

Full Length Research Paper

Antihyperglycemic activity of methanol and aqueous extracts of *Pergularia daemia* Linn.

A. Doss* and S. P. Anand

Department of Botany, National College (Autonomous), Trichy, Tamilnadu, South India.

Accepted 12 December, 2013

The objective of this study was to evaluate the antidiabetic activities of methanol and aqueous extracts of *Pergularia daemia* in alloxan induced diabetic rats. Methods used was methanol and aqueous extracts (250 mg/kg b.w.) of *P. daemia* was administered to alloxan induced diabetic mice for 21 days and blood glucose levels of the diabetic rats were monitored at intervals of hours and days throughout the duration of the experiments. Results shows that oral administration of alcoholic extract of *P. daemia* leaves to diabetic rats for 21 days significantly reduced the levels of blood glucose levels in both acute and sub acute study. In conclusion, these results suggest that the methanol extract of *P. daemia* possess antidiabetic effect on alloxan induced diabetic rats and it can be recommended for the prevention of diabetes mellitus.

Key words: Alloxan monohydrate, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), urea, methanol, haemoglobin.

INTRODUCTION

Diabetes mellitus is one of the most common chronic diseases in the whole world. It is a complex, multifactorial disease which affects the quality, quantity and style of an individual's life. The fact confirmed by reports from the World Health Organization (WHO) shows that India has the largest number of diabetic subjects in the world (Anil et al., 2013). It has been suggested that diabetes is the third leading cause of death due to high incidence of morbidity and mortality after cancer and cardiovascular disorders. Complications such as renal failure, coronary artery disorder, cerebro-vascular disease, neurological complications, blindness, limb amputation, long term damage, dysfunctions and failure of various organs and eventually premature death are associated with chronic hyperglycemia (Kumudhavalli and Jaykar, 2012). The number of people suffering from the disease worldwide is increasing at an alarming rate with a projected 366 million peoples likely to be diabetic by the year 2030 as against 191 million estimated in 2000 (Wild et al., 2004). India

has today become the diabetic capital of the world with over 20 million diabetics and this number is set to increase to 57 million by 2025 (Sharma et al., 2010). The presently available synthetic drugs such as sulfonylureas and biguanides have serious side effects and thus searching for a new class of compounds is essential to overcome diabetic problem (Noor et al., 2008). Management of diabetes without any side effect is still a challenge to the medical community. There is continuous search for alternative drugs. For centuries, medicinal plants have been used to treat various human diseases. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological activities, higher safety margins and lesser costs. After the recommendations made by WHO on diabetes mellitus, investigations on hypoglycemic agents from medicinal plants has become more important. According to world ethnobotanical information reports, almost 800 plants may possess anti-

*Corresponding author. E-mail: androdoss@gmail.com.

diabetic potential. In India, a number of plants are mentioned in ancient literature (Ayurveda) for the cure of diabetic conditions.

Medicinal plants like *Trigonella foenum graecum*, *Allium sativum*, *Gymnema sylvestris* and *Syzigium cumini* have been studied (Grover et al., 2002) for treatment of diabetes mellitus. However, a scientific proof of the anti-diabetic activity of medicinal plants and phytopharmaceuticals with fewer side effects is still lacking (Prasad et al., 2009). *Pergularia daemia* (forsk.) Chiov (Asclepiadaceae), commonly known as utaran (Hindi), Dustapuchettu (Telugu), Uttamarani (Sanskrit) is a slender, hispid, fetid smelling laticiferous twiner found in the plains throughout the hot parts of India. *P. daemia* is said to have more magical application than medical application as it possess diverse healing potential for a wide range of illnesses. Some of the folklore people use this plant to treat Jaundice, as laxative, anti-pyretic, expectorants and also in infantile diarrhea. The leaf latex is locally used as pain killers and for relief from toothache (Hebbbar et al., 2010), the sap expressed from the leaves are held to cure sore eyes in Ghana. The plant reduces the incidence of convulsion and asthma. It is used to regulate the menstrual cycle and intestinal functions. The root is useful in treating leprosy, mental disorders, anemia and piles (Omale et al., 2011).

Therefore, the present study aimed to investigate the antidiabetic activity of methanolic and aqueous extracts of *P. daemia*.

