CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Available online at: http://www.iajps.com *Research Article*

PHYTOCHEMICAL SCREENING AND *IN-VIVO* ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF *MORINGA OLEIFERA*

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Article Received: July 2022 **Accepted:** July 2022 **Published:** August 2022

Abstract
Diabetes mellitus (DM) is a global health problem and the incidence of DM is increasing at alarming rate all over the world. Many Indian medicinal plants have been reported to possess potential antidiabetic activity and could play important role in the management diabetes. Moringa oleifera (M. oleifera) popularly known as miracle tree belongs to the family Moringaceae. It is a medicinal plant in which the leaves are the most nutritious part, being a significant source of vitamins and protein among others. This study was conceived and designed based on the gaps in the research that has been performed and what is known about the plant. This study investigated the protective and ameliorative potential of ethanol extract of M. oleifera leaf (50-200mg/kg) on alloxan-induced diabetes in Wistar rats. Qualitative analysis of various phytochemical constituents was determined by the well-known test protocol available in the literature. Alloxan-induced diabetic rats were orally pretreated with extract (50, 100 or 200 mg/kg) or post-treated with extract (50, 100 or 200 mg/kg/day), glibenclamide (2.5 mg/kg/day) or metformin (200 mg/kg/day) for two weeks. Other animals received only extract, alloxan (diabetic control) or vehicle (control). Blood glucose concentrations were measured at the beginning and twice weekly. Glucose levels in extract pretreated rats were lower than alloxan-induced levels, but when compared with control, were higher except that glucose level was normalized from the 11th day in 200 mg/kg extract pretreated rats. Glucose concentrations in extract, glibenclamide or metformin post-alloxan administered rats were decreased compared to diabetic rats. But they were higher than control, except by day 14 wherein glucose level was normal or lower. When compared with glibenclamide or metformin, glucose levels of extract treated animals were higher on the 7th day, but lower at the end of treatment. In addition, extract treatment caused hypoglycemia after fourteen days of treatment in normal rats. The results demonstrate that M. oleifera leaf possesses protective and ameliorative antidiabetic potential in rats.

Keywords: *Diabetes mellitus, Moringa oleifera, Phytochemical screening, Alloxan monohydrate.*

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Please cite this article in press Megha Shrivastava et al, ***Phytochemical Screening And In-Vivo Antidiabetic Activity Of Ethanolic Extract Of Leaves Of Moringa Oleifera****., Indo Am. J. P. Sci, 2022; 09(8).*

INTRODUCTION:

Diabetes mellitus is an epidemic occurring in adults throughout the world and is the leading cause of kidney failure, heart attack, blindness and lower limb amputation. It is the fourth main cause of death in most developed countries. The prevalence of diabetes is estimated to reach 330 million by the year 2025, according to International Diabetes Federation, with the greatest potential increase being in Africa and Asia. This numerical increase will occur in developing countries. By the year 2025, over 75% of people with diabetes will reside in developing countries, as compared to 62% in 1995[1]. Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides and glinides. Many of them have a number of serious adverse effects; therefore, the search for more effective and safer hypoglycemic agents is one of the important areas of investigations [2]. Many herbs and plants have been described as possessing hypoglycemic activity when taken orally [3]. According to the World Health Organization, there are more than 1200 plant species worldwide used in the treatment of diabetes mellitus and substantial number of plant showed effective hypoglycemic activity after laboratory testing [4]. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically in antidiabetic and antihyperlipidemic remedies. More than 400 plant species having hypoglycemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus. Most of the plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids etc. that are frequently implicated as having antidiabetic effect [5]. However, the study of plant for hypoglycemic, antioxidant and hypolipidemic activities may give new pharmacological approaches in the treatment of diabetes mellitus [6]. 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 1diabetes 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 [7].*M. oleifera* is one of the most widely distributed species of the Moringaceae family throughout the World, especially in Asian countries, having a remarkable range of pharmacological properties in addition to significant nutritional value. Moringa derives from the Tamil word murungai referring to a twisted pod found in young fruit [8]. *M. oleifera* is a fast-growing, deciduous tree that can reach a height of 10- 12m (32-40 ft) and trunk diameter of 45 cm (1.5 ft). The bark has a whitish-grey color and is surrounded by thick cork while the shoots have purplish or greenish -white, hairy bark. The tree has an open crown of drooping and fragile branches. The flowers are fragrant and bisexual, surrounded by five unequal, thinly veined, yellowish - white petals. The fruit is a hanging, three-sided brown capsule of 20-45 cm size which holds dark brown, globular seeds with a diameter around 1cm. The seeds have three whitish papery wings and are dispersed by wind and water [9]. The leaves are the most nutritious part of the plant, being a significant source of B vitamins, vitamin C, pro-vitamin A as beta-carotene, vitamin K, manganese, and protein, among other essential utrients [10]. The therapeutic use of *M. oleifera* leaves has been evaluated in diabetes because of their possible capacity to decrease blood glucose concentrations after ingestion because they contains polyphenols such as quercetin-3-glycoside, rutin, kaempferol and glycosides [11-13]. Several biological activities of the *M. oleifera* leaves have been reported such as anti-septic, antioxidant, antihypertensive, larvicidal, fungicidal, hyperlipidemic amongs others [14]. Several studies have shown that *M. oleifera* leaves presented anti-diabetic properties [15]. Other parts of the plant such as the pods and seed have also shown to exhibit anti-diabetic property [16]. This study was therefore intended to evaluate the protective and ameliorative potential of low dose levels of *M. oleifera* ethanol leaf extract (50-200 mg/kg) on alloxan induced diabetes in Wistar rats.

