

EVALUATION OF ANTIHYPERLIPIDEMIC AND ANTIDIABETIC ACTIVITY OF HYDRO-ALCOHOLIC EXTRACTS OF *MORINGA OLIEFERA* SEEDS IN HIGH FAT DIET INDUCED RAT MODEL

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ABSTRACT

The aim of this study was to investigate the Antihyperglycemic and antihyperlipidemic activities of aqueous and ethanol extracts of seeds of *Moringa oleifera* in alloxan (ALX) induced diabetic rats. Diabetes was confirmed after 5 days of single intraperitoneal injection of ALX (150 mg/kg) in albino Wister rats. Aqueous and ethanol extracts of seeds of *Moringa oleifera* (200 and 400 mg/kg) and Glibenclamide (10 mg/kg, p.o.) orally administered daily for 15 days, blood was withdrawn for glucose determination on 0, 1, 10 and 15 days respectively. Five days before the termination of the experiment, the oral glucose tolerance test (OGTT) was performed to assess the glucose tolerance. For this purpose, overnight fasted rats were fed glucose (2 g/kg) orally and blood was collected at 0, 30, 60 and 120 min interval from orbital sinus for glucose estimation. On the 15th day, overnight fasted rats were sacrificed and blood was collected for the determination of high density lipoproteins cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), total cholesterol (TC), total glycerides (TG) and total proteins (TP). Aqueous and ethanol extracts of seeds of *Moringa oleifera* at doses of 200 and 400 mg/kg showed significant reduction in blood glucose, lipid when compared to diabetic control group. We concluded that aqueous and ethanol extracts of seeds of *Moringa oleifera* possess antihyperglycemic, antihyperlipidemic activities. The histopathology study of pancreas was also performed which showed hypoglycemic effect.

KEYWORDS: *Moringa oleifera*, antihyperglycemic, antihyperlipidemic.**INTRODUCTION**

Diabetes mellitus is a common and very prevalent disease affecting the citizens of both developed and developing countries. It is estimated that 25% of the world population is affected by this disease. Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin.

Many Indian plants have been investigated for their beneficial use in different types of diabetes and reports occur in numerous scientific journals. Ayurveda and other traditional medicinal system for the treatment of diabetes describe a number of plants used as herbal drugs. Hence, they play an important role as alternative medicine due to less side effects and low cost. The active principles present in medicinal plants have been reported to possess pancreatic beta cells re-generating, insulin releasing and fighting the problem of insulin resistance.

Moringa oleifera is the most widely cultivated species in the genus *Moringa*, the only genus in the plant family

Moringaceae. Common names include moringa, drumstick tree (from the long, slender, triangular seed-pods), horseradish tree (from the taste of the roots, which resembles horseradish), and ben oil tree or benoil tree (from the oil which is derived from the seeds).

M. oleifera is a fast-growing, drought-resistant tree, native to the southern foothills of the Western Ghats in southwestern India, and widely cultivated in tropical and subtropical areas where its young seed pods and leaves are used as vegetables, and many parts of the tree are used in traditional herbal medicine. It can also be used for water purification.^[1]

MATERIAL AND METHODS**Plant material**

The seeds of *Moringa Oleifera* were collected from the local area of Allahabad District, Uttar Pradesh, India in the month of June-July 2017.

Preparation of plant extract

The seeds were air dried at room temperature and the dried seeds were grind into fine powder with an auto-mix blender. The powdered part was kept in a deep freezer until the time use. Powder was further extracted by ethanol by maceration technique.

Animals

Healthy, adult Albino Wistar rats (180-200gm) of either sex were purchased from the National Center for Laboratory Animal sciences, Hyderabad used for study. Housed individually in polypropylene cages, maintained under standard conditions (12 h light; and 12 h dark cycle; 23±2° C, 50± 5%, relative humidity), they were fed with standard rat pellet diet (Hindustan Lever Ltd; Mumbai, India) and were ad libitum. The Institutional Animal Ethics Committee approved the study.

Acute toxicity study

The acute oral toxicity study has to be carried out as per the guidelines set by OECD, revised draft guidelines 423, received from CPCSEA, ministry of social justice and empowerment, Govt of India.

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The test substance is administered in a single dose by using a stomach tube or a suitable intubation canula. In the unusual circumstance that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours.

Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. The flow charts of Annex 2 describe the procedure that should be followed for each of the starting doses.

Preparation of extracts dosage form

Extract dose of crude drug were freshly prepared as a fine homogenized suspension in aqueous.

Antidiabetic activity

The rats were randomized into seven groups comprising of six animals in each groups as given below.

Group I: normal control rats were given 0.5% Tween 80 for 15 days.

Group II: Diabetic controls have been given 0.5% Tween 80 for 15 days, 5 days after alloxan (150mg/kg, i.p.) treatment.

