The Journal of Phytopharmacology 2014; 3(1): 29-34
Online at: www.phytopharmajournal.com

Research Article

ISSN 2230-480X
JPHYTO 2014; 3(1): 29-34
January- February
© 2014, All rights reserved

P.Vinoth Kumar
Departement of Biotechnology, J.J. College of Arts and Science, Pudukottai-622404, India

N.Ramesh
Departement of Biotechnology, J.J. College of Arts and Science, Pudukottai-62240, India

Correspondence:
N.Ramesh
Departement of Biotechnology, J.J. College of Arts and Science, Pudukottai 622404, India
E-mail:
rameshbiotechno@gmail.com

Anti-hyperglycaemic activity of *Pergularia daemia* (Forssk.) Chiov

*P.Vinoth Kumar, N.Ramesh**

Abstract

Pergularia daemia (Forssk.) Chiov. is commonly known as 'Velipparuthi' and its leaves are traditionally used to treat fevers, rheumatism, liver disorders, emetic and expectorant. The present work is aimed to evaluate the effect of leaves of *P. daemia* on blood glucose level status in streptozotocin induced diabetic rats. STZ diabetic rats showed decreased levels of blood glucose as compared to normal. Daibetic animals were treated with various extract of *P. daemia* leaves (100, 200 and 300 mg/kg b.w) for 21 days. Oral administration of *P. daemia* showed the marked reduction in elevated level of serum glucose as compared with diabetic group at a dose of 100, 200 and 300 mg/kg. The results suggested that oral administration of *P. daemia* possesses significant antidiabetic potential. It was concluded that antidiabetic effect of *P. daemia* may due to its bioactive compounds responsible for antidiabetic activity present in the leaves extract.

Keywords: Diabetes mellitus, *Pergularia daemia*, Explant, Leaf, Streptozotocin, Serum glucose.

Introduction

Diabetes mellitus is a chronic metabolic disorder that affects human body in terms of physical, psychological and social health. It is defined as a group of disorders characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins.[1, 2] It is becoming the third "killer" of the health of mankind along with cancer, cardiovascular and cerebrovascular diseases.[3] The prevalence of diabetes mellitus is expected to reach up to 4.4% in 2030, and the occurrence was found to be high in India, China, and USA.

Pergularia daemia (Forssk.) Chiov. Syn. - Daemia extensa R. Br. (Family-Asclepiadaceae) is distributed throughout the hotter parts of India. It is also seen in the Himalayas up to an altitude of 3500m in the hills of Assam and Bihar and is also found in Sri Lanka and Afghanistan.[4, 5] Pergularia daemia has a vast of applications in different folk medicines including the Ayurveda system and are believed to increase defence against various diseases. It is used as pungent, cooling, anthelmintic, laxative and antipyretic agents. It cures biliousness, asthma, ulcers. It is useful in eye complaints, urinary discharges, leucoderma, uterine complaints, inflammation and facilitates parturation. Decoction and juice of the leaves are reputed to be a cure for snake bite.[6]

Pergularia daemia has a general reputation as an expectorant, emetic and also to infantile diarrhea.[7] It is commonly used among the tribes of sherveroy hills of Tamil Nadu as a substitute for *Gymnema sylvestre* in the treatment of diabetes.[8] But, its antidiabetic activity is yet to be experimented on researched for its full potential.[9] Therefore, it was thought worthwhile to evaluate its antidiabetic effects using Streptozotocin (STZ) induced experimental rats.

Materials and Methods

Explant collection

Pergularia daemia was collected from the Southern parts of Pudukkottai district, Tamil Nadu, India, and planted in the J.J. College Botanical Garden. The plants were raised in pots containing mixture of soil and farmyard manure in the ratio of 1:1. Small disease free tender leaf were collected from 5-6 months grown plants, cut into 0.5-1.0 cm segments and used as explants for callus induction.

