International Journal of Current Trends in Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijctpr

Research Article Open Access

Antidiabetic activity and phytochemical screening of crude extracts of *Diospyros malabarica* (DM) on Alloxan-induced diabetic Rats

A. Ravi kumar[1]*, S. Satyanarayana[1], V. Govardhani[1], Gireesh Jampana[1], Matle Naga Priyanka[2]

[1]*Celon Laboratories Private Limited, Aleap Industrial Estate, Near Pragathinagar, Gajularamaram, R.R. Dist. 500090, Telgana, India*
[2]*Department of Pharmaceutical Analysis, Narayana Pharmacy College, Nellore, Andhra Pradesh, India*

A B S T R A C T

The aim of present study was to evaluate antidiabetic activity of Hydro alcoholic extract of *Diospyros malabarica (DM)* family: Ebenaceae, leaves in Alloxan induced diabetic rats. The Hydro alcoholic extract of *Diospyros malabarica (DM)* was studied for antidiabetic activity in Alloxan induced diabetic rats by oral administration of extract 200,400mg/kg body weight for 28 days. The effect was compared with oral dose of 150mg/kg Glibenclamide. The determination of blood glucose level by GOD-POD kit method. The result shows the Hydro alcoholic extract of *Diospyros malabarica (DM)* leaves significantly lowered the blood glucose of hyperglycemic rats. From the toxicity study it was observed that Hydro alcoholic extract of *Diospyros malabarica (DM)* was nontoxic up to 2g/kg body weight and phytochemical study showed the presence of phytosterols, flavonoids and glycosides. It is concluded that Hibiscus Cannabinus leaf extract has significant antidiabetic activity, which lowered the fasting blood glucose level in Alloxan induced diabetic rats.

Keywords: Anti-Diabetic activity, Diospyros malabarica, glibenclamide, Wistar rats, hyperglycemic activity, high fat diet, Alloxan

A R T I C L E I N F O

CONTENTS

Article History: Received 10 May 2016, **Accepted** 26 June 2016, **Available Online** 15 July 2016

***Corresponding Author**
A. Ravi kumar
Celon Laboratories Private Limited,
Aleap Industrial Estate, Near
Pragathinagar, Gajularamaram,
Manuscript ID: IJCTPR3068

PAPER-QR CODE

Citation: A. Ravi kumar, *et al.* Antidiabetic activity and phytochemical screening of crude extracts of *Diospyros malabarica* (DM) on Alloxan-induced diabetic Rats. *Int. J. Curnt. Tren. Pharm, Res.*, 2016, 4(4): 203-207.

1. Introduction

Diabetes mellitus (DM), often simply referred to as diabetes, is a group of metabolic diseases in which a patient has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced.[1] This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger).[2] It is characterized by hyperglycaemia due to defective insulin action, insulin secretion or both. Several medicinal plants are used in the management of diabetes mellitus. According to the World Health Organization (WHO).[3] Evaluations of plant products to treat diabetes mellitus is of growing interest as they contain many bioactive substances with therapeutic potential. In recent years, several authors evaluated and identified the antidiabetic potential of traditionally used Indian medicinal plants using experimental animals.[4] Previous studies confirmed the efficacy of several medicinal plants in diabetes mellitus. The ***Diospyros malabarica*** (Family: Ebenaceae), commonly known as Bandadamara in Tirumala hills, is a succulent, prostrate herb, with fleshy leaves. [5] It is found growing abundantly all over India and is eaten as a leafy vegetable. It contains more omega-3-fatty acids than any other leafy vegetable plant. Besides vitamins (mainly vitamin C, vitamin B), carotenoids and dietary minerals have been reported. Some alkaloid igments large amounts of Norepinephrine, a neurohormone that has vasopressin and hypertensive activities have been reported in this plant. Other constituents include dopamine, coumarins, Flavanoids, saponins and urea**.[6]** analgesic. The pharmacological studies have shown Tumor inhibiting and wound healing activity. *Diospyros malabarica* has been claimed to possess anti diabetic activity in traditional system of medicine but no systematic, scientific work has been done to prove this activity.[7]

2. Materials and Methods

Collection and authentication: The leaves of *Diospyros Malabarica* were collected in the month of January 2014 from forest area of Tirumala Hills, Thirupathi, and Chittor district. Andhra Pradesh. It was shade dried away from sunlight and stored suitably. The plant material was taxonomically identified by the Dr. K. Madhava Chetty, Assistant professor, Department of Botany, Sri Venkateshwara University, Thirupathi, Andhra Pradesh, India.