Group III: Rats have been given Glibenclamide (10mg/kg/day, p.o.) for 15 days, 5 days after alloxan (150mg/kg, i.p.) treatment.

Group IV: Test rats have been given ethanol extract of *Moringa oliefera* (200mg/kg, p.o.) for 15 days, 5 days after alloxan (150mg/kg, i.p.) treatment.

Group V: Test rats have been given ethanol extract of *Moringa oliefera* (400mg/kg, p.o.) for 15 days, 5 days after alloxan (150mg/kg, i.p.) treatment.

Group VI: Test rats have been given aqueous extract of *Moringa oliefera* (200mg/kg, p.o.) for 15 days, 5 days after alloxan (150mg/kg, i.p.) treatment.

Group VII: Test rats have been given aqueous extract of *Moringa oliefera* (400mg/kg, p.o.) for 15 days, 5 days after alloxan (150mg/kg, i.p.) treatment.

Oral glucose tolerance test

Five days before the termination of the experiment, the oral glucose tolerance test (OGTT) was performed to assess the glucose tolerance. For this purpose, overnight (18 h) fasted rats were fed glucose (2 gm/kg) orally and blood was collected at 0, 30, 60 and 120 minute interval from orbital sinus for glucose estimation.

Assessment of Oral glucose tolerance test

Blood samples were collected from tail puncturing of each rat at 0 minute, 30 minute, 60 minute and 120 minute and blood glucose was estimated by glucose estimation kit. Percent reduction in blood glucose was calculated with respect to the initial level.

Assessment of Anti-diabetic activity

Blood samples were collected from tail puncturing of each rat at 0 day, 1st day, 10th day and 15th day and blood glucose was estimated by glucose estimation kit. Percent reduction in blood glucose was calculated with respect to the initial level.

Assessment of Antihyperlipidemic activity

At the end of 15th day, blood was collected by heart puncture and serum was separated for the estimation of total cholesterol, HDL, LDL, VLDL, total Glycerides, total serum protein, albumin, globulin etc and the liver was isolated from the rats of all the groups and kept in 10% formalin solution and hence send for histopathological investigation.

RESULTS

Table 1.1: Effect of ethanol and aqueous extracts of seeds of *Moringa oleifera* on lipid profiles and total proteins in diabetic rats.

Parameter	Normal	High Cholesterol Diet Control	High cholesterol diet treated with <i>Moringa oleifera</i> Extract (200-400 mg/kg b.w.,p.o.)
Cholesterol (mg/dl)	65.82 ± 1.90	378.73 ± 4.99	282.02 ± 6.38
Triglyceride (mg/dl)	77.83 ± 7.89	181.79 ± 10.24	125.89 ± 05.23
HDL-C (mg/dl)	2.08 ± 1.20	1.56 ± 0.90	1.54 ± 0.89
LDL (mg/dl)	6.21 ± 6.57	447.38 ± 21.65	321.17 ± 26.10
VLDL (mg/dl)	17.48 ± 0.48	36.35 ± 1.29	25.17 ± 0.50
HDL-ratio	178.49 ± 14.52	4.47 ± 0.35	13.1 ± 00.61
Atherogenic Index	2.08 ± 0.10	8.56 ± 0.09	4.25 ± 0.20

Values are expressed as mean ± SEM for (n=6) rats in each group, when compared to control **p<0.01, *p<0.05 and ^{ns}p>0.05.

Table 1.2: Effect of ethanol and aqueous extract of SEEDS of *Moringa oleifera* on OGTT of diabetic rats.

Groups	Treatment / mg/kg	Blood glucose levels (mg/dl)			
		0 min	30 min	60 min	120 min
I	Normal control	90 ± 1.3	126 ± 1.2 (↑40.0%)	117 ± 1.4 (↓30.0%)	93 ± 0.9 (↓13.33%)
II	Glibenclamide, 10 mg/kg	234 ± 1.9	295 ± 2.26 (↑26.06%)	291 ± 1.5 (↑24.35%)	287.4 ± 2.6 (↓22.64%)
III	Control, 0.5% Tween 80	179.2 ± 0.8	208.9 ± 1.6 (↑16.57%)	222 ± 1.02 (↑23.88%)	186.1 ± 0.8 (↓13.85%)
IV	Ethanol extract 200 mg/kg	166.3 ± 0.9	212.6 ± 1.09 (↑27.84%) _a	239.9 ± 0.9 (↑44.25%)	196.1 ± 0.78 (↓17.91%)
V	ethanol extract 400 mg/kg	183.3 ± 0.6	212.6 ± 1.05 (↑15.98%) _a	248.3 ± 0.76 (↑35.46%)	189.9 ± 1.5 (↓13.6%) _a
VI	Aqueous extract 200 mg/kg	209.1 ± 1.4	241.5 ± 0.6 (↑15.49%)	261.6 ± 0.56 (↑25.10%)	192 ± 0.96 (↓8.18%)
VII	Aqueous extract 400 mg/kg	195.5 ± 2.1	^s 227.7 ± 0.76 (↑16.47%) _a	236 ± 0.11 (↑20.71%)	180.1 ± 0.7 (↓12.77%)

Table 1.3: Effect of ethanol and aqueous extracts of seeds of *Moringa oleifera* on blood glucose level in alloxan induced diabetic rats.