Media preparation, callus induction and its proliferation

Murashige and Skoog[10] medium supplemented with auxins viz., 2,4-D, NAA, IAA and cytokinins viz., BAP and Kin alone at different concentrations was used for callus initiation. The cultures were incubated under fluorescent lights with 1500-2000 lux for 16 h at 25±1°C and 80±10 relative humidity. Each experiment had 20 replicates and was repeated thrice. The proliferated callus culture was sub cultured and maintained for 45-50 days on the same medium supplemented with the same growth regulator.

Preparation of extracts

The leaves and calli were dried under shade, coarsely powdered, and extracted with chloroform (60-80°C) followed by alcohol, and then water using Soxhlet apparatus. The extracts so collected were distilled off on a Water bath at atmospheric pressure and the last traces of the solvents were removed in vacuo. [11]

Animals

In the present study healthy, matured male albino rats (wistar strain) were used. Rats weighing 180-230 g were obtained from the Periyar College of Pharmaceutical Sciences, Tiruchirapalli, Tamil Nadu, India and kept in plastic animal cages with 12 h light and dark cycle in the institutional animal house. The animals were fed with standard rodent diet and provided water ad libitum. After one week of acclimatization the animals were used for the further experiments. Approval from the Institutional Animal Ethical Committee for the usage of animals in the experiments was obtained as per the Indian CPCSEA guidelines.

Chemicals

Streptozotocin (STZ) and glibenclamide were purchased from Sigma Aldrich, St. Louis, MO, USA. All other chemicals and solvents used were of Analytical Grade obtained from E-Merck and Himedia, Mumbai, India.

Acute toxicity study

Acute toxicity studies were carried out using Acute Toxic Class Method as per OECD-423 Guideliness.[12] Chloroform leaf extract; ethanol leaf extract, aqueous leaf extract and ethanol leaf callus extract of *P. daema* were administered at a starting dose of 2000 mg/kg b.w of orally to 4 male rats. The animals were observed for mortality and behavioral changes during 48 h.

Experimental induction of diabetes

Diabetes was induced in a group of rats after an overnight fast by single intraperitoneal injection of STZ, which was freshly dissolved in 0.1M citrate buffer (pH 4.5). The dose was 40 mg/kg b.w. STZ treated animals were allowed to drink 5% glucose solution overnight to overcome drug induced hypoglycemia. After 48 h of STZ administration, the blood glucose ranges above 200-300 mg/dl was considered as diabetic rats and used for the experiment.

Experimental design

In the experiment, a total of 162 rats were used, randomly divided into 27 groups of 6 animals each and treatments continued in an aqueous solution daily using an intragastric tube for 21 days.

Group of Animals:

Group-I	:	Normal rats received 3% gum acacia
Group-II, III, IV	:	Leaf chloroform extract (100, 200, 300 mg/kg b.w.)
Group-V, VI, VII	:	Leaf ethanol extract (100, 200, 300 mg/kg b.w.)
Group-VIII, IX, X	:	Leaf aqueous extract (100, 200, 300 mg/kg b.w.)
Group-XI, XII, XIII	:	Leaf ethanol callus extract (100, 200, 300 mg/kg b.w.)
Group-XIV	:	Streptozotocin (STZ) 40 mg/kg b.w. (Diabetic control)
Group-XV, XVI, XVII	:	STZ+Leaf chloroform extract (100, 200, 300 mg/kg b.w.)
Group-XVIII, XIX, XX	:	STZ+Leaf ethanol extract (100, 200, 300 mg/kg b.w.)
Group-XXI, XXII, XXIII	:	STZ+Leaf aqueous extract (100, 200, 300 mg/kg b.w.)
Group-XXIV, XXV, XXVI	:	STZ+Leaf ethanol callus extract (100, 200, 300 mg/kg b.w.)
Group-XXVII	:	STZ+Glibenclamide (600 µg/kg b.w.)

