Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 5, Issue 1, 2012 ISSN - 0974-2441

Research Article

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

SAI MANGALA DIVI [1*], RAMESH BELLAMKONDA[2], SARALA KUMARI DASIREDDY [3]

[1*]**Department of Biochemistry, Sri Sathya Sai Institute of Higher Medical Sciences, Prasanthigram, Puttaparthy, Prasanthi Nilayam 515134 , Andhra Pradesh, India, [2] Department of Biochemistry, Sri Venkateswara University, Tirupati 517502, Andhra Pradesh, India,[3]Department of Biochemistry, Sri Krishnadevaraya University, Anantapur 515003,Andhra Pradesh, India. Email**: smdivi@gmail.com

Received: 17 September 2011, Revised and Accepted: 8 November 2011

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 hyper lipedemia, 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 antihyperlipedemic 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 2030[1]. 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 unknown[2].

Moringa oleifera Lam (syn Pteriogosperma Geartn), belongs to the monogeneric family Moringaceae and it is one of the best known, most widely distributed and naturalized species[3]. 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 medicines[4] . Many parts of this plant *i.e*., leaves, immature pods, flowers and fruits are edible and are used as a highly nutritive vegetable in many countries[5]. 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[6] and are vey rare in nature[7]. The leaves exhibit strong antioxidant property expressed in terms of free radical scavenging activity and reducing power[8]. Niazimicin, a compound from the leaves has been proposed to be a potent chemoprotective agent in chemical carcinogenesis[9] and Niazimicin (9+10), a thiocarbamate from the leaves of M. oleifera, exhibits inhibition of tumour promoter induced Epstein-Barr virus activation[10]. 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 [11], hepatoprotective activity[12]and found to preserve and enhance the process of spermatogenesis in mice [13]. The aqueous extract of leaves of M. oleifera has shown to lower the blood sugar in diabetic rats [14,15]. The fresh leaf juice was found to inhibit the growth of microorganisms, staphylococcus aureus and Pseudomonas aeruginosa, which are pathogenic to humans [16].

Earlier studies on antihyperglycemic and antihyperlipidemic 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 assaying plasma glucose, triacylglycerols, cholesterol. Direct HDL-C and LDL-C kits were procured form 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 10:1. The extract was dissolved in distilled water prior to use.

Animals

Male albino Wistar rats were procured from Sri Venkateswara Enterprizes, 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 intraperitonial injection of freshly prepared Streptozotocin (STZ) in 0.05M citrate buffer P^H 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, anthracene glycosides, flavonoids, gallic tannins, catecholic compounds, phenols, saponins, steroids and triterpines was performed by following the standard methodology [17,18,19,20].

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 [21] as follows:

VLDL-C = TAG/5

The values were expressed as mg/dl.

The antiatherogenic index (AAI) was calculated according to the method of Guido and Joseph [22], from total cholesterol and HDL-C as follows:

AAI= HDL-C X 100 / TC - HDL-C

The values were expressed as percentage.

Plasma Insulin was determined by radioimmunoassay kit (RIAK-I) provided by Bhabha Atomic Research Center (Mumbai, India). The insulin resistance index was calculated according to the Homeostasis Model of Assessment (HOMA-IR) by the following formula [23]:

Fasting plasma insulin (µU/ml) x Fasting glucose (mmol/L) / 22.5

Statistical analysis

Data were expressed as the mean ± SEM for the number (n= 5) of animals in the group. The data were subjected to statistical analysis by Duncan's Multiple Range (DMR) test [24]. Values of $p < 0.05$ were considered statistically significant.

RESULTS

The phytochemical screening of AEMO revealed the presence of alkaloids, flavonoids, gallic tannins, phenols, saponins and catecholic compounds and steroids indicating the presence of pharmacologically important phytochemicals.

General observations

During the experimental period including the window period about 30% mortality was observed in STZ induced diabetic group, whereas no mortality was observed in the remaining groups. 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).

