ISSN: 2278-5213

RESEARCH ARTICLE

Impact of *Moringa oleifera* Lam. leaf powder on the altered lipid profile of diabetic mice

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Abstract

Effect of leaf powder of *Moringa oleifera* Lam. on lipid profile in normal and diabetic mice was investigated. Diabetes was induced in female mice (25 ± 5 g) by intraperitoneal injection of alloxan monohydrate. Both normal and diabetic mice were fed with standard animal food containing 40% of *M. oleifera* leaf powder for two weeks. In control mice fed with leaf powder of *M. oleifera* significantly reduced Low Density Lipoprotein-Cholesterol (LDL-C) from 26.9 ± 3.2 (mg/dL) to 15.1 ± 2.2 (mg/dL) and significant increase of High Density Lipoprotein Cholesterol (HDL-C) from 17.6 ± 0.9 (mg/dL) to 19.2 ± 0.3 (mg/dL). In diabetic mice, reduction was recorded in serum triglyceride (TG) from 156.2 ± 3.8 (mg/dL) to 101.2 ± 2.2 (mg/dL) levels and decrease in total cholesterol from 188.2 ± 2.8 (mg/dL) to 122.2 ± 2.2(mg/dL) when mice fed with *M. oleifera* leaf powder. These results indicate that *M. oleifera* leaf powder improved serum lipid profile in normal and diabetic mice by decreasing TG, LDL-C and increasing serum HDL-C and may be of great value in managing the diabetic complications.

Keywords: *Moringa oleifera*, diabetes, serum triglyceride, total cholesterol, diabetic complications.

Introduction

Diabetes mellitus is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and protein and an increased risk of complication of vascular diseases (Rodrigues and McNeill, 1992; Pickup and Williams, 1991). The minimum defining characteristic feature to identify diabetes mellitus is chronic and substantiated elevation of circulating glucose concentration (Levy *et al.,* 2000). Diabetes mellitus is associated with a large number of lipid abnormalities. Emerging evidence confirms the crucial role of hyperlipidemia, mainly elevated blood cholesterol, particularly LDL cholesterol and VLDL cholesterol in the development of atherosclerosis-related disease (Rang *et al.,* 2003). Significant abnormalities in lipid metabolism and lipoproteins in diabetes are evident which in turn depend on the extent of insulin deficiency, insulin resistance obesity (Pessin and Saltiel, 2000), diet and the presence of associated primary and other secondary causes of hyperlipidemia (Ohno *et al,* 2000). In diabetic hyperlipidemia, a series of peculiar lipoproteins and other lipids appear and interaction of this with oxidative stress (Rao *et al.,* 2003) and free radicals leads to enhanced lipid peroxidation in plasma, tissues and membranes, causing extensive tissue damage. It is well known that lipid peroxidation provides a continuous supply of free radicals that play an important role in etiopathogenesis of diabetes and its complications (Wild *et al.,* 2004). *Moringa oleifera*, locally known as shajna, belongs to the monogeneric family Moringaceae and is widely distributed in the Indo-Bangla subcontinent and cultivated throughout the tropical belt (Nikkon *et al.,* 2003).

Different parts of this plant are used in the indigenous systems of human medicine for the treatment of a variety of human aliments. Ethanolic leaf extract of *M. oleifera* is used as a hypotensive agent (Akhtar and Ahmad, 1995). The leaves of *M. oleifera* are reported to be used as a hypocholesterolemic and hypoglycemic agent (Dangi *et al.,* 2002). *Moringa oleifera* leaves are highly nutritious, being a significant source of β-carotene, vitamin C, protein, iron and potassium. The leaves are cooked and used like spinach. In addition to being used fresh as a substitute for spinach, its leaves are commonly dried and crushed into a powder and used in soups and sauces. Amino acids in green leafy vegetables vary considerably and many that are staples are low in the sulphur bearing amino acids namely methionine and cystine (Gassenschmidt *et al.,* 1995).

Various therapeutic methods used in diabetes treatment available today achieve transiently regulated euglycemia but fail to prevent lipid and lipoprotein alterations, ultimately, exposing the diabetic humans and animals to cardiovascular complications (Grant *et al.,* 1995). Moreover, many of these drugs exert various side/toxic effects such as hepatotoxicity or cardiac failure (Une *et al.,* 2008). Concurrently, phytochemicals identified from traditional medicinal plants are presenting an exciting opportunity for the development of new types of therapeutics. This has accelerated the global effort to harness and harvest those medicinal plants that bear substantial amount of potential phytochemicals showing multiple beneficial effects in combating diabetes and the related complications without causing side effects (Palanduz *et al.,* 2001).

