www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.1, pp 351-357, Jan-Mar 2012

Antidiabetic and Antihyperlipidemic Activities of *Malvastrum coromandelianum* Linn leaves in Alloxan induced Diabetic Rats

Amol Bhalchandra Deore*, Preetam Nivrutti Chavan, Vinayak Dnyandev Sapakal, Nilofar S. Naikwade

Department of Pharmacology, Appasaheb Birnale College of Pharmacy, South Shivajinagar, Sangli-416416. India

Corres.author: amoldeore22@gmail.com

Abstract: In present study the aqueous extract of *Malvastrum coromandelianum* Linn leaves was evaluated for antidiabetic and antihyperlipidemic activities on alloxan induced diabetic rats in acute and chronic study. The aqueous extracts **of** *Malvastrum coromandelianum* leaves showed significant ($P<0.01$) reduction in fasting blood glucose level and normalize the lipid profile (total cholesterol, HDL cholesterol and triglyceride) in alloxan induced diabetic rats on 21 day of the treatment.

Key words: Alloxan; total cholesterol, HDL cholesterol, triglyceride, fasting blood glucose.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by alteration in carbohydrate, protein and fat metabolism resulting from inherited and or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Insulin deficiency results in hyperglycemia which triggers complications involving dyslipidemia, macroangiopathy, retinopathy, cataract formation, peripheral nerve damage, nephropathy, endothelial dysfunction and others [1-5]. It is projected that incidence of diabetes is on rise. Present number of diabetics worldwide is 150 million and this is likely to increase to 300 million or more by the year 2025 [6]. Reasons for this rise include increase in sedentary lifestyle, consumption of energy rich diet, obesity, higher life span, etc[7]. Insulin and oral hypoglycemic agent have certain effects like causing severe hypoglycemia at higher doses, insulin allergy, resistance, edema, lipoatrophy, lipohypertrophy, liver problems, cardiotoxicity, lactic acidosis and diarrhoea. It is apparent that due to the side effects of the currently used drugs, there is a need for a safe agent with minimal adverse effects, which can be taken for long durations [8,9].

The medicinal plants might provide a useful source of new oral hypoglycemic compounds for development of pharmaceutical entities or as a dietary adjunct to existing therapies[10]. *Malvastrum coromandelianum* is used in traditional medicine as an antiinflammatory, analgesic, antidysenteric plant[11-13].Various extracts of the aerial parts of *Malvastrum coromandelianum* showed antinociceptive activity[14]. Rattanajarasroj was demonstrated the hypoglycemic effect of extract of *Malvastrum coromandelianum* leaves[15].

Malvastrum coromandelianum (L.) Garcke has been claimed to treat diabetes mellitus. Alloxan hydrate treated animals exhibit severe hyperglycemia, glycosuria, hyperlipidemia, polyphagia, polydypsia and other symptoms of uncontrolled diabetes[16]. We have investigated effect of *Malvastrum coromandelianum* Linn leaves extracts on fasting blood sugar level and lipid profile in alloxan induced diabetic rats.

MATERIALS AND METHODS

Chemicals

Alloxan hydrate was purchased from Research Lab Fine Chem Industries, Mumbai. Blood glucose was assayed using kits from Span Diagnostics Ltd, Surat, India. Total cholesterol, HDL cholesterol, triglyceride were assayed using kits from Agappe Diagnostics Ltd, Ernaculam, Keala. One touch glucometer (Accu-check sensor) of Roche Diagnostics, Germany and Uristix was purchased from Bayer Diagnostics India Ltd.

Plant material

The leaves of Malvastrum coromandelianum Linn were collected from the Sangli, authenticated from Dr. Yadav,Department of Botany, Willington college,Sangli. (Voucher specimen no. BN122)

Preparation of aqueous extract of Malvastrum coromandelianum Linn leaves

The leaves of Malvastrum coromandelianum were allowed to dry in the shade. The dried leaves were subjected to size reduction to a coarse powder by using mixer grinder. The coarsely powdered form of shade dried fruit rinds was placed in a conical flask containing distilled water and closed with cotton plug for 7 days at room temperature. Then it was filtered using a piece of clean, sterile, white cotton cloth and evaporated to dryness to yield extract. The solid extract obtained was stored in an airtight container in refrigerator for further use. 20 gm of dried leaves powder used for extraction that has given 1.4 gm of water extract (7%). The suspension of aqueous extract was prepared by using 0.5% w/v carboxymethylcellulose (cmc) for experiment.