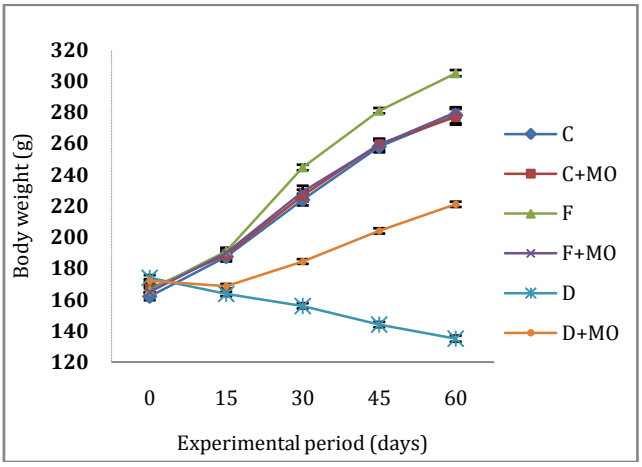

Fig 1: Effect of AEMO on body weight of fructose fed IR and STZ induced diabetic rats.

At the end of 60 days, F group showed 9.7% increase and D group showed 51.4% 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. The observation reveals AEMO administration completely prevented fructose induced weight gain in F group and partially prevented the weight loss observed in STZ induced diabetic rats.

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.

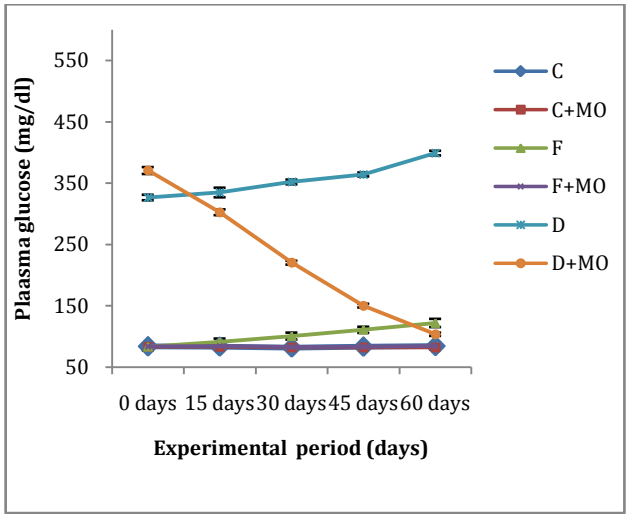

Fig 2: Effect of AEMO on fasting plasma glucose levels in fructose fed IR and STZ induced diabetic rats

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 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 355% recovery of HOMA in F+MO and D+MO groups respectively (Fig 4).

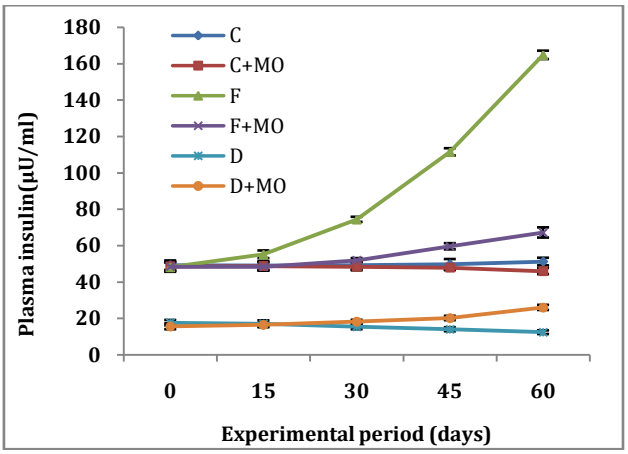

Fig 3: Effect of AEMO on fasting plasma insulin levels in fructose fed IR and STZ induced diabetic rats