MATERIAL AND METHOD:

Plant material

Plant material (leaves part) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade. Alloxan (Central Drug House Pvt.Ltd.,India), Metformin, Glibenclamide tablets were procured from the authorized distributor of the company other chemicals and solvent obtained from Qualigens, India were used.

Extraction procedure

Defatting of plant material

Leaves of *M. oleifera* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction

200gm of dried plant material were exhaustively extracted by refluxing in ethanol at room temperature. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts and preserved in a refrigerated at 4oC for the experiments.

Phytochemical screening

Phytochemical screening to detect the presence of bioactive agents was performed by standard procedures [17, 18]. After the addition of specific reagents to the solution, the tests were detected by visual observation of color change or by precipitate formation.

Animals

Wistar rats (150–250 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute oral toxicity

Acute toxicity study of the prepared extracts was carried out according to the Organization for Economic Co-Operation and Development (OECD) Guidelines-423 [19] the animals were fasted for 4 h, but allowed free access to water throughout. As per the OECD recommendations, the starting dose level should be that which is most likely to produce mortality in some of the dosed animals; and when there is no information available on a substance to be tested in this regard; for animal welfare reasons, The hydroalcoholic extract of *Euphorbia neriifolia* (50, 100, 150, 200, 300 mg/kg/day) was administered orally for 4 days of six groups of rats (n=6) and the animals were kept under examination for mortality as well as any behavioral changes. Acute toxicity was determined as per reported method [20].

Induction of diabetes in rats

After fasting, diabetes was induced by a single intraperitoneal injection of 120 mg/kg body weight of 'Alloxan monohydrate' in distilled water. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycaemia. These animals were tested for diabetes after 15 days and animals with blood glucose (fasting) were selected for experimentation [21, 22].

Experimental protocol

Animals were divided into five groups of 6 rats each.
Group I: Rats served as normal-control and received the vehicle (0.5 ml distilled water/day/rat)
Group II: Rats (diabetic) were administered Metformin / Glibenclamide (600µg/kg p.o.) for 14 days.
Group III: Rats (diabetic) were administered ethanolic extract of ***M. oleifera*** (50 mg/kg p.o.) for 14 days.
Group IV: Rats (diabetic) were administered ethanolic extract of ***M. oleifera*** (100 mg/kg p.o.) for 14 days.
Group V: Rats (diabetic) were administered ethanolic extract of ***M. oleifera*** (100 mg/kg p.o.) for 14 days.