Animals

Male/female Wistar albino rats weighing 180-200 g, procured from the animal house of Pharmacology department, Appasaheb Birnale College of Pharmacy, Sangli were used with the approval of The Institute Animal Ethics Committee. During the complete course of the experiment, rats were maintained at room temperature in the animal house. The animals had free access to food pellets (Amrut Laboratories animal feed, Sangli) and water ad libitum. Each group of animals was housed separately with a distinct identity throughout the study. Throughout, internationally accepted ethical guidelines for the care of laboratory animals were followed in the study period.

Induction of diabetes in experimental rats

The alloxan hydrate solution was prepared freshly in saline solution, kept on ice and injected immediately. Male/female Wistar Albino rats, weighing between 180-200 g, were selected and marked for individual identification. Rats were fasted for at least 16 hrs because fasted animals are more susceptible to alloxan [17,18].

Hyperglycemia was induced by injecting alloxan hydrate at a dose of 130 mg/kg intraperitoneally showed low mortality in rats[18,19]. 10% dextrose was there after administered orally since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release[20,21]. Hyperglycemia was checked by using Uristix. Rats that died during the experiment were excluded from the analysis. Five days after alloxan injection, blood glucose levels were determined by using Accu check glucometer to confirm the development of diabetes and rats with fasting blood glucose level >300 mg/dl were included in further study. The mortality rate of alloxan induced diabetic rats was found up to 30% during the present study. Based on that we have taken 40% extra animals for inducing diabetes.

Experimental design

All the diabetic animals were divided in to the four groups and non-diabetic animals included in fifth group with six animals in each group. Group A, B, and C were served as vehicle control (non-diabetic animals), diabetic control and standard drug (Glibenclamide, 10 mg/kg per day p.o.) respectively. Groups D and E were treated with oral administration of the aqueous extract 200 mg/kg and 400 mg/kg respectively, given for 21 days. Daily treatment with plant extract and standard drug were started 5 days after alloxan injection for 21 days.

Assessment of biochemical parameters

In acute study, fasting blood glucose levels were tested at 0, 2, 4 and 6 hrs after the administration of extract on 1st day of the treatment. On 1st,7th,14th and 21stday of treatment of the study, blood sample was collected from retro orbital plexus with the help of a capillary tube under ether anesthesia from overnight fasted rats and fasting blood glucose was estimated. Serum was separated by centrifugation and analyzed for total cholesterol[22], triglyceride[23] by enzymatic GPO-AP-ESPAS colorimetric method, HDL cholesterol[24].

Statistical analysis

All the values of fasting blood glucose and biochemical estimations were expressed as mean ± standard error of mean (S.E.M.) and statistical analysis was performed by using Dunnett's t-test. $P < 0.01$ was considered as the criterion of significance.

Table 1: Effect of oral administration of aqueous extract of Malvastrum coromandelianum Linn on fasting blood glucose level in diabetic rats in acute study.

Groups	Dose mg/kg	Post treatment levels (hours)			
		0	2	4	6
Vehicle control	10ml	85 ±3.15**	84.16 ± 2.01**	84 ± 2.06**	83.66 ± 3.05**
Diabetic control	10ml	363 ± 3.31	375.17 ± 4.96	368.5 ± 5.13	377.67 ± 4.55
Glb	10	383.17 ± 6.1**	245.50 ± 5.89**	182.67 ± 4.06**	105.67 ± 3.00**
AE 200	200	375.67 ±5.81**	282.17 ± 4.04**	224.17 ± 4.55**	160.17 ± 2.79**
AE 400	400	380.17 ± 5.91*	262.67 ± 5.74**	199.5 ± 3.8**	111.67 ± 2.29**

Vehicle, 0.5% carboxymethylcellulose suspension in normal saline; AE200, aqueous extract of *Malvastrum coromandelianum* Linn (200 mg/kg); AE400, aqueous extract of *Malvastrum coromandelianum* Linn fruit rind (400 mg/kg); Glb , Glibenclamide (10 mg/kg).