MATERIALS AND METHODS

Collection of plant materials

Fresh plant parts (*P. daemia*) were collected randomly from the gardens and villages of Trichy district, Tamilnadu from the natural stands. The botanical identity of this plant was confirmed by Dr. V. Sampath Kumar, Scientist - C, Botanical Survey of India (Southern Circle), Coimbatore, Tamilnadu. A voucher specimen has been deposited at the Department of Botany, National College (Autonomous), Tiruchirapalli-620 001, Tamilnadu, India.

Preparation of extracts

Aqueous extraction

One hundred grams (100 g) of dried powder were extracted in distilled water for 6 h at slow heat. Every 2 h it was filtered through Whatman No. 1 filter paper and centrifuged at 5000 g for 15 min. The supernatant was collected. This procedure was repeated twice and after 6 h the supernatant was concentrated to make the final volume one-fifth of the original volume.

Solvent extraction

One hundred grams (100 g) of dried plant powdered samples were extracted with 200 ml of methanol kept on a rotary shaker for 24 h. Thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make

the final volume one-fifth of the original volume. It was stored at 4°C in airtight bottles for further studies.

Animals

The animals of both sexes were used for these experiments. They were obtained from Animal House, RVS Pharmaceutical Sciences, Coimbatore, Tamilnadu. The animals were housed in standard cages and were maintained on a standard pelleted feed and water was given *ad libitum*. All the experiments were carried out according to the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments of Animals (CPCSEA), Government of India.

Induction of diabetes

The animals were fasted for 24 h and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of alloxan monohydrate (150 mg/kg b.w.) in sterile normal saline. 72 h later, rats with blood glucose (BGL) levels above 200 mg/dl were considered diabetic and selected for the experiment.

Experimental design: The animals were randomly divided into five groups with 6 rats in each group and treated as follows:

Group I: Normal control (saline) (by using an intragastric catheter tube (IGC)).

Group II: Diabetic control.

Group III: Diabetic rats received *P. daemia* methanol extract (250 mg/kg b.w.) for 21 days by IGC.

Group IV: Diabetic rats received *P. daemia* aqueous extract (250 mg/kg b.w.) for 21 days by IGC.

Group V: Diabetic rats received glibenclamide (2 mg/kg b.w.) daily orally for 21 days by IGC.

The change in body weight and fasting plasma glucose (FPG) levels of all the rats were recorded at regular intervals during the experimental period. For acute toxicity study, FPG were monitored after 30, 60, 120 and 180 min of administration of single dose of the extracts and at the end of 0, 7th, 14th and 21st days for sub acute study. Blood was drawn from the ventricles and centrifuged.

Biochemical analysis

Blood samples were taken into centrifuged at 3000 rpm for 15 min. Serum biochemical parameter such as blood glucose (Sasaki et al., 1972), plasma insulin (Anderson et al., 1993), glycosylated haemoglobin (Karunanayake and Chandrasekharan, 1985), creatine (Owen et al., 1954) and urea (Varley, 1976).

Statistical analysis

All the data were subjected to Duncan's multiple range test (DMRT) was done by using the SPSS version 2007 WINSAT software.

RESULTS

The methanol and aqueous extracts of *P. daemia* administered orally to the rats at the doses of 100, 200, 400, 800, 1200 and 1600 mg/kg b.wt did not produce any significant changes in the autonomic, behavioural or neurolo-

Table 1. Effect of methanol and aqueous extracts of *P. daemia* on body weights of alloxan-induced diabetic mice.

Treatment	Day 0 (g)	Day 7 (g)	Day 14 (g)	Day 21 (g)
Normal control	172.2 ± 2.07	165.25 ± 0.45	169.68 ± 0.15	170.44 ± 3.55
Diabetic control	164.66 ± 1.28	159.37 ± 2.23	158.65 ± 0.05**	158.18 ± 1.28**
PDME (250 mg/kg b.w.)	164.58 ± 2.23	165.94 ± 0.03	167.52 ± 0.25 ^a	169.07 ± 2.83 ^{aa}
PDAE (250 mg/kg b.w.)	161.35 ± 1.05	164.8 ± 1.45	165.17 ± 3.20 ^a	165.91 ± 0.56 ^a
Glibenclamide (10 mg/kg b.w.)	164.63 ± 1.02	165.48 ± 0.032	165.95 ± 0.45 ^a	171.47 ± 1.25 ^{aa}

Each value is SEM ± 5 individual observations; * P < 0.05, ** P<0.01, *** P<0.001 compared normal control versus diabetic mice; a - P < 0.05, aa - P<0.01 compared -diabetic mice versus drug treated.