Extraction

Diospyros Malabarica was used in the form of crude 50% of Hydro alcoholic extract and this extract was prepared according to the traditional system of medicine. The shade dried and a coarsely powdered leaf (1kg) was extracted with 50% Hydro alcoholic compounds in the cold for 72 hours. The extract was filtered and distilled on water bath, a reddish brown syrupy mass was obtained and it was finally dried at low temperature under reduced pressure in a rotary evaporator. A crude residue (75g) was obtained giving a yield of 7.5%. The antidiabetic effects were evaluated by oral administration of the extract to the Alloxan induced diabetic rats.[9]

Preliminary phytochemical screening

The hydro alcoholic extract of *Diospyros malabarica*. Was tested for phytoconstituents such as carbohydrates, Alkaloids, Tannins, Triterpenoids, Amino acids phenolic compounds, glycosides, saponins using standard phytochemical methods.[10]

Animals:

Experimental animals of male Wistar albino rats weighing 150-200g were obtained from Raghavendra enterprises. The animals were housed in stainless steel cages at a controlled room temperature of 24C, under a 12 h light and 12 h dark cycle. After one week of acclimatization, the experimental animals were divided randomly in to 5 groups (n=6). The experimental protocol was approved by the Institutional Animal Ethical Committee.[11]

Chemicals: Glibenclamide and Alloxan were obtained from Sigma-Aldrich, Bangalore. Petroleum ether and Ethanol, chloroform and all other reagents used were of analytical grade. Sod, Catalase, GP_X were obtained from thermal diagnostics Ltd., and Span diagnostics Ltd.

Instruments

UV-Visible spectrophotometer (Analytical systems, Model no: AUV 2060), electronic balance (Shimadzu, Model no: DS-852 J), homogenizer (Ever shine, Model no: 607) and Cooling centrifuge (Remi, Model no: C-24 BL)..

Acute toxicity study

Acute toxicity studies were conducted by following OECD 423 guidelines for safe dose administration to animals the results of cute toxicity study revealed that LD_{50} values of hydro alcoholic extract of *Diospyros malabarica* were high and apparently showed the safety of extracts. The treatment of rats with hydro alcoholic extract of *Diospyros malabarica* did not change the autonomic or behavior response in rats.[12] the zero % mortality for hydro alcoholic extract of *Diospyros malabarica* was found at the doses of 2000mg/kg. Hence $1/10^{th}$ of the dose was considered as therapeutic dose for the evaluation of antidiabetic activity of Evaluation of Anti-Diabetic Activity of *Diospyros malabarica* Leaves In Low Dose Alloxan And High Fat Diet Induced Diabetes In Wistar Rats.[13]

Alloxan Induced diabetes: Diabetes was induced by a single IP dose of 100-120 mg/kg of Alloxan (2, 4, 5,6 tetraoxypyrimidine; 2,4,5,6- pyrimidinetetrone) (S.D Fine – Chem. Ltd., Mumbai, India), in sterile saline. Induces permanent diabetes. In rats used in the dose of 150 mg/kg.[14]

Experimental procedure

Adult Wistar rats weighing 150- 200g were used for the study. The animals were divided in to 5 groups of 6 each. Group -I served as normal healthy control. Group-II (Diseased control). Group-III (Standard) diabetic rats given Glibenclamide (150mg/kg i.p). Group-IV(Test-1) diabetic rats given *Diospyros malabarica* extract(HAEM) (200mg/kg body weight). Group-V control rats given *Diospyros malabarica* extract (HAEM) (400mg/kg body weight).. The crude extract was administered for a period of 30 days.[15]