Biochemical assays

After the separation of plasma, from blood samples on 21st days of the treated animals the buffy coat was removed and the packed erythrocytes were washed thrice with cold physiological saline. A known volume of the erythrocyte was lysed with cold hypotonic phosphate buffer at pH 7.4. The hemolysate was separated by centrifugation at 2000 rpm for 10 min and the supernatant was used for the estimation of blood glucose. Blood glucose level was estimated by the method of Sasaki *et al*.[13]

Statistical analysis

Statistical analysis was performed using SPSS Software Package, version 11.5. The values were analyzed by One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). All these results were expressed as mean ±SD for six rats in each group, p-values <0.05 were considered as significant.[14]

Results and discussion

Callus was initiated from stem and leaf explants of *P. daemia* on basal MS medium supplemented with 2,4-D, α-NAA, BAP, KIN and IBA at different concentrations 1.0 - 3.0 mg/l. The maximum induction rate was observed at 2, 4-D 2.0 mg/l combination with 0.5mg/l BAP and 1.0 mg/l α-NAA.[15]

The acute toxicity study revealed the non-toxic nature of the chloroform, ethanol, aqueous and ethanol callus extracts at the tested concentrations. No lethal toxic reactions were observed until the end of the experiment.

The biochemical parameters were recorded from the test animals. The blood glucose levels (Table 1) of the normal animals were 89.12 mg/dl on the 0thday and 89.06 mg/dl on the 21st day. The streptozotocin treated control showed 88.40 mg/dl on the 0th day and 348.21 mg/dl on the 21st day. Diabetic animals treated with 300 mg/kg of the chloroform leaf extract showed 82.02 mg/dl on the 0th day and 160.68 mg/dl on the 21st day. The scores of the diabetic animals treated with 300 mg /kg of the ethanol extract were 87.22 mg/dl on the 0th day and 132.61 ml/dl on the 21st day. Diabetic animals treated with 300 mg /kg of the aqueous extract showed 89.33 mg/dl on the 0th day and 152.80 mg/dl on the 21st day. Diabetic animals treated with 300 mg /kg of the ethanol extract of callus showed 83.30 mg/dl on the 0th day and 123.26 mg/dl on the 21st day. Diabetic animal treated with 600 µg /kg of glibenclamide (standard diabetic drug) registered 86.23 mg/dl on the 0th day and 194.6 mg/dl of blood glucose on the 21st day.