Values are expressed as mean ± S.E.M. Statistical analysis were performed using Dunnett's test. $p<0.01$ was taken as the criterion of significance. **$p<0.01$ when compared with diabetic control and when diabetic control compared with vehicle control.

Table 2: Effect of daily oral administration of aqueous extract of Malvastrum coromandelianum Linn on fasting blood glucose level in diabetic rats for 21 days.

Groups	Dose mg/kg	Post treatment levels (Days)			
		1	7	14	21
Vehicle control	10ml	88 ± 1.52**	84 ± 0.57**	84 ± 0.57**	85 ± 2.5**
Diabetic control	10ml	370 ± 4.16	350 ± 5.68	360 ± 3.78	355 ± 2.08
Glb	10	367 ± 6.60**	217 ± 4.73**	109 ± 4.57**	85 ± 3.15**
AE 200	200	350 ± 4.58**	266 ± 3.51**	195 ± 3.51**	115 ± 4.58**
AE 400	400	345 ± 1.52**	229 ± 4.04**	141 ± 2.51**	97 ± 3.21**

Values are expressed as mean ± S.E.M. Statistical analysis were performed using Dunnett's test. $p<0.01$ was taken as the criterion of significance. **$p<0.01$ when compared with diabetic control and when diabetic control compared with vehicle control.

RESULTS

Fasting blood glucose level

The effect of aqueous extract of Malavastrum coromandelianum on fasting blood sugar level was assessed in alloxan induced diabetic rats at various time intervals (Table 1). In acute study, The fasting blood glucose level of diabetic rats significantly ($P<0.01$) reduced from 375 and 383 mg% to 160 and 105 mg%, 6 h after administration of aqueous of Malavastrum coromandelianum Linn at the dosed of 200 and 400 mg/kg body weight respectively which is comparable to that of effect of 100 mg/kg of pioglitazone hydrochoride ($P<0.01$). In chronic study (Table 2), Treatment on severely (Fasting Blood Glucose >250 mg/dl) diabetic rats for 21 days at doses of 200 and 400 mg/kg of aqueous extract normalized the fasting blood glucose from 350 mg/dl to 115 mg/dl and 345 mg/dl to 97mg/dl respectively.

Lipid profile

The mean values of lipid profile are presented in table 3. Serum total cholesterol and triglyceride levels were significantly higher in untreated diabetic control rats compared with the diabetic rats administered with Malavastrum coromandelianum Linn extract. Serum cholesterol, serum triglyceride levels were decreased significantly ($P<0.01$) by Glibenclamide and the extract of Malavastrum coromandelianum Linn due to 21 days of treatment. HDL levels were increased by Glibenclamide and aqueous extract.

Table 3: Effect of daily oral administration of aqueous extract of Malvastrum coromandelianum Linn on serum lipid profile in diabetic rats for 21 days.