Collection of blood samples

The blood samples were collected from the retro-orbital venous plexus of rats without any coagulant for the

separation of serum. After collecting the blood in micro centrifuge tubes they were kept for 1 hr at room temperature and serum was separated by centrifugation at 2500 rpm for 15 min and stored until analyzed for various biochemical parameters.[16]

Estimation of parameters

Serum glucose was estimated by GOD/POD method of Bio chemical parameters total glucose levels, LDL level, HDL levels, Triglycerides were assayed

3. Results and discussions

For the present study, the rats were divided into five groups and treatment was given for a period of 28days. Diabetes mellitus was induced by using High fat diet and low dose of Alloxan (100mg/kg, i.p) at last two consecutive days. After 48hrs, of induction the biochemical parameters were assessed and the results obtained are discussed below. The preliminary phytochemical tests performed on HAEDM were found to be Tannins, phytosterols, saponins and Alkaloids, phenols, proteins, steroids.

Table 1: Results for phytochemical screening

Phyto-constituents	Hydro alcohol extract of *Diospyros malabarica*
Alkaloids	+
Tannins	+
Carbohydrates	+
Proteins	+
Flavanoids	-
Steroids	+
Saponins	+
Phenols	+
Fats & oils	-
Triterpenoids	+
Amino acids	+

+ Present; - Absent.

The results indicated the presence of alkaloids, carbohydrates, Tanins, Triterpenoids, saponins, phenols, amino acids & proteins and phytosterols in Hydro alcoholic extract of *Diospyros malabarica (DM)*.

Table 2: Effect of HAEDM on glucose levels Alloxan& High Fat Diet Induced Diabetic Rats

Group	Treatment	0 day	7th day	14th day	21st day	28th day
Group 1	Control	84.54	86.32	88.3	88.43	91.23
Group2	Diabetic Control	271.13	280.45	292.3	315.3##	324.1###
Group 3	Alloxan+ Glibenclamide	271.23	265.4	224.5	125.2**	99.3***
Group4	ALLOXAN+HAEDM+HFD 200 Mg/Kg	291.3	221.3	193.8	142.2**	123.2***
Group 5	ALLOXAN+HAEDM+HFD 400 Mg/Kg	287.7	204.2	170.3*	127.5**	104.8***

Values were expressed as mean ± S.E.M of 6 observations,### Indicates p<0.001 when compared to respective normal group,*** Indicates p<0.001 when compared to respective disease control group,**Indicates p<0.01 when compared to respective disease control group.

