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Antidiabetic activity of *Moringa oleifera* Lam.

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Abstract: Diabetes mellitus is a clinical syndrome with insufficient insulin secretion, abnormal glucose tolerance, universal, microangiopathia, neuropathia, accentuated atherosclerotic changes. *Moringa oleifera* Lam (Moringaceae) leaves are emetic and their juice with black pepper is used in headache. The leaves are anthelmintic, aphrodisiac, cures hallucinations, antidiabetic, dry tumors, cough and asthma. Different extracts of *Moringa oleifera* prepared in different solvents and used in alloxan induced diabetic rats. Total alcohol, Successive alcohol and Successive chloroform significantly decreases the serum cholesterol, serum triglyceride, serum VLDL, serum LDL and Total alcohol extract increases the serum HDL level in alloxan induced diabetic rats. The plant is worth for antidiabetic activity. So this result provides a platform for the drug designers and gives them an opportunity to prepare such herbal formulations which can be used as to treatment on diabetes.

Keywords: *Moringa oleifera, Antidiabetic,* Diabetes mellitus

I. Introduction

Diabetes mellitus is a clinical syndrome with insufficient insulin secretion, abnormal glucose tolerance, universal, microangiopathia, neuropathia, accentuated atherosclerotic changes [1]. There are more than 125 million people with diabetes in the world today, and by 2010 this number is expected to approach 220 million. It is also estimated that there are 30 to 33 million diabetes in India now, and every fourth diabetes in the world today is an Indian. Indians are genetically more susceptible to diabetes and the World Health Organization (W.H.O) predicts the number of diabetes in India would go up to 50 million by 2010 and 80 million by 2030 [2]. Herbal remedies for diabetes have been recorded in ancient medical literature. Plants hold definite promises in the management of diabetes mellitus. Diabetes mellitus is a common chronic endocrine disorder. Since ancient time a number of herbal medicines have been used in the treatment of this disease [3]. Many studies have been carried out in search of a suitable plant drug that would be effective in Diabetes mellitus. *Moringa oleifera* Lam (Moringaceae) leaves are emetic and their juice with black pepper is used in headache [4]. The leaves are anthelmintic, aphrodisiac, cures hallucinations, antidiabetic, dry tumors, hic cough and asthma [5]. Alloxan produces selective necrosis of β-cells of langerhans of pancreas and it leads to the deficiency of insulin. Insulin deficiency produces elevation of blood glucose levels and also causes excessive catabolism of protein & the amino acids that are released, utilized for gluconeogenesis & it also stimulate lipolysis in the adipose tissue and leads to hyperlipidemia that can be used to evaluate the Antidiabetic activity by measuring the, Total Cholesterol, Total glyceride, HDL, LDL, and VLDL levels[6]. By this reference the plant *Moringa oleifera* is selected for scientific validation for the antidiabetic activity in alloxan induced model.

II. Experimental Section

II. 1. Collection and authentication of plant

The leaves of *Moringa oleifera* Lam. were collected in July 2008, from road side of Modasa, Gujarat, India Identification of plant was carried out by Dr. M. S. Jangid, Botanist, Sir P. T. Science College, Modasa. Voucher specimens were deposited in Pharmacognosy museum, department of Pharmacognosy, Shri. B. M. Shah College of Pharmaceutical Education and Research, Modasa.

II. 2. Preparation of Extracts

The shade dried leaves of *M. oleifera* were reduced to fine powder (# 40 size mesh) and around two portions of 200 gm each were weighed. One portion was subjected to successive hot continuous extraction (soxhlet) with petroleum ether (40°-60°C), chloroform and alcohol. Finally the drug was macerated with chloroform water. Each time before extracting with the next solvent the powdered material was air dried. After the effective extraction, the solvents were distilled off, the extract was then concentrated on water bath, and second portion was subjected to hot continuous extraction (soxhlet) with ethanol only, the extract obtained was concentrated. Now each extract was weighed. Its percentage was being calculated in terms of air dried weight of plant material. The characteristics were noted color and consistency etc. All the extracts were concentrated under reduced pressure using rotary evaporator and the residue was dried in desiccators over anhydrous calcium chloride.