Groups	level Blood sugar in Group (15 days) mg/dL (mean ± SD)				
	Initial	Day 1	Day 5	Day 10	Day 15
Normal	70.78 ± 7.03	65.05 ± 9.33	66.70 ± 9.85	67.00 ± 7.41	65.48 ± 5.88
Control Glibenclamide, 10 mg/kg	249.76 ± 8.85	262.28 ± 14.75	285.85 ± 4.78	309.20 ± 8.09	180.21 ± 8.68
Control, 0.5% Tween 80	250.85 ± 8.40	252.49 ± 5.57	239.23 ± 8.42	204.38 ± 5.84	313.28 ± 4.73
Ethanol extract 200 mg/kg	248.04 ± 3.89	249.65 ± 7.85	221.24 ± 5.41	189.10 ± 8.22	178.14 ± 9.30
Ethanol extract 400 mg/kg	249.70 ± 8.85	256.08 ± 4.98	239.88 ± 8.84	214.23 ± 3.33	154.85 ± 10.24
Aqueous extract 200 mg/kg	251.84 ± 4.90	256.57 ± 5.57	233.45 ± 6.30	192.77 ± 4.89	192.03 ± 5.80
Aqueous extract 400 mg/kg	248.38 ± 3.50	251.17 ± 8.14	217.97 ± 4.52	190.10 ± 7.91	182.85 ± 4.58

Mean ± SEM for (n=6) rats in each group, when compared to control **p<0.01 and ^{ns}p>0.05.

Table 1.4: Effect of ethanol and aqueous extracts of seeds of *Moringa oleifera* on lipid profiles and total proteins in diabetic rats.

Groups	Biochemical parameters (mg/dl)						
	TC	HDL-C	LDL-C	VLDL-C	TG	Total protein	Albumin
Normal control	118.5 ± 0.2236**	44.5 ± 0.2236**	48.5 ± 0.2236*	24.5 ± 0.2236**	121.5 ± 0.2236**	6.245 ± 0.0022**	3.335 ± 0.0022**
Glibenclamide 10 mg/kg	122.5 ± 0.2236**	46.5 ± 0.2236**	46.5 ± 0.2236**	20.5 ± 0.2236**	145.5 ± 0.2236**	6.45 ± 0.0224**	3.35 ± 0.0224**
Diabetic Control	140.5 ± 0.2236	41.5 ± 0.2236	66.5 ± 0.2236	32.5 ± 0.2236	157.5 ± 0.2236	5.65 ± 0.0224	5.25 ± 0.0224
Ethanol extract 100 mg/kg	120.5 ± 0.2236**	43.5 ± 0.2236**	45.5 ± 0.2236**	28.5 ± 0.2236**	144.5 ± 0.2236**	6.205 ± 0.0022**	3.345 ± 0.0024**
Ethanol extract 200 mg/kg	115.5 ± 0.2236**	45 ± 0.4472**	48.5 ± 0.2236**	30.35 ± 0.02236**	148.5 ± 0.2236**	6.75 ± 0.0224**	3.315 ± 0.0024**
Aqueous extract 100 mg/kg	125.5 ± 0.2236**	41.5 ± 0.2236 ^{ns}	44.5 ± 0.2236**	24.25 ± 0.02236**	120.5 ± 0.2236**	6.65 ± 0.0224**	3.65 ± 0.0224**
Aqueous extract 200 mg/kg	117.5 ± 0.2236**	43.5 ± 0.2236**	50.5 ± 0.2236**	22.5 ± 0.2236**	115.5 ± 0.2236**	6.45 ± 0.0224**	2.75 ± 0.0224**

Values are expressed as mean ± SEM for (n=6) rats in each group, when compared to control **p<0.01 and ^{ns}p>0.05.

Diabetes mellitus is a common and very prevalent disease affecting the citizens of both developed and developing countries. It is estimated that 25% of the world population is affected by this disease. Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. The herbal drugs with antidiabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine.

