lower (20.5%) body weight than C group, it showed a significantly higher (63.7%) body weight when compared to D group. No visible side effects and variation in animal behaviour (respiratory distress, abnormal locomotion and catalepsy) were observed in C+MO group indicating the non-toxic nature of AEMO. A significantly higher intake of food and water was observed in F group from 10th day onwards of the experimental period compared to C group. Rats in F group seem to be obese when compared with the remaining five groups. Group D rats showed the characteristic signs of diabetes such as polyuria, polydipsia and polyphagia and failure to gain weight.

Effect of AEMO on body weight

F group animals as a model of insulin resistance showed excess gain in body weight where as STZ diabetic rats as a model of type -1 diabetes, showed loss of body weight when compared to C group (Fig 1).

Effect of AEMO on fasting Plasma glucose

The plasma glucose levels of F group and D group increased gradually and at the end of the experimental period the % of increase was 45 and 372.9 respectively when compared to C.
Obviously the intensity of hyperglycemia was more prominent in D group than in F (Fig 2). However, administration of AEMO showed beneficial effect which was reflected by the un deviated plasma glucose in F+MO group and only 23.22% increase in D+MO compared to C. Group C+MO remained persistently euglycemic and the plasma glucose levels of C+MO, F+MO and D+MO during the experimental period clearly indicated that AEMO did not exhibit hypoglycemic activity; instead, it showed antihyperglycemic effect. D showed 14.7% increase compared to C group. AEMO ameliorated the insulin sensitivity both in F+MO and D+MO groups as was evident from 91.3% and 35% recovery of HOMA in F+MO and D+MO groups respectively (Fig 4).

Effect of AEMO on plasma insulin
F group showed 222% increase whereas D group showed 75.8% decrease in fasting plasma insulin when compared to C (Fig 3). Administration of AEMO led to protection from fructose induced hyperinsulinemia and STZ induced insulin depletion which was evident from significantly lower (59.2%) insulin level in F+MO group than F and significantly higher insulin levels (109.6%) in D+MO group than D. However, the recovery was partial as the insulin levels in F+MO and D+MO did not reach C levels. Enhanced insulin sensitivity in C+MO group was evident from its persistant euglycemic state at lower insulin levels.

Effect of AEMO on HOMA
In clinical research, HOMA is widely used to assess insulin sensitivity. Both F and D groups exhibited insulin resistance but the severity was more in F group than in D. At the end of the experimental period, F group showed 365.9% increase in HOMA and D+MO groups respectively (Fig 4).

Effect of AEMO on lipids
Administration of AEMO led to protection from fructose feed induced and STZ induced diabetic rats plasma TAG, TC, HDL, LDL, VLDL and AAI of experimental groups are presented in Table 1.

Table 1: Effect of AEMO on lipid profile and AAI in fructose fed IR and STZ induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TAG</th>
<th>TC</th>
<th>HDL-C</th>
<th>LDL-C</th>
<th>VLDL-C</th>
<th>AAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>82.92±0.17a</td>
<td>66.04±0.16a</td>
<td>26.98±0.17a</td>
<td>18.09±0.14c</td>
<td>16.57±0.03c</td>
<td>69.11±0.97c</td>
</tr>
<tr>
<td>C+MO</td>
<td>71.28±0.34a</td>
<td>58.12±0.19b</td>
<td>32.28±0.17b</td>
<td>15.01±0.09b</td>
<td>14.22±0.07b</td>
<td>124.94±1.03b</td>
</tr>
<tr>
<td>F</td>
<td>204.04±5.22c</td>
<td>98.46±0.24c</td>
<td>34.48±0.11c</td>
<td>27.22±0.14c</td>
<td>40.80±0.10c</td>
<td>53.90±0.49c</td>
</tr>
<tr>
<td>F+MO</td>
<td>81.50±0.16b</td>
<td>70.06±0.35d</td>
<td>30.94±0.12d</td>
<td>18.73±0.21d</td>
<td>16.16±0.02d</td>
<td>79.13±0.97d</td>
</tr>
<tr>
<td>D</td>
<td>185.50±1.06b</td>
<td>83.33±0.27c</td>
<td>17.74±0.11c</td>
<td>21.60±0.17c</td>
<td>37.10±0.20c</td>
<td>27.07±0.30c</td>
</tr>
<tr>
<td>D+MO</td>
<td>80.88±0.22a</td>
<td>68.30±0.39c</td>
<td>29.76±0.11c</td>
<td>18.59±0.14d</td>
<td>16.15±0.03d</td>
<td>77.24±0.50d</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n=5 animals). Values with different superscripts within the row are significantly different at P < 0.05 (Duncan's multiple range test).