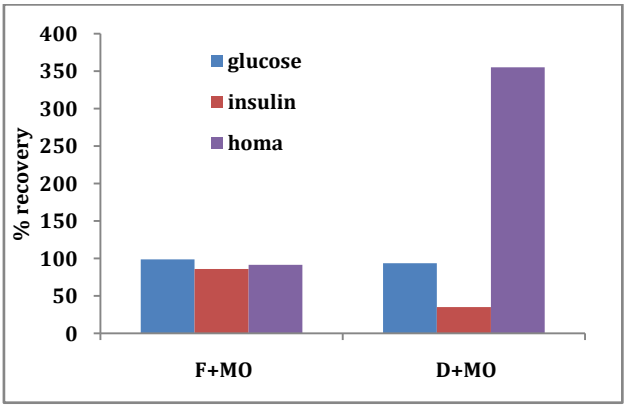

Fig 4 Per cent recovery from fructose feed induced and diabetic induced alterations in plasma glucose, plasma insulin and HOMA values by AEMO administration in F+MO and D+MO groups.

Effect of AEMO on lipids

The plasma concentrations of TAG, TC, HDL, LDL, VLDL and AAI of six 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.

Groups	TAG	TC	HDL-C	LDL-C	VLDL-C	AAI
C	82.92±0.17^{a}	66.04±0.16^{a}	26.98±0.17^{a}	18.09±0.14^{a}	16.57±0.03^{a}	69.11±0.97^{a}
C+MO	71.28±0.34^{b}	58.12±0.19^{b}	32.28±017^{b}	15.01±0.09^{b}	14.22±0.07^{b}	124.94±1.03^{b}
F	204.04±5.2^{c}	98.46±0.24^{c}	34.48±0.11^{c}	27.22±0.14^{c}	40.80±0.10^{c}	53.90±0.49^{c}
F+MO	81.50±0.16^{a}	70.06±0.35^{d}	30.94±0.12^{d}	18.73±0.21^{d}	16.16±0.02^{d}	79.13±0.97^{d}
D	185.50±1.06^{d}	83.30±0.27^{e}	17.74±0.11^{e}	21.60±0.17^{e}	37.10±0.20^{e}	27.07±0.30^{c}
D+MO	80.88±0.22^{a}	68.30±0.39^{f}	29.76±0.11^{f}	18.59±0.14^{d}	16.15±0.03^{d}	77.24±0.50^{d}

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 (14.0%), TC (12.0%), LDL (17.1%) and VLDL (14.2%) and increasedlevels 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

	Plasma glucose (mg/dl)					
Time↓	**C**	**C+MO**	**F**	**F+MO**	**D**	**D+MO**
0 min	84.4±1.34[a]	82.8±1.59[a]	122.4±1.38[b]	84.8±1.24[a]	399.2±1.71[c]	104.0±1.17[d]
30 min	120.4±1.29[a]	116.0±1.58[a]	141.0±2.06[b]	120.4±1.54[a]	449.0±1.36[c]	172.0±0.90[d]
60 min	143.8±1.48[a]	138.4±0.83[b]	163.0±2.43[c]	143.2±1.48[a]	584.4±1.61[d]	248.8±1.25[e]
120 min	86.4±1.00[a]	83.8±1.59[a]	128.2±1.15[b]	87.6±1.37[a]	568.8±2.53[c]	128.2±2.04[b]

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 ($AUC_{glucose}$) compared to F+MO and D+MO respectively (Fig 5).