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (that is beta cells) when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type 1 diabetes in humans. Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid.^[2] The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction. One study suggests that alloxan does not cause diabetes in humans. Others found a significant difference in alloxan plasma levels in children with and without diabetes Type 1.^[3]

Because it selectively kills the insulin-producing beta-cells found in the pancreas, alloxan is used to induce diabetes in laboratory animals.^[4] This occurs most likely because of selective uptake of the compound due to its structural similarity to glucose as well as the beta-cell's highly efficient uptake mechanism (GLUT2).^[5] Some studies have shown that alloxan is not toxic to the human beta-cell, even in very high doses, probably because of differing glucose uptake mechanisms in humans and rodents. Alloxan is, however, toxic to the liver and the kidneys in high doses.^[6]

Insulin is also the principal control signal for conversion of glucose to glycogen for internal storage in liver and muscle cells. Lowered glucose levels result both in the reduced release of insulin from the β -cells and in the reverse conversion of glycogen to glucose when glucose levels fall. This is mainly controlled by the hormone glucagon, which acts in the opposite manner to insulin. Glucose thus forcibly produced from internal liver cell stores (as glycogen) re-enters the bloodstream; muscle cells lack the necessary export mechanism. Normally, liver cells do this when the level of insulin is low (which normally correlates with low levels of blood glucose).^[7]

Phytochemical studies have revealed the presence of several phytochemicals including alkaloids, glycosides, flavonoids, steroids, phenolic compounds and tannins. The percentage yield of aqueous and ethanol extracts of seeds and seeds were found to be more than the other extracts.^[8] Polyphenoles are the major plant compounds with high level of antioxidant activity due to their ability to absorb, neutralize and to quench free radicals as well as their redox properties presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation. Results obtained in the present study revealed that the levels of these phenolic compounds in the aqueous and ethanol extracts of seeds and seeds of *Moringa oleifera* were considerable.^[9]

Higher insulin levels increase some anabolic ("building up") processes, such as cell growth and duplication, protein synthesis, and fat storage. Insulin (or its lack) is the principal signal in converting many of the bidirectional processes of metabolism from a catabolic to an anabolic direction, and *vice versa*. In particular, a low insulin level is the trigger for entering or leaving ketosis (the fat-burning metabolic phase).

The diabetogenic agent Alloxan is a hydrophilic and chemically unstable pyrimidine derivative, which is toxic to pancreatic β -cells because it can generate toxic free oxygen radicals during redox cycling in the presence of reducing agents such as glutathione and cysteine. The increase in oxygen free radicals in diabetes could be due to increase in blood glucose levels, which generates free radicals due to auto oxidation. In the present work, involvement of free radicals in progression of disease and protective effects of *zygium cuminis* has been examined. Administration of ethanol and aqueous extracts of *Moringa oleifera* for 15 days showed significant antidiabetic, antihyperlipidemic and antioxidant activities in Alloxan induced diabetic rats.^[10]

Hyperlipidemia is one of the major cardiovascular risk factors. It has been demonstrated that insulin deficiency in diabetes mellitus leads to a variety of derangements in metabolic and regulatory processes, which in turns leads to accumulation of lipids such as Total Glycerides and total cholesterol in diabetic patient, diabetes mellitus alters the normal metabolism of cholesterol and triglycerides showed an increase in alloxan induced diabetic rats. Under normal conditions, the enzyme lipoprotein lipase hydrolyses triglycerides. Diabetes mellitus results in failure to activate this enzyme thereby causing hypertriglyceridemia. Our data showed in line with notion as the Alloxan (140mg/kg, i.p.) treated diabetic rats exhibited clear cut abnormalities in lipid metabolism as evidenced from the significant elevation of serum TG, TC, LDL-C, VLDL-C and reduction of HDL-C levels. Treatment with ethanol and aqueous extracts of *Moringa oleifera* for 15 days was sufficient to produce a significant reduction in the TG, TC, LDL-C, VLDL-C and significant increase in HDL-C levels in

diabetic rats. These results indicate that ethanolic and aqueous extracts of *Moringa oleifera* has a lipid lowering effect on the diabetic rats.^[10]

The findings of the present study shows a number of positive effects of *Moringa oleifera* on rats with Alloxan induced disturbances in glucose tolerance and lipoprotein profile. Thus, ethanolic and aqueous extracts of *Moringa oleifera* are beneficial in the control of diabetes and abnormalities in lipid profiles. These beneficial effects of *Moringa oleifera* are specially promising in the light of preventing lifestyle disease of the cardiovascular systems.^[5,8]

The histopathology study of pancreas was also performed which showed hypoglycemic effect. The study reveals that in glucose-fed rats, the maximum hypoglycemic effect was produced within one hour during glucose tolerance test, this indicates that it takes about one hour for the active ingredient(s) or its (their) metabolites in the ethanol and aqueous extracts of seeds of *Moringa oleifera* to enter into the circulation and target tissues to bring about hypoglycemic effect.^[10]

In conclusion the result of the present study indicates that *Moringa oleifera* may have active principle(s) that exerts antidiabetic and antihyperlipidemic activities.

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