Table 1: Changes in the blood glucose level of normal and experimental animals

Control & Treatment		Groups	0 day (mg/dl)	7th day (mg/dl)	14th day (mg/dl)	21th day (mg/dl)
Normal		I	89.12±4.12	90.46±2.12	90.98±2.52	89.06±2.55
Chloroform	100 mg/kg	II	90.50±1.25	90.99±0.43	87.48±0.31	90.46±2.16
	200 mg/kg	III	90.89±1.48	87.74±0.71	87.76±0.31	88.28±1.49
	300 mg/kg	1V	85.64±2.73	88.95±0.22	84.61±0.39	84.51±1.91
Ethanol	100 mg/kg	V	90.40±5.01	92.44±4.13	91.63±3.49	91.52±3.09
	200 mg/kg	VI	91.60±8.40	93.06±6.23	94.72±4.87	90.65±8.14
	300 mg/kg	VII	89.15±4.19	90.82±2.39	92.76±3.78	90.82±8.96
Aqueous	100 mg/kg	VIII	98.41±1.25	86.6±1.97	84.3±5.11	81.97±2.68
	200 mg/kg	IX	88.44±1.79	82.67±1.20	87.67±1.60	89.31±2.54
	300 mg/kg	X	83.51±1.79	84.66±1.65	81.25±2.36	89.16±1.37
Ethanol callus	100 mg/kg	XI	89.00±4.36	90.00±0.25	85.66±1.90	88.65±1.06
	200 mg/kg	XII	85.52±4.33	92.15±2.37	90.33±2.49	89.17±2.35
	300 mg/kg	XIII	80.25±0.31	90.11±2.25	85.17±2.86	91.33±2.24
Diabetic (STZ)	40 mg/kg	XIV	88.40±4.17*	320.5±4.86*	337.9±4.50*	348.21±3.23*
STZ + Chloroform	100 mg/kg	XV	89.72±5.46#	255.49±5.24#	220.31±6.87#	172.28±2.24#
	200 mg/kg	XVI	89.90±6.56#	251.49±5.43#	215.8±5.72#	165.01±5.73#
	300 mg/kg	XVII	82.02±5.32#	248.34±5.36#	212.8±5.31#	160.68±7.16#
STZ + Ethanol	100 mg/kg	XVIII	84.12±0.47#	241.04±0.52#	202.10±0.2#	149.4±0.61#
	200 mg/kg	XIX	86.65±0.39#	236.3±3.38#	200.68±1.35#	141.78±0.84#
	300 mg/kg	XX	87.22±1.01#	233.15±5.23#	194.23±1.42#	132.61±1.46#
STZ + Aqueous	100 mg/kg	XXI	84.16±1.39#	246.63±5.41#	210.32±5.85#	158.56±1.36#
	200 mg/kg	XXII	85.43±1.01#	244.16±5.94#	205.36±5.72#	153.42±1.82#
	300 mg/kg	XXIII	89.33±1.56#	243.15±1.39#	203.17±6.97#	152.80±2.86#
STZ + Ethanol callus	100 mg/kg	XXIV	83.01±1.92#	230.0±1.57#	186.31±2.21#	128.62±1.46#
	200 mg/kg	XXV	85.16±1.86#	230.06±1.18#	185.80±0.42#	125.15±1.44#
	300 mg/kg	XXVI	83.30±2.16#	223.98±0.79#	177.84±1.03#	123.26±1.13#
STZ + Glibenclamide	600 µg/kg	XXVII	86.23±1.08#	210.97±5.04#	204.05±3.06#	194.6±6.41#

Values are expressed as mean±SD (n=6).
Diabetic control is compared with normal; *Values are statistically significant at P* <0.05 compared to normal.
Treated groups are compared with diabetic control; # Values are statistically significant at P# <0.05 compared to diabetic control.

Many of the oral anti diabetic agents have a number of serious side effects; so managing diabetes without any side effects is still a challenge.[16] Therefore a search for a more and safer hypoglycemic agent is still a challenge and a vital area of research.

The solvent extract at different doses in *P. daemia* have been studied extensively chloroform, ethanol, aqueous and ethanol callus extracts of *P. daemia* were tested on diabetic rats. They were proved to have significant hypoglycemic properties. These extracts improved the biochemical parameters assessed, also brought about regeneration of β-cells of the pancreas and increased insulin levels.

Streptozotocin at high doses selectively destroys the pancreatic insulin-secreting β-cells, leaving less functional cells resulting in diabetes mellitus.[17-20] In most of the experimental studies hyperglycemia was induced by streptozotocin or alloxan.[21, 22]

Conclusion

In the present investigation, daily administration of chloroform, ethanol, aqueous, ethanol callus extracts of *P. daemia* led to decrease in blood glucose levels in STZ-induced diabetic rats. Though all the four extracts proved to be effective, the ethanol callus leaf extract and ethanol leaf extract of *P. daemia* had satisfactory capacity to restore glucose levels to near normal. *P. daemia* is claimed to be useful in diabetes in folklore medicine. The results of the present study indicate that the plant extract was found to reduce the blood glucose level in STZ-induced diabetic rats.

References

1. Patel D.K., Kumar R., Prasad S.K., Sairam K., Hemalatha S. Anti-diabetic and in vitro antioxidant potential of Hybanthus enneaspermus (Linn) F. Muell in streptozotocin-induced diabetic rats. Asi Pac J Trop Biomed. 2011; 1(4): 316-322.

2. Warjeet Singh L. 2011. Traditional medicinal plants of Manipur as anti-diabetics. J Med Plant Reser. 2011; 5(5): 677-687.