II. 3 Qualitative chemical tests

All the extracts like alcohol, water and succesive petroleum ether, chloroform, methanol, water extracts were tested in pharmacognosy & Phytochemical research laboratory in our college to know the different constituents present in them by the standard procedures. The extracts were tested for different phytoconstituents like sterols [7], alkaloids [8] triterpenes, saponins [9], flavonoids [10] and carbohydrates [11].

II. 4 Chemicals

Alloxan monohydrate was procured from Loba chemie Pvt. Ltd, Mumbai, Maharashtra, India. Glibenclamide tablet (Daionil brand) manufactured by Aventis Pharmaceuticals, Mumbai, Maharashtra, India. CONTOUR™ TS" Glucometer and Diastix urine sugar strip was procured from Bayer healthcare, India.

II. 5 Anti-diabetic activity

II. 5.1. Selection of animal

Healthy albino rats of wistar strain, weighing 150-250gm of either sex were used for the study. The animals were housed in a two rat per polypropylene cages, maintained under controlled condition of temperature (25±1^{O}C), humidity (55±5 ^{O}C) and 12-hr/12-hr light/dark cycle. Animals had free access to standard palette diet and purified drinking water *ad* libitium. All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of B.M.C.P.E.R, Modasa and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

II. 5.2. Antidiabetic Activity

The albino rats 150-200 gm either sex, were allowed to fast for 24hrs prior to experimentation and rendered diabetic by injection a single dose of Alloxan monohydrate 120mg/kg body weight prepared in normal saline; by I.P. route. Since alloxan could evoke fatal hypoglycemia as a result of massive insulin release, rats were received 20% of glucose solution for first 6 hr then simple tape water was

given. The rats were then kept for next 24 hr with free access of 5 % glucose solution to prevent hypoglycemia. After a forth night, alloxan monohydrate treated rats were tested for the evidence of diabetes by estimating their blood glucose level by using "CONTOUR™ TS" Glucometer. Blood samples were obtained through retro-orbital plexus. After 18hrs of injection of Alloxan, Diabetes was confirmed by testing urine sugar with Diastix. The animals with sugar level more than 200mg/dl were selected. Animals were maintained for four days in diabetic condition for well establishment of diabetes [12, 13, 14].

II. 5.3. Extracts and Standard Drug Used

Various extracts of the leaves of *Moringa oleifera* Lam. suspended with Polyethylene glycol-400 was employed for assessing Antidiabetic activity as follows.

- **Extracts preparation:** Total alcoholic extract, successive petroleum ether extract, successive chloroform extract, successive ethanolic extract, successive water extract of *Moringa oleifera* Lam were used (250 mg/kg Body weight).

- **Standard preparation:** Glibenclamide tablet (250 mg/kg Body weight) (Daionil brand) was used as a standard drug. It was purchased from local market weighing (5mg each tablet) 150mg and made into fine powder in a mortar and to it 30ml of vehicle was added and triturated to make a fine solution. Dose was calculated on the basis of daily doses (10mg) for a man of 75kg was used as a reference.

II. 5.4. Experimental Design

Animals were divided into eight groups of six each.

***Table 1**: Experimental Design of Animals for Antidiabetic activity*

Groups	Condition of Groups
I.	Healthy normal animals received only the vehicle served as Normal control.
II.	Untreated diabetes induced animals served as a Diabetic control.
III.	Diabetic induced animals and treated with standard drug- Glibenclamide (10mg/kg) served as Reference standard treated.
IV.	Diabetic induced animals and treated with Total alcoholic (250mg/kg) served as Total alcoholic extract treated.
V.	Diabetic induced animals and treated with Successive Petroleum Ether extract (250mg/kg) served as Successive Petroleum Ether extract treated.
VI.	Diabetic induced animals and treated with Successive Chloroform extract (250mg/kg) served as Successive Chloroform extract treated.
VII.	Diabetic induced animals and treated with Successive ethanolic extract (250mg/kg) served as Successive ethanolic extract treated.
VIII.	Diabetic induced animals and treated with Successive water extract (250mg/kg) served as Successive water extract treated.