In recent years, search for natural dietary therapeutic methods for controlling diabetes are much active as diet plays a key role in the treatment of diabetes (Levy *et al.,* 2000). The protective effect of leaf powder of *M. oleifera* in controlling the complications of diabetes related to hyperlipidemia has not been worked out extensively. Against these backdrops, in this study, the effect of leaf powder of *Moringa oleifera* on diabetes related to hyperlipidemia has been investigated by evaluating its hypolipidemic properties in alloxan induced diabetic mice.

Materials and methods

Experimental animals: Three month old female Swiss Albino mice (Body weight: 25 ± 5 g) were obtained from CDRI Lucknow were maintained at the animal house of University Dept. of Zoology, Bhagalpur, Bihar. They were separated and kept in stainless steel cages in a temperature and humidity controlled with 12 h light/dark cycle. Food and water were given to the animal according to their need. All the animals were kept as accepted principles for laboratory animal use and care as per the guidelines of CPCSEA. The mice were acclimatized for one week before the experiment and then used in experiment at about 12 weeks of age.

Leaf powder and extract preparation: Powder product of *Moringa oleifera* Lam. leaves were purchased from (Sanjeevani Herbals), Salem-Impex, B-39-TNHB, Salem, Tamil Nadu. This company sold product commercially for medicinal and nutritional purposes. It is a spray dried product in powder form, standard in quality and a part of bulk production by the industry. The typical nutrition profile of *Moringa oleifera* Lam. leaves is given in Table 1 and 2.

Drugs and chemicals: The drug alloxan monohydrate (Loba chemical, Mumbai) was purchased from commercial sources. All other chemicals were analytical grade and used as such without further testing.

Induction of diabetes: Experimental animals were kept on fast for 18 h prior to induction of diabetes. Diabetes was induced by intra-peritoneal administration of Alloxan monohydrate (Rodriguez *et al.,* 1999). The total dose of Alloxan-monohydrate (450 mg/kg/bw) was administered in three injections at intervals of 48 h (150 mg/kg/bw each time).

Table 1. Nutrient profile of *Moringa oleifera* Lam. leaf powder (100 g).

Nutrient	Fresh leaves	Dry leaves	Nearest products
Vitamin A (mg)	6.8	18	Carrot: 1.8 mg
Vitamin C (mg)	220	15	Orange :30 mg
Calcium (mg)	440	2000	Milk: 120 mg
Iron (mg)	0.85	28.2	Spinach: 1.14 mg
Potassium (mg)	259	1320	Banana: 88 mg
Protein (g)	6.7	27.9	Yogurt: 3.1 g

Table 2. Trace elements profile of *Moringa oleifera* Lam. leaf powder (100 g).

Trace element	Amount
Fiber (g)	4.8
Carbohydrates (g)	3.7
Protein (g)	2.5
Minerals (g)	2.0
Fat (g)	0.1
Chlorine (mg)	423
Potassium (mg)	259
Vitamin C (mg)	120
Calcium (mg)	30
Magnesium (mg)	28
Calories	26
Niacin (mg)	0.2
Iron (mg)	0.18
Zinc (mcg)	0.16
Copper (mcg)	0.01
Manganese (mcg)	0.05
Thiamine (mg)	0.05
Riboflavin (mg)	0.07
Phosphorus (mg)	110
Carotene (mg)	110
Chromium (mcg)	0.003
Sodium (g)	0

Experimental design: Experimental animals were divided in to four groups of 6 animals in each group. Group-I (Control), Group-II (Diabetic control), Group-III (Diabetic control mice fed with leaf powder of *Moringa oleifera* Lam.), Group-IV (Control mice fed with leaf powder of *Moringa oleifera* Lam.). The total experimental protocol was maintained for 14 d after induction of diabetes (Nambiar and Seshadri, 2001).

Biological assays: Blood from mice was collected by cardiac puncture every time after the completion of treatment period. Blood was collected in the vancutainer tubes containing no anti-coagulant. Blood (1.5 mL) were centrifuged for 15 min at 1500 rpm speed and supernatant (serum) was carefully aspirated at room temperature and used for the further experiment (Henry, 1979). Serum was used for the estimation of lipid profile. Total cholesterol (TC) and HDL (direct method), LDL and VLDL levels were calculated as per Fridevald's equation (Friedevald *et al.,* 1972).

VLDL cholesterol = Serum triglyceride/5

LDL = Total cholesterol – (HDL-C + VLDL-C).