Groups	Dose mg/kg	Post treatment levels (Days)			
		1	7	14	21
Total cholesterol (mg/dl)					
Vehicle control	05ml	126 ± 1.15**	120 ± 1.52**	122 ± 3.05**	120 ± 1.00**
Diabetic control	10ml	170 ± 2.30	175 ± 1.00	179 ± 1.73	180 ± 1.00
Glb	30	184 ± 1.52**	141 ± 2.30**	128 ± 0.57**	115 ± 2.08**
AE200	200	170 ± 2.30**	155 ± 2.08**	135 ± 1.15**	121 ± 3.05**
AE400	400	168 ± 4.00**	146 ± 2.64**	132 ± 2.08**	118 ± 1.15**
HDL cholesterol (mg/dl)					
Vehicle control	05ml	38 ± 2.27**	39 ± 1.93**	39 ± 3.1**	40 ± 2.58**
Diabetic control	10ml	27 ± 1.29	21 ± 1.58	16 ± 1.03	12 ± 0.91
Glb	30	23 ± 0.94**	35 ± 1.07**	42 ± 3.04**	45 ± 1.58**
AE 200	200	21 ± 1.22**	29 ± 0.77**	36 ± 0.91**	41 ± 0.94**
AE 400	400	22 ± 1.78**	32 ± 0.95**	39 ± 2.19**	43 ± 1.82**
Triglyceride (mg/dl)					
Vehicle control	05ml	84 ± 1.65**	29 ± 3.70**	88 ± 3.70**	90 ± 5.76**
Diabetic control	10ml	224 ± 3.15	216 ± 5.95	208 ± 5.35	205 ± 7.61
Glb	30	204 ± 3.61**	150 ± 4.56**	110 ± 6.67**	75 ± 6.22**
AE 200	200	215 ± 2.98**	178 ± 4.33**	136 ± 4.32**	89 ± 3.64**
AE 400	400	210 ± 3.53**	164 ± 5.68**	125 ± 5.78**	82 ± 6.67**

HDL, high-density lipoprotein.

Values are expressed as mean ± S.E.M. Statistical analysis were performed using Dunnett's test. $p<0.01$ was taken as the criterion of significance. **$p<0.01$ when compared with diabetic control and when diabetic control compared with vehicle control.

Where, AE 200: Aqueous extract of *M. coromandelianum* Linn (200 mg/kg)

AE 400 : Aqueous extract of *M. coromandelianum* Linn (400 mg/kg)

Glb: Glibenclamide (10 mg/kg)

Fig1: Effect of oral administration of aqueous of Malvastrum coromandelianum Linn on fasting blood glucose level in diabetic rats in acute study.

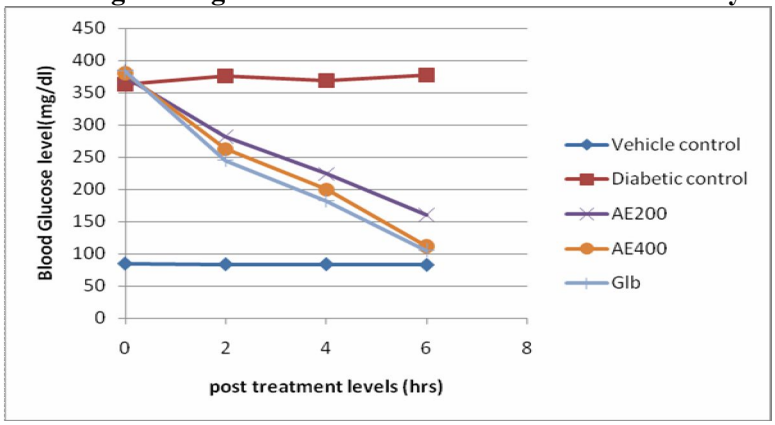
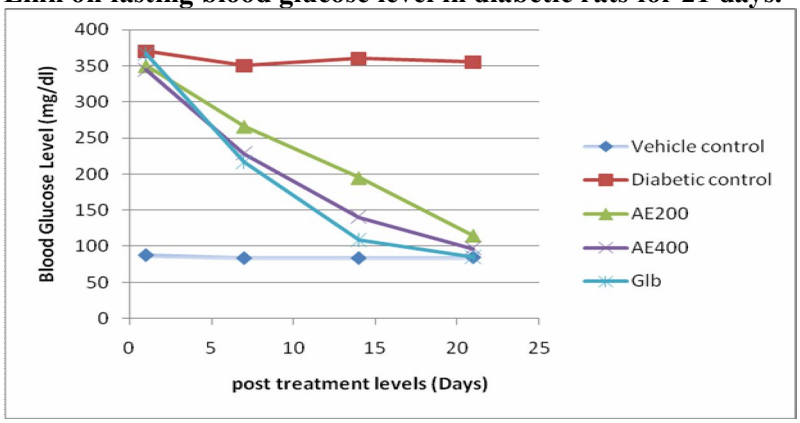


Fig2: Effect of daily oral administration of aqueous extract of Malvastrum coromandelianum Linn on fasting blood glucose level in diabetic rats for 21 days.