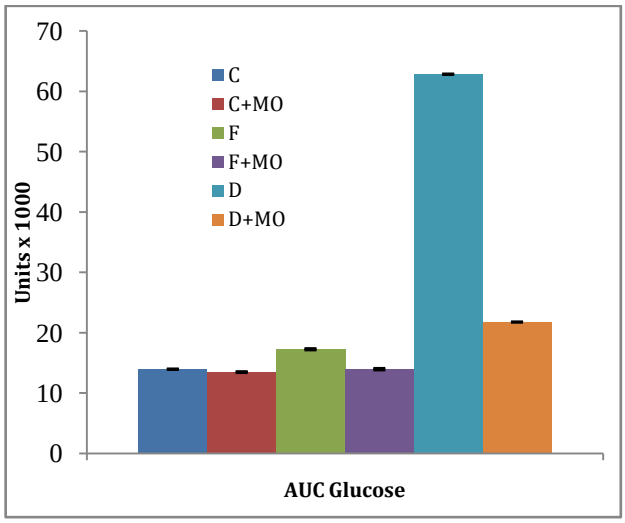

Fig 5: Effect of AEMO on AUC $_{glucose}$ during OGTT in fructose fed IR and STZ induced diabetic rats

DISCUSSION

There are reports indicating that increased fructose consumption increases bodyweight and adiposity in Hamsters[25] and rats [26]. Hepatic metabolism of fructose favors *de novo* lipogenesis, and this may be linked with both hyperlipidemia and increased body fat stores[27]. Obesity is almost invariably associated with insulin resistance. One of the consequences of dietary fructose induced insulin resistance is impaired glucose tolerance[28]. 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[29] as well as experimental animal IR models[30]. In addition, dietary fructose metabolism leads to high concentration of FFA in liver, which in turn enhances hepatic gluconeogenesis[28]. 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[31]. 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.

In spite of polyphagia, the loss of body weight observed in D group of rats was possible due to defect in glucose metabolism and increased muscle wasting due to excessive break down of tissue proteins. Muscle wasting, negative nitrogen balance and enhanced gluconeogenesis are characteristic features of uncontrolled diabetes[32]. D group showed severe hyperglycemia and hypoinsulinemia. STZ is a diabetogenic agent and it selectively destroys the insulin producing β- cells of pancreas by inducing necrosis . This was evident from marked depletion of plasma insulin levels in D group which ultimately led to hyperglycemia. AEMO administration resulted in elevation of plasma insulin levels with near normal glucose levels in D+MO group. In the present study, the elevation in plasma insulin levels in D+MO group may be due to the substances present in the plant extract which stimulate insulin secretion or which protect the intact functional β-cells from further deterioration or due to regeneration of STZ destructed β-cells. This is probably because the pancreas contains stable (quiescent) cells which have the capacity of regeneration[33]

Our results are in agreement with earlier reports that many traditional medicinal plants like Diabecon [34], Dioscorea [35], Ocimum sanctum [26] have successfully prevented fructose induced insulin resistance and Commophora showed antihyperglycemic activity in STZ induced diabetic rats[36]. Earlier reports revealed the glucose intolerance ameliorating effect of Moringa oleifera leaves in spontaneously diabetic Goto Kakazaki rats [14]and STZ diabetic rats[15].

Several mechanisms have been suggested to explain dyslipidemia in fructose fed conditions, which include enhanced hepatic lipogenesis, over production of VLDL and impairment in their peripheral catabolism[37]. Fructose is more lipogenic than glucose because fructose bypasses the rate limiting step catalysed by phosphofructokinase (PFK) and enters the glycolytic pathway, providing carbon atoms for both the glycerol and acyl portions of TAG. In addition, unlike glucose, which stimulates both TAG production and TAG removal, fructose impairs removal of TAG creating the known dyslipedemic profile[38]. Several investigators have noted that diets containing 60% fructose elevate plasma TAG levels in hamsters and cause insulin resistance[39]

Hypertriacylglycerolemia may be secondary in increasing the very low density lipoprotein-triacylglycerols (VLDL-TAG) secretion rate, since elevations in plasma triacylglycerols have been correlated with increase in this ratio[40]. In insulin resistance, increased efflux of NEFA from adipose tissue, and impaired insulin mediated skeletal muscle uptake of NEFA, increase hepatic NEFA concentrations[41]. The increased NEFA concentrations enhance hepatic TAG synthesis and ultimately VLDL synthesis in liver, which in turn leads to hypercholesterolemia.