Both F and D groups showed dyslipidemia i.e., increased levels of plasma TAG (146.1and 123.7%), TC (49.1 and 26.1%), VLDL-C (146.2 and 123.9%) and LDL-C (50.5 and 19.5%) compared to C. However, F group showed increased HDL-C (27.8%) and D group showed decreased HDL-C (34.2%) than C group. In spite of higher HDL-C, F group showed significantly lower AAI (22%) than C group. The intensity of hyperlipidemia was more pronounced in F group than in D group. Administration of AEMO showed beneficial effects in both F+MO and D+MO groups by the restoration of TAG, TC, LDL, VLDL and AAI towards C levels. Significantly decreased levels of TAG (14%), TC (12%), LDL (17.1%) and VLDL (14.2%) and increased levels of AAI (80.8%) in C+MO group compared to C indicate the beneficial effect of AEMO on age related atherogenecity too.
Table 2 Effect of AEMO treatment on plasma glucose during OGTT in fructose fed IR and STZ diabetic rats

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>C</th>
<th>C+MO</th>
<th>F</th>
<th>F+MO</th>
<th>D</th>
<th>D+MO</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td>143.8±1.48</td>
<td>138.4±0.83</td>
<td>163.0±2.43</td>
<td>143.2±1.48</td>
<td>584.4±1.61</td>
<td>248.8±1.25</td>
</tr>
<tr>
<td>60 min</td>
<td>120.4±1.29</td>
<td>116.0±1.58</td>
<td>141.0±2.06</td>
<td>120.4±1.54</td>
<td>449.0±1.36</td>
<td>172.0±0.90</td>
</tr>
<tr>
<td>120 min</td>
<td>84.4±1.00</td>
<td>83.8±1.59</td>
<td>128.2±1.15</td>
<td>87.6±1.37</td>
<td>568.8±2.53</td>
<td>128.2±0.49</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., (n = 5 animals). Values with different superscripts within the row are significantly different at P < 0.05 (Duncan’s multiple range test).

**Effect of AEMO on OGTT**

Changes in plasma glucose levels after an oral glucose load are shown in Table 2. During OGTT the plasma glucose values of all experimental groups reached maximum value by 60 min after glucose challenge which was significantly higher in F and D groups compared to C. Except in D group, the raised plasma glucose values in remaining five groups returned to their corresponding basal / near basal levels by 120 min. F and D groups showed increased Area Under Curve for glucose \((AU_{Glucose})\) compared to F+MO and D+MO respectively (Fig 5).