DISCUSSION

Alloxan produces selective cytotoxicity in pancreatic β-cells through the generation of reactive oxygen species resulting in reduced synthesis and release of insulin[29]. Single administration of alloxan hydrate (130 mg/kg, i.p.) led to 5-6 fold elevation of fasting blood glucose levels, which was maintained over a period of 3 weeks in experimental rats. Daily treatment for three weeks of aqueous extract of Malvastrum coromandelianum Linn at the dose 400 mg/kg, p.o. lowered the fasting blood glucose level in significantly bringing it nearly back to normal on 21st day (Figs 1 and 2). In present study, both aqueous extract and glibenclamide showed rapid normalization of blood glucose levels while hyperglycemia was maintained in diabetic control group throughout the total duration of the study (Fig 1). It is possible that both aqueous extract 400m/kg and glibenclamide bring about release of insulin from the surviving β-cells, thereby, resulting in normalization of blood glucose levels. Diabetes mellitus is often linked with abnormal lipid metabolism. The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma[25]. Increased production of very low density lipoprotein (LDL) by the liver results from increased delivery of fatty acids because of decreased utilization by muscle and increased delivery of fatty acids from visceral abdominal fat to the liver via the portal circulation. Decreased catabolism of postprandial triglyceride rich lipoprotein particles because of reduced lipoprotein lipase activity accentuates diabetic dyslipidemia[26]. The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents the risk factor for coronary heart diseases[27]. Lowering of serum lipids concentration through dietary or drug therapy seems to be associated with a decrease in the risk of vascular diseases[28]. The results of this study reveal that a regular administration of *Malvastrum coromandelianum* Linn extracts for 21 days nearly normalized lipid profile in diabetic animals. The dose of 400 mg/kg of aqueous extract not only lowered total cholesterol and triglyceride level but also enhanced the cardioprotective lipid HDL.

In present study the aqueous extracts of *Malvastrum coromandelianum* Linn showed significant ($P<0.01$) reduction in blood glucose level, total cholesterol, triglyceride level in alloxan induced diabetic rats.

CONCLUSION

From this study, we can conclusively state that aqueous extract of leaves of *Malvastrum coromandelianum* Linn have beneficial effects on blood glucose level as well as improving hyperlipidemia due to diabetes. Further pharmacological and biochemical investigations are underway to elucidate the mechanism of the antidiabetic and hypolipidemic potential.

ACKNOWLEDGMENT

Authors want to expressed gratitude to Dr. Magdum, Vice Principal, Appasaheb Birnale college of pharmacy,Sangli for their constant guidance, discussion and also for providing digital library for the present research