Similar to our observation in the present study, increased plasma HDL-C was found in high fructose fed animals i.e., male Sprague–Dawley rats[42], Hamsters[43], and Wistar rats[44]. Higher plasma HDL-C was found in rabbits fed with high cholesterol diet[45]. In spite of higher HDL-C, F group rats showed lower AAI when compared to C and F+MO groups. Epidemiological studies as well as studies in animal models of atherosclerosis support the cardio protective role of HDL-C[46]. However, functional defects in HDL may also contribute to atherosclerotic cardiovascular diseases. HDL does not prevent oxidation of LDL as well in diabetic patients, as it does in non-diabetics and HDL isolated from subjects with NIDDM exhibited a decreased capacity to induce cholesterol efflex[47]. Such type of functional defects of HDL-C may exist in fructose fed condition thus leading to the lower AAI in F group. But administration of AEMO

corrected dislipidemia and improved the AAI probably by rectifying the functional defects of HDL-C.

The dyslipidemia 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[48]. 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, glucagon, 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[49]. This explains the role of LDL in premature development of atherosclerosis under diabetic conditions[50]. Atherogenic Index indicates deposition of foam cells, plaque, fatty infiltration or lipids in heart, coronaries, aorta, liver and kidney. The higher the atherosclerotic index the greater the risk of these organs to oxidative damage. The protection by the administration of AEMO against the atherogenecity 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 atherogenecity in D group compared to F group can also be attributed to increased oxidative insult under hyperglycemic conditions than under IR conditions. The antihyperlipidemic and anti atherogenic 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 atherosclerotic plaque formation in artery and also possess lipid lowering activity in rabbits, fed with high cholesterol diet[45]. The hydroalcoholic extract of M. oleifera leaves exert notable cardio protective effects on myocardial infarction and possess myocardial preservative actions[51]. The crude extract of Moringa leaves has been reported to exhibit cholesterol lowering effect in high fat diet fed[11] and iron deficient rats[52] and in hyperlipedemics[53].

Numerous epidemiological studies suggest that herbs/diets rich in phytochemicals and antioxidants execute a protective role in health and disease[54]. Flavonoids, sterols, triterpenoids, alkaloids, saponins and phenolics are reported as bioactive antidiabetic principles[55,56]. Flavonoids can regenerate damaged β-cells in the alloxan induced diabetic rats[57]. Polyphenols inhibit lipid peroxidation by acting as chain breaking peroxyl radical scavengers and can protect LDL from oxidation[58] and also inhibit hepatic lipid synthesis[59]. Thus AEMO with its treasure of phytochemicals exhibited a protective role as antidiabetic and anti hyperlipidimic , 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.