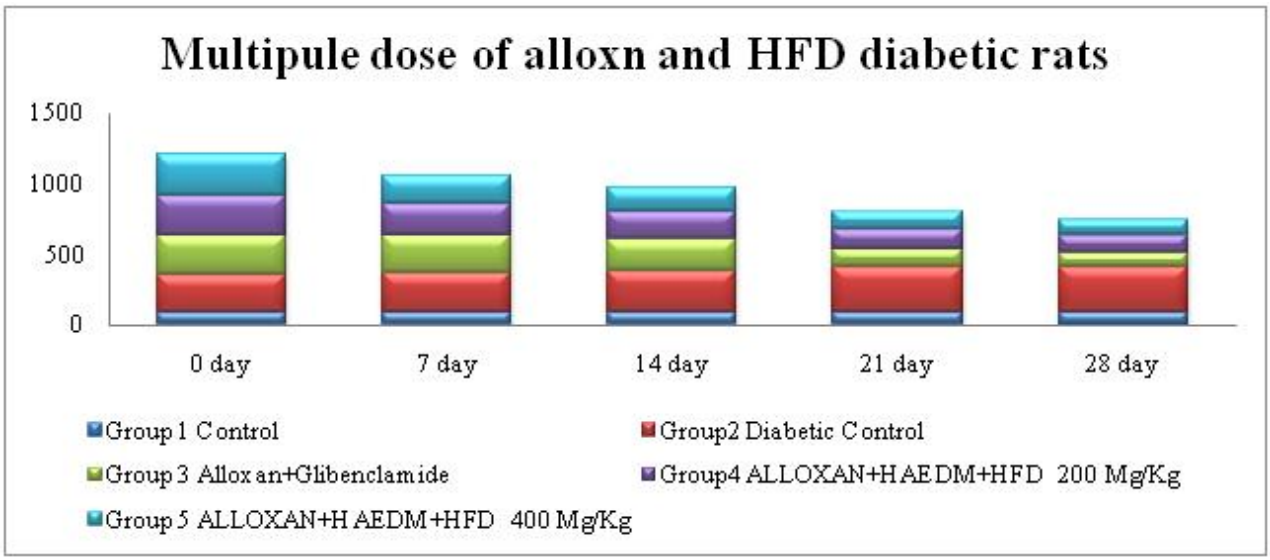

Figure 1: Multiple Doses of Alloxan HFD Diabetic Rats

In the anti-diabetic activity, the HAEDM effects of extract on body weight is measured on 7th, 14th,21st and 28th day of post induction and were compared with normal and diabetic control groups. The values are shown in Table No-1alloxan induced diabetic rats showed a significant decrease (P<0.001) in body weight compared to normal rats. Oral administration of leaf

extract at the dose of 400mg/kg showed a significant) increase (P<0.001) in body weight on 21st and 28th day of post induction when compared to untreated diabetic rats.[17]

Table 2: Effect of HAEDM on body weight in Alloxan& high fat diet induced diabetic rat

Group	Treatment	Body weight (gm)				
		0 day	7th day	14th day	21st day	28th day
Group 1	Control	183.4	187.3	190.2	192.4	194.5
Group2	Diabetic Control	180.4	176.4	164.5	159.3	151.3
Group 3	Alloxan+ Glibenclamide	187.3	183.2	184.6	186.8***	191.5***
Group4	ALLOXAN+HAEDM+HFD200Mg/Kg	89.3	184.3	172.3**	177.8**	181.5*
Group 5	ALLOXAN+HAEDM+HFD 400 Mg/Kg	187.3	179.2	174.5*	181.21**	183.5***

Values were expressed as mean ± S.E.M of 6 observations,### Indicates p<0.001 when compared to respective normal group,*** Indicates p<0.001 when compared to respective disease control group,**Indicates p<0.01 when compared to respective disease control group.

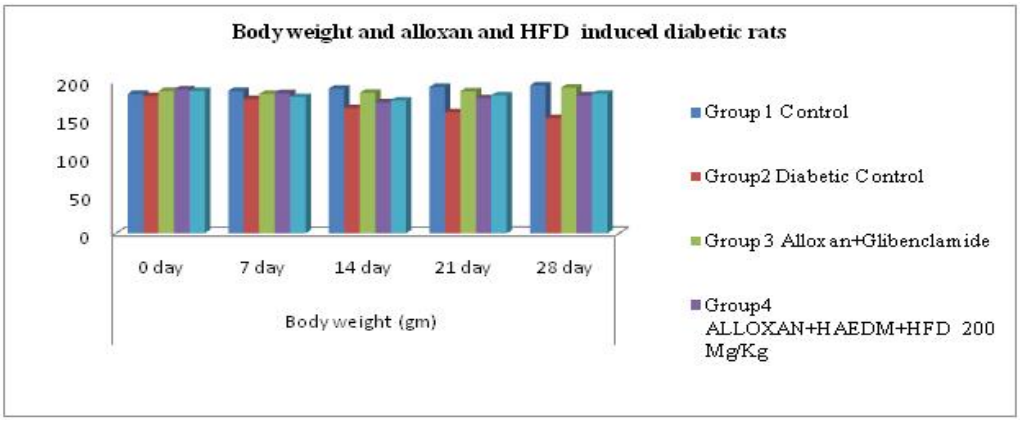

Figure 2: Body weight Alloxan and hfd induced diabetic rats

The effect HAEDM leaf extract on fasting blood glucose level is measured on 7th, 14th,21st and 28th day of post induction and compared with normal and diabetic control groups. The values are shown in table No-2. Alloxan induced rats showed a significant increase (P<0.001) in fasting blood glucose level compared to normal rats. Oral administration of leaf extract at the dose of 400mg/kg body weight showed a significant.[18]