3. Chauhan A., Sharma, P.K., Srivastava, P., Kumar, N., Dudhe, R. Plants having potential anti-diabetic activity: A review. Der Pharm Lett. 2010; 2(3): 369-387.

4. Chopra R.N., Nayar S.L., Chopra I.C. Glossary of Indian Medicinal Plants. Council of Scient and Indust Reser. 1956; Pp 188

5. Gamble J.S. Flora of the Presidency of Madras, Botanical Survey of India: Calcutta. 1957; 2: Pp 588.

6. Kirtikar K.R., Basu, B.D. Indian Medicinal Plants, Dehardun: International Book Distributors. 1999; 3: Pp 1616–1617.

7. Sathyavathi G.V., Raina M.K., Sharma M. Medicinal Plants of India. Ind Coun Med Reser.. 1976; Pp 326-327.

8. Raghunathan K., Mitra R. Pharmacognosy of Indigenous Drugs. Centr Coun of Reser in Ayur & Sidd. 1982; 982-996.

9. Wahi A.K., Ravi J., Hemalatha S. and Singh P.N. Anti diabetic activity of Daemia extensa. J Nat Remed. 2002; 2: 80-83.

10. Murashige T., Skoog, F. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physio Plant. 1962; 15: 473-497.

11. Merlin N.J., Parthasarathy V., Manavalan R., Kumaravel, S. Chemical investigation of aerial parts of Gmenlina asiatica Linn by GC-MS. Pharmaco Reser. 2009; 1(3): 152-156.

12. OECD- Acute oral toxicity- Acute toxic class method guidelines-423. Paris. 1996.

13. Sasaki T., Matsy S., Sorae, A. Effect of acetic acid concentration on the colour reaction in the o-toluidine boric acid method for blood glucose estimation. Rins Kagar. 1972; 1: 346-53.

14. Duncan B.D. Multiple range test for corleted and hetroscedastic means. Biometrics. 1957; 13: 359-364.

15. Vinoth Kumar P., Ramesh N. and Siva Subramanian S. In Vitro Studies on Callus Induction and Suspension Culture Biomass Yield From Pergularia daemia (Forssk) Chiov. International Journal of Pharmacy and Pharmaceutical Sciences. 2012; 4 (1): 518-521.

16. Radermecker R.P., Scheen A.J. Allergy reactions to insulin: Effects of continuous subcutaneous insulin infusion and insulin analogues. Diabet Meta Res & Rev. 2007; 23(5): 348-355.

17. Kamtchouing P., Sokeng D.S., Moundipa P.F., Pierre W., Jatsa B.H., Lontsi D. Protective role of Anacardium occidentale extract against sreptozotocin induced diabetes in rats. J Ethnopharm. 1998; 62: 95-99.

18. Pushparaj P.N., Low H.K., Manikandan J., Tan B.K., Tan, C.H. Antidiabetic effects of Cichorium intybus in streptozotocin-induced diabetic rats. J Ethnopharmaco. 2006; 111(2-4): 430-434.

19. Skyler J.S. Prediction and prevention of Type I Diabetes: Progress, Problems and Prospects. Clin Pharma & Thera. 2007; 81(5): 768-771.

20. Sridhar S.B., Sheetal U.D., Pai M.R.S.M., Shastri M.S. Preclinical evaluation of the antidiabetic effect of Eugenia

jambolina seed powder in streptozotocin induced diabetic rats. Braz J Med & Biol Reser. 2005; 38(3): 463-468.

21. Leatherdale B.A., Panesar R.K., Singh G., Atkins T.W., Bailey C.J. and Bignell A.H.C. Improvement in glucose tolerance due to Momordica charantia. Brit Med J. 1981; 282 (6279): 1823-1824.

22. Hamada Y., Kitazawa S., Kitazawa R., Fujii H., Kasuga M. and Fukagawa M. Histomorphometric analysis of diabetic osteopenia in streptozotocin-induced diabetic mice. A Possible Role of Oxidative Stress. 2006; 40(5): 1408-1414.