REFERENCES

1. Baynes J.W., Role of oxidative stress in development of complications in diabetes, Diabetes. 1991,40, 405-412.
2. Brownlee M., and Cerami A., The biochemistry of the complications of diabetes mellitus, Ann. Rev. Biochem. 1981,50, 385-432.
3. Meinders A.E., and Kuyvenhoven J.P., Review article Oxidative stress and diabetes mellitus Pathogenesis of long-term complications, Eur. J Inter. Med. 1999,10, 9-19.
4. Nagappa A.N., Thakurdesai P.A., Venkat Rao N., and Jiwan Singh, Antidiabetic activity of Terminalia catappa Linn fruits, J Ethnopharmacol. 2003,88, 45-50.
5. O'Brien R.M., and Granner D.K., Regulation of gene expression by insulin, Phys. Rev. 1996,76, 1109-1161.
6. King et al., Global burden of diabetes, 1995-2025, prevalence, numerical estimates, and projections, Diabetes Care. 1998, 21, 1414-1431.
7. Yajnik C.S., The insulin resistance epidemic in India, fetal origins, later lifestyle, or both? Nutr. Rev. 2001,59, 1-9.
8. Brunton L.L., Parker K.L., Blumenthal D.K., and Buxton L.O. Insulin, oral hypoglycemic agents and pharmacology of endocrine pancreas, Goodman and Gillman's Manual of pharmacology and therapeutics, The McGraw-Hill Companies, USA, 2008,1048-1068.
9. Katzung B.G., Pancreatic hormone and antidiabetic drugs. Basic & Clinical Pharmacology, edited by Bertram G. Katzung, 10th Edition, McGraw Hill,USA,2007, 683-705.
10. Bailey L.J., and Day C., Traditional plant medicine as treatment for diabetes, Diabetes Care. 1989,12, 553-564.
11. Fyson P.F., The flora of the Nilgiris and Pulney Hill Tops, Volume I. 1st ed. Dehra Dun, India, Bishen singh Mahendra Pal Singh, 1974.
12. Kirtikar K.R., and Basu B.D., Indian medicinal plants, International Book Distributors and Publishers, Dehra Dun, India, 1995.
13. Khonsung P., Nantsupawat S., Jesadanont S. N., Chantharateptawan V., and Panthong A., Antiinflammatory and analgesic activities of water extract of Malvastrum coromandelianum (L.) Garcke, Thai. J Pharmacol. 2006, 28(03).
14. Reddy Y. S. R., Sama V., and Suresh B., Antinociceptive activity of Malvastrum coromandelinum, Fitoterapia. 2001, 72(3),278-280.
15. Rattanajarasroj S., Bansiddhi J., Kun-Anek A., and Chaorai B., Hypoglycemic activity of daikhad (Malvastrum coromandelianum (L.) Garcke) in rats, Bull. Dept. Med. Sci., 2005,180.
16. Srinivasan K., and Ramarao P., Animal models in type 2 diabetes research, an overview, Ind. J. Med. Res. 2007,125,451-472.
17. Katsumata K., Katsumata K., and Katsumata Y. Protective effect of diltiazem hydrochloride on the occurrence of alloxan or streptozotocin induced diabetes in rats, Hor. Met. Res. J., 1992, 24, 508-510.
18. Szkudelski T., The Mechanism of Alloxan and Streptozotocin Action in B Cells of the Rat Pancreas, Phys. Res. 50, 2001,536-546.
19. Kannur D. M., Hukkeri V. I., and Akki K. S., Antidiabetic activity of Caesalpinia bonducella seed extracts in rats, Fitoterapia 2006,77, 546-549.
20. Kliber A., Szkudelski T., and Chichlowska J., Alloxan stimulation and subsequent inhibition of insulin release rom in situ perfused rat pancreas, J. Phys. Pharmacol, 1996,47,321-328.
21. Sushruta K., Satyanarayana S., and Srinivas N., Evaluation of the blood-glucose reducing effects of aqueous extracts of the selected umbelliferous fruits used in culinary practices, Trop. J. Pharma. Res. 2006,5(2), 613-617.
22. Roeschlau P., Bernt E., and Gruber W., Enzymatic determination of total cholesterol in serum, Zeitschrift fur Klinische Chemie und Klinische Biochemie. 1974,12, 226.
23. Buccolo G., and David M., Quantitative determination of serum triglycerides by use of enzyme, Clin. Chem. 1973,19, 476-482.
24. Friedwald J., Levy Y.R., and Friedrickson S.D., Estimation of concentration of low-density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge, Clin. Chem. 1972,18, 499-502.
25. Punitha R., and Manoharan S., Antihyperglycemic and antilipidperoxidative effects of Pongamia pinnata (Linn.) Pierre flowers in alloxan induced diabetic rats, J. Ethnopharmacol. 2006,105, 39-46.
26. Braunwald E., Zipes D.P., and Libby P., Heart disease, Textbook of cardiovascular medicine, 8th edition, Saunders,2008.

27. Davidson M.B., Diabtes Mellitus Diagnosis and Treatment, Wiley, New York, 1981.
28. Rhoads G.G., Gulbrandse C.L., and Kagen A. Serum lipoprotein and coronary artery disease in a population study of Hawaiian Japanese men, N. Eng. J. Med. 1976, 294,293-298.
29. Sakurai K., Katoh M., Someno K., and Fujimoto Y., Apoptosis and mitochondrial damage in INS-1 cells treated with alloxan, Bio. Pharm. Bull. 2001, 24, 876-882.

*****