REFERENCES

1. WHO, WHO Expert Committee on Diabetes Mellitus. Second Report Geneva. World Health Org 2000.
2. Valiathan MS. Healing plants. Current science 1998; 75: 112-26.
3. Nadkarni AK. Indian Materia Medica. Vol 1 Popular Prakashan: Bombay, 810-816.
4. Mughal MH, Ali G, Srivastava PS, Iqbal M. Improvement of drumstic (Moringa Pterygosperma Gaertn)- a unique source of food and medicine through tissue culture. Hamdard Med 1999; 42:37-42.
5 Anwar F, Bhanger MI. Analytical characterization of Moringra oleifera seed oils grown in temperate regions of Pakistan. J Agric Food Chem 2003; 51: 6558-6563.
6 Faizi S, siddiqui BS, Saleem R,Saddiqui S, Aftab K.Isolation and structure elucidation of new nitrile and mustard oil glycosides from Moringa oleifera and their effect on blood pressure. J Nat Prod 1994 (a): 57: 1256-1261.
7 Faizi S, siddiqui BS, Saleem R, Siddiqui S, Aftab K, Gilani AH. Fully acetylated carbamate and hypotensive thiocarbamate glycosides from Moringa oleifera. Phytochemistry1995; 38:957-963.
8 Pari L, Karamac M, Kosinska A, Rybarezyk A amarowicz R. Antioxidant activity of the crude extract of drumstick tree (Moringa oleifera) and sweet Broomweed (Scoparia dulcis) leaves. Polish Journal of Food and Nutrition Sciences 2007; 57:203-208.
9 Guevara AP, Vargas C, Sakurai H, et al. An antitumor promoter from Moringa oeifera Lam. Mutat Res1999; 440: 181-188.
10 Murakami A, Kitazona Y, Jiwajinda S, Koshimizu K, Ohigashi H. Niaziminin, a thiocarbamate from the leaves of Moringa oleifera , holds a srict structural requirement for inhibition of tumor-induced Epstein-Barr virus activation. Planta Med 1998; 64: 319-323.
11 Ghasi S, Nwobodo E, Ofili JO. Hypocholesterolemic effects of crude extract of leaf of Moringa oleifera Lam in high-fat diet fed Wistar rats. J. Ethnopharmacol 2000; 69:21-25.
12 Fakurazi S, Nanthini U, Hairuszah I. Hepatoprotective and Antioxidant action of Moringa oliefera Lam, against Acetaminophen induced hepatotoxicity in rats. International Journal of Pharmacology 2008; 4 Suppl 4: 270-275.
13 Lilibeth AC, and Glorina LP. Effects of Moringa oleifera Lam on the reproduction of male mice. Journal of Medicinal Plants Research 2010; 4 (120): 1115-1121.
14 Ndong M, Uhera M, Katsumata S, Suzuku K. Effects of oral administration of Moringa oleifera Lam on Glucose Tolerance in Goto- Kakizaki and Wistar rats. J Clin Biochem Nutr 2007; 40 (3):229-233.
15 Jaiswal D, Kumar Rai P, Kumar A, Mehta S, Watal G. Effect of Moringa oleifera Lam leaves aqueous extract therapy on hyperglycemic rats. J. Ethnopharmacology 2009; 123(3): 392-396.
16 Caceres A, Cabrera O, Morales O, Mollinedo P, Mendia P. Pharmacological properties of Moringa oleifera. Preliminary screening for antimicrobial activity. J Ethnopharmacol 1991; 33:213-216.
17 Brain KR and Turner TD . The practical evaluation of Phyto pharmaceuticals. Wright Scientechnica. Bristol, Britain 1975; p 56-64.
18 Sofowora A. Medicinal plants and traditional medicines in Africa. POPLINE Document no : 018476. John Wiley and sons Ltd (1982). New York.
19 Trease GE and Evans WC (1983). Pharmacognosy 12 th Ed. Bailer Tindall, London
20 Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis.Chapman and Hall. London 1976; 99-100,109-110, 144-147, 185-188.
21 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499-502
22 Guido S, Joseph J. Effect of chemically different calcium antagonists on lipid profile in rats fed on a high fat diet. Indian Journal of Experimental Biology 1992; 30: 292-294.
23 Pickavance LC, Tadayyon M, Widdowson PS, Buckingham RE, Wilding JP. Therapeutic index for rosiglitazone in dietary obese rats. Separation of efficacy and haemodiluation. Br J Pharmacol 1999; 128: 1570-76.
24 Duncan DB. Multiple range and multiple tests, Biometrics 1955; 42:1-42.