Results and discussion

Among diabetic complications, the abnormalities produced in lipids and proteins are the major etiologic factors. In diabetic patients, extracellular and long lived proteins, such as elastin, laminin and collagen are the major targets of free radicals. These proteins are modified to form glycoproteins due to hyperglycemia. The modification of these proteins present in tissues such as lens, vascular wall and basement membranes are associated with the development of complications of diabetes such as cataracts, microangiopathy, atherosclerosis and nephropathy.

Table 3. Effect of treatment with leaf powder of *Moringa oleifera* Lam. for two weeks on lipid profile parameters.

Parameters	Period (d)	Group-I	Group-II	Group-III	Group-IV
Total cholesterol (mg/dL)	0	80.30 ± 1.8	188.2 ± 2.8	188.2 ± 2.8	80.28 ± 1.7
	7	81.28 ± 1.9	191.2 ± 1.7	160.1 ± 1.6	80.32 ± 2.1
	14	81.8 ± 2.1	193.7 ± 2.2	122.2 ± 2.2	80.40 ± 1.2
Triglyceride (mg/dL)	0	71.8 ± 2.9	156.2 ± 3.8	156.2 ± 3.8	71.8 ± 2.8
	7	71.7 ± 3.1	156.3 ± 2.2	130.1 ± 2.7	71.6 ± 2.2
	14	72.1 ± 2.8	155.8 ± 2.1	101.2 ± 2.2	71.8 ± 2.1
HDL (mg/dL)	0	26.9 ± 3.2	15.6 ± 0.9	15.6 ± 0.9	26.8 ± 3.3
	7	26.8 ± 2.6	15.2 ± 2.1	18.6 ± 0.7	26.6 ± 3.2
	14	26.6 ± 2.2	15.1 ± 2.2	20.2 ± 0.3	26.6 ± 3.3
LDL (mg/dL)	0	39.04 ± 0.5	141.36 ± 0.9	141.36 ± 0.8	39.12 ± 0.5
	7	40.14 ± 0.2	144.74 ± 0.8	115.48 ± 0.8	39.4 ± 0.8
	14	40.78 ± 0.8	147.44 ± 0.6	81.76 ± 0.3	39.44 ± 0.6
VLDL (mg/dL)	0	14.36 ± 3.2	31.24 ± 2.9	31.24 ± 2.9	14.36 ± 3.2
	7	14.34 ± 3.1	31.26 ± 2.7	26.02 ± 2.1	14.32 ± 2.8
	14	14.42 ± 3.2	31.16 ± 2.8	20.24 ± 1.8	14.36 ± 2.7

During diabetes, lipoproteins are oxidized by free radicals. There are also multiple abnormalities of lipoprotein metabolism in very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) in diabetes. Lipid peroxidation is enhanced due to increased oxidative stress in diabetic condition (Glugliano *et al.,* 1996). In this study, serum triglycerides, phospholipids, cholesterol, LDL cholesterol, VLDL cholesterol and atherogenic index were significantly elevated while HDL cholesterol were significantly decreased in diabetic mice (Table 3). Interestingly, the results further indicated that all these lipid and lipoprotein abnormalities were countered by leaf powder of *Moringa oleifera* Lam. in diabetic mice. Glycemic control appears to be the major determinant of total and VLDL triglyceride concentrations (Kumari *et al.,* 1995). Diabetes is a multi-factorial disease leading to several complications and therefore demands a multiple therapeutic approach. Patients of diabetes either do not make enough insulin or their cells do not respond to insulin. In case of total lack of insulin, patients are given insulin injections whereas, in case of those where cells do not respond to insulin many different drugs are developed taking into consideration possible disturbances in carbohydrate and lipid metabolism. For example, to manage post-prandial hyper-lipidemia at digestive level, glucosidase inhibitors such as acarbose, miglitol and voglibose are used and these inhibit degradation of carbohydrates thereby reducing the glucose absorption by the cells. To enhance glucose uptake by peripheral cells, biguanide such as metphormine is used. Sulphonylureas like glibenclamide is insulinotropic and works as secretogogue for pancreatic cells. Although several therapies are in use for treatment, there are certain limitations due to high cost and side effects such as development of hypoglycemia, weight gain, gastrointestinal disturbances and liver toxicity (Dey *et al.,* 2002).