**DISCUSSION**

There are reports indicating that increased fructose consumption increases bodyweight and adiposity in hamsters and rats. Hepatic metabolism of fructose favors \(\text{de novo} \) lipogenesis, and this may be linked with both hyperlipidemia and increased body fat stores. Obesity is almost invariably associated with insulin resistance. One of the consequences of dietary fructose induced insulin resistance is impaired glucose tolerance. Insulin resistance can be attributed to molecular defects like defects in the insulin binding, signal transduction, or post receptor defects. These defects have been widely characterized in humans with type 2 diabetes as well as experimental animal IR models. In addition, dietary fructose metabolism leads to high concentration of FFA in liver, which in turn enhances hepatic gluconeogenesis. Thus plasma glucose levels increase by the increased dietary fructose. Glucose, produced as a result of fructose metabolism stimulates insulin release but the fructose induced insulin resistance prevents the insulin from effectively metabolizing glucose, resulting in hyperglycemia. Insulin resistance also leads to compensatory hyperinsulinemia, where the body attempts to balance the reduced effect of insulin by producing and releasing. Administration of AEMO prevented the increase in blood glucose level in F+MO group and maintained normoglycemia throughout the experimental period. Enhanced insulin sensitivity by AEMO administration is evident from significantly decreased HOMA values and plasma insulin levels in F+MO group compared to F group.
corrected dislipidemia and improved the AAI probably by rectifying the functional defects of HDL-C. The dislipidemia observed in D group was characterized by higher TC, TAG, VLDL-C, LDL-C and a lowered HDL-C levels than C group, a pattern strongly correlating cardiovascular risk. Dyslipidemia is a frequent complication noted in chemical induced diabetes. The abnormally high concentrations of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from peripheral depots, since insulin inhibits the hormone sensitive lipase. On the other hand, gluagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of uninhibited action of lipolytic hormone on fat depots. In contrast to F group, D group showed a lower HDL-C levels than all other experimental groups with a lowered AAI. Severe hyperglycemia observed in STZ diabetic rats is favourable for increased non-enzymic glycation of LDL and HDL. There are reports that LDL-C increases its atherogenic potential after chemical modification including glycation. This explains the role of LDL in premature development of atherosclerosis under diabetic conditions. Athrogenic Index indicates deposition of foam cells, plaque, fatty infiltration or lipids in heart, coronaries, aorta, liver and kidney. The higher the athrogenic index the greater the risk of these organs to oxidative damage. The protection by the administration of AEMO against the athrogenicity in IR/insulin deficient conditions can be attributed to its hypolipidemic and antioxidant properties. In spite of more pronounced dyslipidemia observed in F group versus D group the AAI was greater in F group than D group. Thus, greater athrogenicity in D group compared to F group can also be attributed to increased oxidative insult under hypolipidemic conditions than under IR conditions. The antihyperlipidemic and anti athrogenic property of AEMO is evident by the corrected dislipidemia and improved AAI both in F+MO and D+MO groups.

Moringa oleifera leaves are used in folk medicine for their lipid lowering effect. Aqueous extract of M. oleifera leaves prevented athrogenic plaque formation in artery and also possess lipid lowering activity in rabbits, fed with high cholesterol diet. The hydroalcoholic extract of M. oleifera leaves exert notable cardio protective effects on myocardial infarction and possess myocardial preservative actions. The crude extract of Moringa leaves has been reported to exhibit cholesterol lowering effect in high fat diet fed and iron deficient rats and in hyperlipidemics. Numerous epidemiological studies suggest that herbs/diets rich in phytochemicals and antioxidants execute a protective role in health and disease. Flavonoids, sterols, triterpenoids, alkaloids, saponins and phenolics are reported as bioactive antidiabetic principles. Flavonoids can regenerate damaged β-cells in the alloxan induced diabetic rats. Polyphenols inhibit lipid peroxidation by acting as chain breaking peroxyl radical scavengers and can protect LDL from oxidation and also inhibit hepatic lipid synthesis. Thus AEMO with its treasure of phytochemicals exhibited a protective role as antidiabetic and anti hyperlipidemic , both in type 2 and type 1 diabetes in experimental rats.

CONCLUSION

The present study reveals that AEMO has some obvious therapeutic implications against insulin resistance, impaired glucose tolerance, hyperglycemia, atherogenic lipoprotein profile and their prevention both in IR and type 1 diabetic animal models. AEMO with its multiple beneficiary properties would seem useful as an adjuvant for the prevention and/or management of diabetes.

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