II. 5.5. Collection of Blood Sample and Analysis

On day zero and after seven days, blood samples were collected from the retro-orbital plexus of 8 hr fasted and anesthetized animals, by slight exposure to diethyl ether in a glass jar, and one drop poured on strip and rest of blood was filled in vial for the estimation of biochemical parameters. Blood glucose level was measured in all groups by using "CONTOUR™ TS" Glucometer in the unit of mg/dl. Biochemical parameters such as Serum Cholesterol, Triglyceride, HDL, VLDL, LDL, Total Protein, Albumin, Creatinine, Urea, SGOT, SGPT, and Bilirubin were estimated by PARAM Pathology laboratory, Himatnagar using Autoanalyser.

II. 5.6. Statistical Analysis

Results are presented as mean ± SEM of 6 animals. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Dunnett test using graph pad Prism software. The significance difference if any among the groups at P value ≤ 0.05 was considered statistically significant.

III. Results and Discussion

Table 2: *Effect of M. oleifera Lam. leaves extracts on blood glucose level (mg/dl) in Alloxan induced diabetic rats.*

Blood Glucose Profile (M±SEM)			**% Change**
Groups	**0- Day**	**7th – Day**	
Normal	78.00±1.958	77.00±3.028	1.28% (↓)
D.C	352.8±4.270	362.0±2.160	2.60% (↑)
R.S	360.3±5.779	219.5±4.770	39.11% (↓)
TA	364.0±5.212	245.0±5.148	32.69% (↓)
S. P	352.8±6.408	346.0±5.598	1.92% (↓)
S.CH	355.8±13.89	317.0±11.48	10.90% (↓)
S.ETH	359.3±6.102	266.5±9.042	25.82% (↓)
S.W	354.8±7.993	355.5±7.900	0.19% (↑)

(↓) Decrease and (↑) Increase

Results indicated that there is significant (p ≤ 0.05) decreases in the Blood glucose level in diabetic animal treated with Total alcohol extract (245.0±5.148), Successive alcohol extract (266.5±9.042) and Successive chloroform extract (266.5±9.042) in comparison with diabetic control (362.0±2.160). This data suggest that, Total alcoholic, Successive alcoholic and Successive chloroform extracts significantly decreases the blood glucose level, i.e.; 32.69%, 25.82% and 10.90% respectively in alloxan induced diabetic rats.

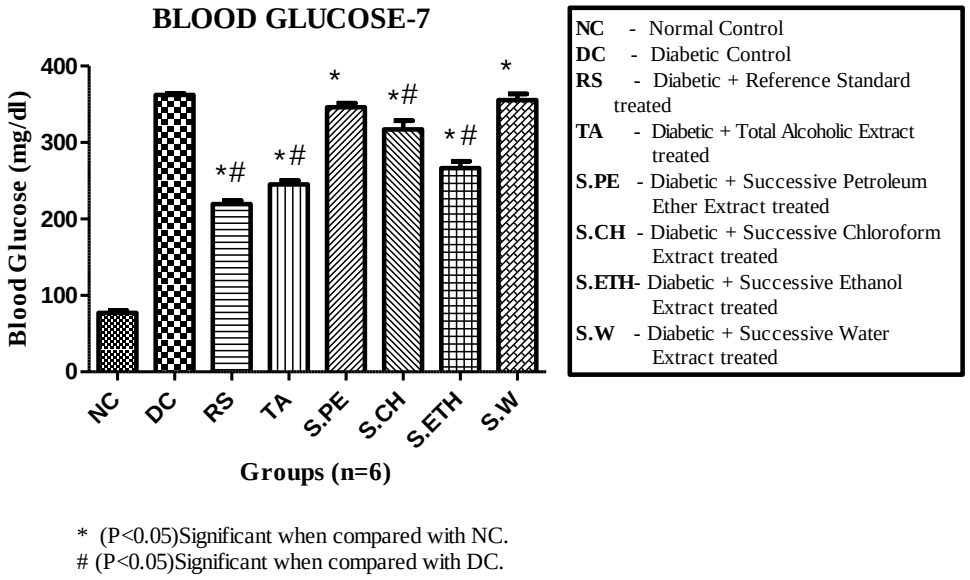

Figure1: *Effect of various extracts of leaves of M. oleifera Lam. on Blood Glucose level in diabetic rats.*

Table 2: *Effect of M. oleifera Lam. leaves extracts on various parameters in Lipid Profile in Alloxan induced diabetic rats.*