Based on recent advances and involvement of oxidative stress in complicating diabetes mellitus, efforts are on to find suitable antidiabetic and antioxidant therapy. In adipocytes, glucose is stored primarily as lipid, owing to increased uptake of glucose and activation of lipid synthetic enzymes, including private dehydrogenase, fatty acid synthase and acetyl-CoA carboxylase. Insulin also profoundly inhibits lipolysis in adipocytes, primarily through inhibition of the enzyme hormone-sensitive lipase (Anthonsen *et al.,* 1998). However, in some cases of diabetes, treatment with insulin with normalization of plasma glucose levels did not restore the HDL-C concentrations to normal implying that, factors in addition to hyperglycemia are causing the lower HDL-C (Arora *et al.,* 2002). Synthesis of VLDL is promoted by an increase in the flux of free fatty acids in liver and ultimately the particles are converted to LDL. The increased circulatory VLDL-C and the associated triglycerides due to defective clearance of these particles from circulation are in agreement with earlier studies of Babu and Srinivasan (1987) and these changes were attributed to the altered activity of lipoprotein lipase. It seems that the changes in adipose tissue lipolysis or intra-hepatic mechanisms involving other changes in fractional esterification of fatty acids are in the assembly or secretion of VLDL is responsible for the increase in triacylglycerol secretion rate. The characteristic lipid abnormality is hypertriglyceridemia with associated increase in plasma cholesterol. Elevated plasma triglyceride concentration is seen in type 2 DM either due to triglyceride over-production and/or underutilization. Lipoprotein lipase activity is markedly impaired, besides, a significant improvement in LDL internalization and degradation suggesting that chemical modification of LDL particle like non-enzymatic glycation of LDL itself might result in its increased incorporation in the arterial wall via a receptor independent pathway.

Studies have strongly suggested an inverse relationship of HDL cholesterol with atherosclerosis to be independent of other lipid abnormalities (Ferretti *et al.,* 2002). Insulin has important effects on key steps in the metabolism of lipids and lipoproteins and alterations in lipid metabolism are common in diabetic population (Lyons, 1992). A decrease in HDL turnover has been shown in diabetes. Some reports revealed that non-enzymatic glycosylation of HDL accelerates its catabolism in guinea pigs and the same mechanism might be responsible for the low levels of HDL in diabetic mice observed in the present study (Gordon *et al.,* 1997). In the present study, cholesterol, triglycerides and free fatty acids were brought down significantly by leaf powder of *Moringa oleifera* Lam. feeding in diabetic mice (Table 3). This effect could be partly due to the control of hyperglycemia. Elevated LDL, VLDL and decreased HDL cholesterol concentrations in diabetic mice appear to be markedly altered favorably by leaf powder of *M. oleifera* leaves supplementation. The entire lipid abnormalities developed in alloxan induced diabetic mice were effectively countered by feeding leaf powder of *M. oleifera* leaves. Certain specific phytochemicals such as flavonoids (Robak and Gryslenski, 1988) and fiber (Madar, 1983) might be playing a role in rectifying the abnormalities. The components of leaf powder of *M. oleifera* leaves might also be influencing lipoprotein associated cholesterol fractions and probably the phytocomponents exert action similar to the drugs cholestyramine, mevanolin, lovastatin and simvastatin that are used for correcting the imbalance in plasma lipoproteins in diabetes. Consumption of plant material like leaf powder of *M. oleifera* containing antioxidants i.e. vitamin (A, C, E), (carotenoids) and (phytonutrients) increases the antioxidant status in mice blood and tissues and these compounds are capable of modulating LDL oxidation through several mechanisms. Studies have demonstrated that vitamin C is more potent in preventing LDL oxidation than vitamin E and a combination of the two vitamins is even more effective than either alone. Levy *et al.* (2000) observed that dietary supplementation of natural β-carotene normalized the elevated LDL cholesterol oxidation and thereby reduced the risk of development of atherosclerosis in diabetes. As β-carotene is the chief constituent in leaf powder of *M. oleifera* it can be assumed that atherosclerotic preventive role was exerted by leaf powder of *M. oleifera* by inhibiting LDL cholesterol oxidation. The crude extract of *Moringa* leaves has a significant cholesterol lowering action in the serum of high fat diet fed mice which might be attributed to the presence of a bioactive phytoconstituent, i.e. sitosterol (Ghasi *et al.,* 2000). *Moringa* fruit has been found to lower the serum cholesterol, phospholipids, triglycerides, low density lipoprotein, very low density lipoprotein cholesterol to phospholipid ratio, atherogenic index lipid and reduced the lipid profile of liver, heart and aorta in hypercholesteremic rabbits and increased the excretion of fecal cholesterol (Mehta *et al.,* 2003).

Conclusion

It is evident from the study, that leaf powder of *Moringa oleifera* Lam. efficiently regulated blood glucose in diabetic mice and very efficiently ameliorated lipid abnormalities associated with diabetes in alloxan induced diabetic mice by virtue of its essential antioxidant, antidiabetic compounds and phytonutrients. The synergistic role played by these compounds is attributed to the protection of diabetic mice against lipid abnormalities.

Acknowledgements

This study has been funded by UGC for UGC-JRF.

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