25 Kasim Karakas SE, Vriend H, Almario R, Chow LC, Goodman MN. Effects of dietary carbohydrates on glucose and lipid metabolism in golden Syrian hamsters. J Lab Ciln Med 1996; 128:208-213.
26 Reddy SS, Karuna R, Saralakumari D.Prevention of insulin resistance by ingesting aqueous extract of Ocimum sanctum to fructose fed rats. Horm Metab Res 2008; 40: 44-49
27 Kok N, Robertroid M, Deizenne N. Dietary oligo fructose modifies the impact of fructose on hepatic triacylglycerol metabolism. Metabolism 1996; 45: 1547-50.
28 Elliott SS, Keim NL, Stem JS, Teff K, Havel PJ. Fructose, weight gain, and the insulin resistance syndrome. Am J Clin Nutr 2002; 76: 911-22.
29 Shulman GL. Cellular machanisms of insulin resistance in humans. Am J Cardiol 1999; 84 (suppl 1A): 3J-10J.
30 Kim JK, Gavrilova O, chen Y,reitman ML,Shulman GI. Mechanism of insulin resistance in A-ZIP/F-1 fatless mice, J Biol Chem 2000; 275:8456-8460.
31 Bezerra RMN, Ueno M, Silva MS, Tavares DQ, Carvalho CRO, Saad MJA.A high fructose diet affects the early steps of insulin action in muscle and liver of rats. J Nutr 2000; 130: 1531-35.
32 Chatterje MN, Shinde R. Text book of medical biochemistry 4 nd edition. Jaypee Brothers medical publishers, New Delhi, 2000.
33 Cano DA, Rulifson IC, Heiser PW, Swigart LB, Pelengaris S, German M, Evan GI, Bluestone JA, Hebrok M. Regulated β-cell regeneration in the adult mouse pancreas. Diabetes 2008; 57: 958-66.
34 Yadav H, Jain S, Prasad GBKS, Yadav M. Preventive effect of Diabecon, a polyherbal preparation during progression of diabetes induced by high fructose feeding in rats. J Pharmacol Sci 2007; 105: 12-21.
35 Hsu JH, Wu YC, Liu IM, Cheng JT. Diascorea as the principal herb of Die-Huang-Wan, a widely used herbal mixture in China for improvement of insulin resistance in fructose rich chow-fed rats. J Ethnopharmacol 2007; 112(3): 577-84.
36 Ramesh B, Karuna R, Reddy SS, Ramesh K, Ramatholisamma P, Appa Rao CH, Saralakumari D. Antihyperglycemic and antioxidant activities of alcoholic extract of Commiphora mukul gum resin in Streptozotocin induced diabetic rats. Pathophysiology 2011; 18:255-261.
37 Busserolles J, Gueux E, Rock E, Mazur A, Rayssiguier Y. Substituting honey for refined carbohydrates protects rats from hypertriglyceridemic and prooxidative effects of fructose. J Nutr 2002; 132: 3379-82.
38 Kazumi T, Odaka H, Hozumi T, Ishida Y, Amano N, Yoshino G. Effects of dietary fructose or glucose on triglyceride production and lipogenic enzyme activities in the liver of Wistar fatty rats, an animal model of NIDDM. Endocr *J* 1997; 44: 239-45.
39 Remillard P, Shen G, Milne R, Maheux P. Induction of cholesterol ester transfer protein in adipose tissue and plasma of the fructose fed hamster . Life Sci 2001; 69: 677- 687.
40 Zavaroni I, Chen YDI, Reaven GM. Studies of the mechanism of fructose-induced hypertriglyceridemia in the rat. Metabolism 1982; 311: 1077-83.
41 Ginsberg HN, Huang LS. The insulin resistance syndrome: impact on lipoprotein metabolism and atherothrombosis. J Cardiovasc Risk 2000; 7: 325-331.
42 Benadoa M, Alcantaraa C, DeLa Rosa R, Ambrosea M, Mosiera K, Kerna M Effects Of various levels of dietary fructose on blood lipids of rats. Nutrition Research 2004; 24(7): 565-571.
43 Limin W, Jhu Y, Rosemary L, Waisem. High carbohydrate diets affect the size and composition of plasma lipoproteins in hamsters (Mesocricetus auratus) Comparative Medicine 2008; 58 (2): 151-160.