RESULTS AND DISCUSSIONS:

The crude extracts so obtained after extraction process was concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of ethanolic extracts was found to be7.8 %. The results of qualitative phytochemical analysis of the crude powder of leaves of *M. oleifera* were shown in Table 1. Ethanolic extracts of *M. oleifera* showed the presence of alkaloids, flavonoids, phenols and saponins. Blood glucose levels in all extract pretreated groups were lower when compared with the levels in rats that received alloxan alone. However, the glucose levels in pretreated groups were higher compared to the control except 200

mg/kg pretreated group where blood glucose levels became comparable with control on days 11 and 14. Intra-group comparison showed that glucose levels in 50 or 100 mg/kg extract administered groups were higher than the 200 mg/kg extract treated group Table 2. Blood glucose levels in rats that were administered extract were decreased dose-dependently over the 14 days treatment period, compared to alloxan alone treated rats. However, glucose levels in extract treated rats on sampling days 3, 7 and 11 were higher than control. On day 14, glucose level in the control (74.00±1.66mg/dl) was comparable with that in group that received 100 mg/kg extract (84.00±2.89 mg/dl), but lower in the group that received 200 mg/kg extract, 42.00±1.73mg/dl. In addition, glucose levels in glibenclamide and metformin administered rats were reduced over the treatment period compared to alloxan alone treated rats. Also, whereas glucose level in glibenclamide treated rats was not significantly different from control on day 14, all other glibenclamide and metformin induced glucose levels were higher than control. When compared with glibenclamide or metformin, glucose levels of extract treated animals were not different on days 3 or 11, but higher on day 7. On the 14th day, glucose level in rats that received the smallest dose of extract (50 mg/kg) was higher; while the level in rats that received the highest dose (200mg/kg) was lower when compared to glibenclamide or metformin Table 3.

Table 1 Result of phytochemical screening of extracts of *M. oleifera*

Phytochemicals	**Ethanol extract**
	Leaves
Alkaloids **Mayer's test Wagner's test**	+ +
Flavanoids **Lead acetate testH2SO4 test**	+ +
Steroids **Libermann-burchard test**	-
Terpenoids **Salkowaski test**	+
Anthroquinone **Borntrager's test**	-
Phenol **Ferric chloride testLead acetate test**	+ +
Saponin	+
Tannin	-
Carbohydrates	+
Protein and amino acidsBiuret test **Ninhydrin test**	+ +
Oils and resins	-

Table 2: Blood glucose levels after injection of alloxan (Allox) in ethanol *Moringa oleifera* leaf extract (EML) pretreated Wistar rats

GROUP	BLOOD GLUCOSE CONCENTRATION (mg/dl)				
	Control	Alloxan	EML (50 mg/kg)+Allox	EML (100 mg/kg)+Allox	EML (200 mg/kg)+Allox
Day 1	73.00±2.46	73.00±1.23	71.00±4.62	74.00±2.89	74.00±8.66
Day 3	72.00±1.00	430.00±7.12	280.00±5.77	277.00±9.81	279.00±5.20
Day 7	70.00±2.66	410.00±3.33	232.00±1.15	229.00±5.20	182.00±1.15
Day 11	69.00±0.33	400.00±0.33	200.00±1.15	180.00±11.55	98.00±11.55
Day 14	74.00±1.66	370.00±2.33	172.00±1.15	133.00±1.73	68.00±2.89

Data expressed as mean±SEM, n=5 rats per group

Table 3: Table 6: Effects of ethanol *Moringa oleifera* leaf extract (EML), metformin (Met) and glibenclamide (Glib) on alloxan (Allox)-induced hyperglycemia in Wistar rats (study onameliorative effect)

GROUP	DAY1	DAY3	DAY7	DAY11
CONTROL	73.00±1.33	72.00±1.00	70.00±2.66	69.00±0.33
ALLOXAN	60.00±1.66	441.00±7.12	421.00±3.33	412.00±0.33
ALLOXAN+EML (50mg/Kg)	69.00±5.20	455.00±3.46	311.00±5.77	200.00±11.55
ALLOXAN+EML (100mg/Kg)	72.00±1.73	451.00±1.15	289.00±2.89	164.00±8.66
ALLOXAN+EML (200mg/Kg)	72.00±3.46	428.00±17.32	283.00±2.31	121.00±11.55 b
ALLOXAN+GLIB	73.00±2.31	475.00±17.32	253.00±2.31	161.00±1.15
ALLOXAN+MET	70.00±0.58	442.00±24.83	265.00±3.46	191.00±1.15

Data expressed as mean±SEM, n=5 rats per group

CONCLUSION:

Results from this study indicate indicates that extracts of *M. oleifera* leaf exerts significant anti-diabetic property in rats. These observations provide a pharmacological basis for the traditional use of *M. oleifera* leaf in the management of diabetes mellitus. However, further studies are required to identify the active ingredient responsible for the anti-diabetic properties of the leaf extract.

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