Lipid Profile (M±SEM)					
Groups	**Cholesterol (mg/dl)**	**Triglycerides (mg/dl)**	**HDL (mg/dl)**	**LDL (mg/dl)**	**VLDL (mg/dl)**
Normal	100.2±4.588	40.75±3.262	45.30±4.049	46.75±4.173	8.150±0.6525
D.C	162.6±2.946	92.33±4.645	34.73±1.238	109.2±2.726	18.47±0.9290
R.S	114.0±4.655	51.98±3.975	44.78±1.540	58.91±5.043	10.40±0.7951
TA	115.7±6.353	57.67±5.939	42.22±2.037	61.92±3.401	11.53±1.188
S. P	149.5±5.923	92.73±3.363	34.69±2.027	96.26±5.128	18.55±0.6726
S.CH	136.6±5.225	55.76±6.374	39.56±1.670	83.09±3.874	13.90±1.074
S.ETH	125.3±5.961	55.76±6.374	39.47±1.079	76.63±7.657	11.15±1.274
S.W	161.6±4.650	80.76±7.099	36.49±2.251	109.0±6.272	16.15±1.418

Results indicate that there is significant ($p \leq 0.05$) decreases in the Serum Cholesterol level in diabetic animal treated with Total alcohol extract (115.7±6.353) , Successive alcohol extract (125.3±5.961) and Successive chloroform extract (136.6±5.225) in comparison with diabetic control (162.6±2.946); Significant ($p \leq 0.05$) decreases in the Serum Triglyceride level in diabetic animal treated with Successive alcohol extract (55.76±6.374), Total alcohol extract (57.67±5.939) and Successive chloroform extract (55.76±6.374) in comparison with diabetic control (92.33±4.645). Total alcohol extract (42.22±2.037) significantly increases the serum HDL level at $p \leq 0.05$ when compare with diabetic control (34.73±1.238). Successive alcohol extract (11.15±1.274), Total alcohol extract (11.53±1.188) and Successive chloroform extract (13.90±1.074) significantly decreases the serum VLDL level at $p \leq 0.05$ when compare with diabetic control (18.47±0.929). Total alcohol extract (61.92±3.401), Successive alcohol extract (76.63±7.657) and Successive chloroform extract (83.09±3.874) significantly decreases the serum LDL level at $p \leq 0.05$ when compare with diabetic control (109.2±2.726).

Table 3: *Effect of M. oleifera Lam. leaves extracts on various parameters in Renal Profile in Alloxan induced diabetic rats.*

Renal Profile (M±SEM)				
Groups	**Urea**	**Creatinine**	**Total Protein**	**Albumin**
Normal	46.50±4.291	0.630±0.08347	6.583±0.05406	4.553±0.1494
D.C	110.3±6.575	1.070±0.1060	4.235±0.02533	2.438±0.1205
R.S	54.00±5.874	0.645±0.02661	5.470±0.07616	3.428±0.09259
TA	61.88±3.416	0.775±0.04518	5.720±0.1800	3.620±0.2542
S. P	99.28±5.098	1.013±0.0825	4.418±0.1928	2.653±0.1982
S.CH	89.83±3.008	0.8825±0.02689	4.840±0.1140	3.070±0.1137
S.ETH	69.84±3.899	0.815±0.07053	5.048±0.06356	3.180±0.1853
S.W	104.5±5.507	1.055±0.06898	4.253±0.04679	2.523±0.1875

Results indicate that there was significant ($p \leq 0.05$) decreases in the Serum Urea level in diabetic animal treated with Total alcohol extract (61.88±3.416), Successive alcohol extract (69.84±3.899) and Successive chloroform extract (89.83±3.008) in comparison with Diabetic control(110.3±6.575).

Significant ($p \leq 0.05$) decrease was found in the Total Creatinine level in diabetic animal treated with Total alcohol extract (0.775±0.04518) in comparison with Diabetic control(1.070±0.1060). Significant ($p \leq 0.05$) increases in the Serum Total protein level in diabetic animal treated with Total alcohol extract (5.720±0.1800), Successive alcohol extract (5.048±0.06356) and Successive chloroform extract (4.840±0.1140) in comparison with Diabetic control(4.253±0.04679).