Table 3: Effect of HAEDM on biochemical parameters of Alloxan & high fat diet induced diabetic rats

Group	Treatment	Serum Albumin	Total Cholesterol	Triglycerides
Group 1	Control	4.63	63.8	76.2
Group2	Diabetic control	1.87	123.5###	140.3###
Group 3	Alloxan + Glibenclamide	4.21	76.42**	77.4***
Group4	ALLOXN+HAEDM+HFD200 mg/kg	2.43	93.5*	91.2**
Group 5	ALLOXN+MEDM+HFD 500 mg/kg	3.08	83.4***	85.3***

Values were expressed as mean ± S.E.M of 6 observations,### Indicates p<0.001 when compared to respective normal group,*** Indicates p<0.001 when compared to respective disease control group,**Indicates p<0.01 when compared to respective disease control group.

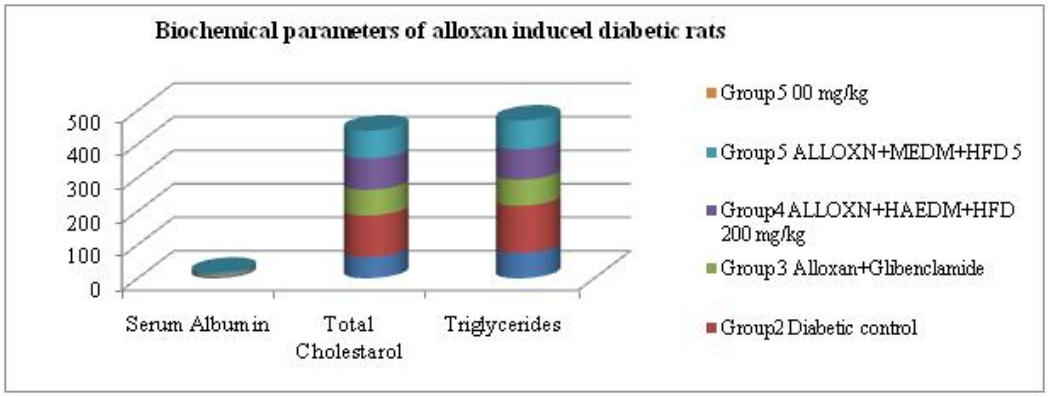

Figure 3: Biochemical parameters of Alloxan induced diabetic rats

The activities of Cholesterol, Triglisarides and HDL,LDL Levels in the liver of control and experimental animals are shown in(Tables 3).The activity of cholesterol, triglycerides and HDL,LDL Levels (liver) were found significantly elevated in diabetic rats when compared with control rats ($p<0.001$). Oral administration of *HAEDM* (250 mg and 400 mg/kg body weight) for 30 days brought back the activity of the above parameters to the near normal level. However, drug alone treated rats (200 and 400mg/kg body weight) did not show any ($p>0.001$) significant changes when compared with control animals. In our study, administration of *HAEDM* extract resulted in a significant reduction in blood glucose level, when compared with diabetic control animals. The extract containing 400 mg/kg body weight showed a better glucose level reduction than 200 mg/kg body weight. The mechanism may be through the stimulation of beta cell for elevated secretion of insulin, there increasing the utilization of glucose, increasing level of HDL, and decreasing level of LDL and Triglycerides in various tissues.[19]

Statistical Analysis

All the data was expressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Bonferroni's test using computer based fitting program (Prism graph pad 6.0). Statistical significance was set accordingly

4. Conclusion

The Hydro alcoholic extract of *Diospyros malabarica* leaf exhibited significant hypoglycemic activity in streptozotocin induced diabetic rats. From the phytochemical analysis it was found that the major chemical constituents of the leaf extract were flavonoids and glycosides.[20] On the basis of above evidence it is possible that the presence of flavonoids may be responsible for the observed antidiabetic activity. Further pharmacological and biochemical investigations are underway to find out the active constituents responsible for antidiabetic activity and to elucidate its mechanism of action.[21]

5. References

[1] Abate, G: In: Demissew, S. (Ed.), Este Debdabe. (1989). Ethiopian Traditional Medicine.