44 Mooradian Ad, Wong NC, Shah GN . Apolipoprotein AI expression in young and aged rats is modulated by dietary carbohydrates. Metabolism 1997; 46:1132-1136.
45 Pilaipark C, Panya K, Yupin S, Srichan P, Noppowan PM, Laddawal PN,Piyani R, Supath S, Klai USP. The in vitro and ex vivo antioxidant properties, hypolipidimic and antiatherosclerotic activities of water extract of Moringa oliefera Lam. Leaves. Journal of Ethnopharmacology 2008; 116:439-446.
46 Turner RC, Millins H, Neil HA, Stratton IM, Manley SE, Matthews DR, Holman RR. Risk factors for coronary artery disease in non insulin dependant diabetes mellitus;United Kingdom prospective Diabetes Study(UKPDS:23) . Br Med J 1998; 316:823-828.
47 Gowri MS, Van der Westhuyzen DR, Bridges SR, Anderson JW. Decreased protection by HDL from poorly controlled type 2 diabetic subjects against LDL oxidation may be due to the abnormal composition of HDL. ArteriosclerThromb Vasc Biol 1999; 19:2226-2233
48 Umesh Yadav CS, Moorthy K, Najma ZB. Combined treatment of sodium orthovanadate and Momordica charantia fruit extract prevents alteration in lipid profile and lipogenic enzymes in alloxan diabetic rats. Molecular and cellular Biochemistry 2005; 268:111-120.
49 Numano F, Tanaka A, Makita T, Kishi Y. Glycated lipoprotein and atherosclerosis. Ann NY Acad Sci 1997; 811: 100-114.
50 Retsky KL, Chen K, Zeind J, Frei B. Inhibition of copper induced LDL oxidation by vitamin C is associated with decreased copper binding to LDL and 2-oxo-histidine formation. Free Radic Biol Med 1999; 26: 90-8.
51 Nandave M, Ojha SK, Joshi S, kumara S and Arya DVS. Moringa oleifera leaf extract prevents Isoproterenol- Induced myocardial Damage in Rats: Evidence for an Antioxidant, Antoperoxidative, and Cardiopretective Intervention. Journal of Medicinal Food 2009; 12(1):47-55.
52 Ndong M, Uhera M, Katsumata S, Sato S, Suzuki K. Preventive effects of Moringa oleifera (Lam) on hyperlipidemia and hepatocyte ultrastuctural changes in iron deficient rats. Biosci Biotechnol Biochem 2007; 71(8): 1826-1833.
53 Nambiar V.S, Guin P, Parnami S, Daniel M. Impact of antioxidants from drumstick leaves on the lipid profile of hyperlipidemics . Journal of Herbal Medicine and Toxicology 2010; 4(1) 165-172.
54 Vinson J, Su X, Zubik L, Bose P. Phenol antioxidant quantity and quality in foods,fruit. J Agric and food Chem 2001; 49(11): 5315-5321.
55 Ivorra MD, Paya M, Villar A. A review of natural products and plants as Potent antidiabetic drugs. J Ethno pharmacol 1989; 27(3): 243-275.
56 Tiwari AK, Madhusudana RJ. Diabetes mellitus and multiple therapeutic approaches of Phytochemicals: present status and future prospects. Current science 2002; 83:30-38.
57 Chakravarthy BK, Gupta S, Gambir SS, Gode KD. Pancreatic beta cell regeneration. A novel antidiabetic mechanism of Pterocarpus marsupium Roxb. Ind J Pharmacol 1980; 12: 123-27.
58 O Byme DJ, Devaraj S, Grundy SM, Jialal L. Comparison of antioxidant effects of Concord grape juice flavanoids and α-tocopherol on markers of oxidative stress in healthy Adults. American Journal of Clinical Nutrition 2002; 76: 1367-1374.
59 Theriault AG, Wang Q, Van Iderstine SC, Chen B, Franke AA, Adeli K. Modulation of hepatic lipoprotein synthesis and secretion by taxifolin, a plant flavanoid. Journal of Lipid Research 2000; 41: 1969-1979.