Significant ($p \leq 0.05$) increases were found in the Serum albumin level in diabetic animal treated with Total alcohol extract (3.620±0.2542) and Successive alcohol of leaves of *M. oleifera* Lam extract (3.180±0.1853) in comparison with Diabetic control(2.438±0.1205)Data indicates that Total alcohol, Successive alcohol and Successive chloroform extract of leaves of *M. oleifera* Lam significantly decreases the Serum Urea, and only Total alcohol extract significantly decreases the Serum Creatinine level.

Table 4: *Effect of M. oleifera Lam. leaves extracts on Hepatic Profile on diabetic rats.*

Hepatic Profile (M±SEM)				
Groups	**SGPT**	**SGOT**	**Alkaline Phosphatase**	**Bilirubin**
Normal	56.75±5.963	63.40±2.495	47.00±1.665	0.1700±0.01291
D.C	149.8±4.090	107.4±3.040	156.3±6.102	0.4700±0.04041
R.S	68.00±2.972	70.00±4.655	52.00±6.795	0.2925±0.01031
TA	76.56±5.586	77.14±3.511	60.82±3.537	0.1973±0.004922
S. P	112.2±7.300	98.86±4.303	91.12±7.852	0.3025±0.06860
S.CH	106.8±11.23	82.23±4.752	93.37±2.876	0.2475±0.03521
S.ETH	87.82±3.482	80.78±6.353	79.75±3.301	0.2075±0.03276
S.W	131.7±5.329	101.5±6.185	103.5±8.703	0.4675±0.02689

Results indicate that there is significant ($p \leq 0.05$) decreases in the Serum SGPT level in diabetic animal treated with Total alcohol extract (76.56±5.586), Successive alcohol extract (87.82±3.482), Successive chloroform extract (106.8±11.23) and Successive Petroleum ether extract of leaves of *M. oleifera* Lam (112.2±7.300) in comparison with Diabetic control (149.8±4.090).
There was significant ($p \leq 0.05$) decreases in the Serum SGOT level in diabetic animal treated with Total alcohol extract (77.14±3.511), Successive alcohol extract (80.78±6.353) and Successive chloroform extract (82.23±4.752) of leaves of *M. oleifera* Lam. in comparison with Diabetic control(107.4±3.040). Significant ($p \leq 0.05$) decreases in the Serum Alkaline Phosphatase level in diabetic animal treated with Total alcohol extract (60.82±3.537), Successive alcohol extract (79.75±3.301), Successive chloroform extract (93.37±2.876) and Successive Petroleum ether extract (91.12±7.852) of leaves of *M. oleifera* Lam in comparison with Diabetic control(156.3±6.102).
There was significant ($p \leq 0.05$) decreases in the Serum Bilirubin level in diabetic animal treated with Total alcohol extract (0.1973±0.004922), Successive alcohol extract (0.2075±0.03276), Successive chloroform extract (0.2475±0.03521) and Successive Petroleum ether extract (0.3025±0.06860) of leaves of *M. oleifera* Lam in comparison with Diabetic control(0.4700±0.04041).

IV. Conclusion

All the results indicates that Total alcohol, Successive alcohol and Successive chloroform significantly decreases the serum cholesterol, serum triglyceride, serum VLDL, serum LDL and Total alcohol extract increases the serum HDL level in alloxan induced diabetic rats. Total alcohol extract and Successive alcohol extract of leaves of *M. oleifera* Lam increases the Serum Protein and Serum Albumin level in alloxan induced diabetic rats. Total alcohol, Successive alcohol, Successive chloroform and successive Petroleum ether extracts of leaves of *M. oleifera* Lam significantly decreases the Serum SGPT, Alkaline Phosphatase and Bilirubin level. Total alcohol extract, Successive alcohol, Successive chloroform of leaves of *M. oleifera* Lam decreases the Serum SGOT level in alloxan induced diabetic rats. It can be concluded from the present research work that the total alcohol, succesive alcohol and chloroform extracts of *Moringa oleifera* Lam works by inhibiting ATP-sensitive potassium channels in pancreatic beta cells. This inhibition causes cell membrane depolarization, which causes voltage-dependent calcium channels to open, which causes an increase in intracellular calcium in the beta cell, which stimulates insulin release. The plant is worth for antidiabetic activity. So this result provides a platform for the drug designers and gives them an opportunity to prepare such herbal formulations which can be used as to treatment on diabetes.

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