[2] Department of Biology, Science Faculty, Addis Ababa University pp: 3-5.

[3] Kameswararao B., Evaluation of antidiabetic effect of Momordica cymbalaria fruit in alloxandiabetic rats. Fitoterapia, 74:7-13, (2003).

[4] Satyanarayana T., Hypoglycemic and antihyperglycemic effect of alcoholic extract of Euphorbia leucophylla and its fractions in normal and in alloxan induced diabetic rats. Pharmacog Mag, 2:244-53,(2006)

[5] Eidi A., Antidiabetic effect of garlic (Allium sativum L.) in normal and streptozotocininduced diabetic rats. Phytomedicine, 13:624-9, (2006)

[6] Sharma UD., Cure of heart diseases with ayurvedic drugs. Sacitra Ayurveda, 47:95-6, (1994). 6. Adedapo, A.A. (2002). Toxicological studies of some plants in the family Euphorbiaceae in rats, Ph.D Thesis, University of Ibadan.

[7] Adedapo, A.A., Adegbayibi, A.Y., Emikpe, B.O. (2005). Some Clinico-pathological Changes associated with the Aqueous Extract of the Leaves of Phyllanthus amarus in Rats. Phytother. Res. 19, 971–976.

[8] Adedapo, A.A., Koduru, S. Jimoh, F.O., Masika, P.J., Afolayan, A.J. (2008a). Evaluation of the medicinal potentials of the methanol extracts of the leaves and stem of Halleria lucida. Bioresource Tech 99(10), 4158-4163.

[9] Bailey, C. J. and Day, C. (1989) Traditional plant medicines as treatment for diabetes, *Diabetes care*. 12(8).

[10] Rajasekaran, S., Sivagnanam, K., Narayanan, V. and Subramanian, S. (2001) Hypoglycemic and hypolipidemic effects of *Aloevera* on experimental rabbits. Publication of *Indian Association of Biomedical Scientists*. 41-45.

[11] Tiwari, A.K., Gode, J.D. and Dubey, G.P. (1989) Effect of *T.arjuna* bark powder on serum lipids and lipoproteins in hypercholesterolemic rabbits. *Indian Drugs.* 26, 664.

[12] Tiwari, A.K., Gode, J.D. and Dubey, G.P. (1990) Effect of *T.arjuna* on lipid profiles of rabbits fed hypercholesterolemic diet. *Int. J. Crude Drugs Res*. 28, 43-47.

[13] Bagepalli S, Kumar A, Prabhakarn V, Nandesh (2008). Pharmacognostical studies of *Portulaca oleracea* Linn. Brazilian J of Pharmacog, 18 (4), 527-531.

[14] Baig NA, Herrine SK, Rubin R (2001). Liver disease and diabetes mellitus. Clin Lab Med, 21,193-207.

[15] Baljinder S, Seena G, Dharmendra K, Vikas G, Bansal P (2010). Pharmacological Potential of *Eriobotrya japonica*- An overview. Inter Res J of Pharm, 1(1), 95-99.

[16] Bancroft JD, Srevens A & Turner DR (1996). Theory and practice of histological Techniques, 4th edn (Churchill Livington, New York) 51.

[17] Banerjee G, Mukherjee A (2002). Biological activity of a common weed: *Portulaca Oleracea L*.-II. Antifungal activity. Acta Botan Hungarica, 44(3-4), 205-208.

[18] Banerjee G, Mukherjee A (2003). Antibacterial activity of a common weed, *Portulaea Oleracea. L*. Geobios (Jodhpur), 30(2-3), 143-144.

[19] Banerjee,Gautam and Mukherjee, Ambarish (1996). *Portulaca oleracea* L: A gem of aliens in India. J of Phytochem Res, 9(2), 111-115.

[20] Kasture VS, Chopde CT, Deshmukh VK. Anticonvulsive activity of Albizzia lebbeck,

[21] Hibiscus rosa sinensis and Butea monosperma in experimental animals. J. Ethnopharmaco.l 71:65-75. (2000).

[22] Nadkarni AK., Indian Materia Medica. Bombay: p. 631, (1954).