Table 2. Antidiabetic effect of methanol and aqueous extracts of *P. daemia* on blood glucose level of alloxan-induced mice during acute study.

Treatment	0 min	30 min	60 min	120 min	180 min
Normal control	102.06 ± 0.40	144.9 ± 0.48	157.86 ± 0.41	173.16 ± 0.47	195.1 ± 0.26
Diabetic control	182.03 ± 0.75	215.8 ± 0.43	225.03 ± 0.25	240.63 ± 0.70	256.86 ± 0.61*
PDME (250 mg/kg b.w.)	197.93 ± 0.30	187.03 ± 0.05	152.13 ± 0.79	143.93 ± 0.30	118.33 ± 1.82
PDAE (250 mg/kg b.w.)	195.16 ± 0.20	183.2 ± 0.91	155.73 ± 0.37	147.8 ± 0.43	121.13 ± 0.61
Glibenclamide (10 mg/kg b.w.)	193.9 ± 0.36	168.23 ± 0.20	142.13 ± 0.32	130.23 ± 0.25*	105.36 ± 0.90*

Each value is SEM ± 5 individual observations; * P < 0.05, aa - P<0.01 compared -diabetic mice versus drug treated.

Table 3. Effect of methanol and aqueous extracts of *P. daemia* on blood glucose level of alloxan-induced mice during sub-acute study.

Treatment	0 day	7 day	14 day	21 day
Normal control	94.16 ± 2.35	99.46 ± 2.40	90.16 ± 1.40	95.26 ± 2.55
Diabetic control	241.33 ± 3.98	262.23 ± 1.51	294.16 ± 2.30*	310.13 ± 4.50*
PDME (250 mg/kg b.w.)	264.66 ± 1.49	192.23 ± 2.40	154.5 ± 4.4*	118.06 ± 1.25*
PDAE (250 mg/kg b.w.)	259.23 ± 2.35	196.3 ± 4.2	160.1 ± 4.75*	125.5 ± 2.30*
Glibenclamide (10 mg/kg b.w.)	278.23 ± 3.32	183.5 ± 6.2	141.4 ± 3.55*	111.16 ± 1.15*

Each value is SEM ± 5 individual observations; * P < 0.05, - P<0.01 compared -diabetic mice versus drug treated.

logical alteration. Acute toxicity studies revealed the non-toxic nature of both extracts of *P. daemia*. The signs and symptoms in all groups were found to be normal. Normal control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight on days 7, 14 and 21. There were observable changes in the body weight of treated diabetic rats. Alloxan caused body weight reduction, which is reversed by alcoholic and aqueous extracts of *P. daemia* after 7, 14 and 21 days of treatment. The same trend was noted in glibenclamide treated groups (Table 1). A dose-dependent reduction in blood glucose levels was observed in alloxan induced diabetic rats treated with aqueous and methanol extracts of *P. daemia*. After a single dose-of the extract given to the alloxan induced diabetic rats, there was a significant P < 0.05 reduction in

blood glucose levels of the diabetic rats within the period of acute study compared to control. The maximum effect was observed at 180 min with the methanol extract exerting comparable to effect of aqueous extract that exerted a more pronounced effect (Table 3). The increased blood glucose level in alloxan induced diabetic rats was significantly P < 0.05 by crude extracts (methanol and aqueous) treatment and it was found to be lowered up to 118.33 and 121.13 at the dose of 250 mg/kg of body weight, respectively (Table 2). The insulin and glycosylated haemoglobin levels of diabetic rats treated with methanol and aqueous extracts of *P. daemia* and glibenclamide, a known hypoglycemic drug, resulted from a significant decrease in glycosylated haemoglobin; whereas increase insulin levels when compared with alloxan alone treated rats. The maximum reverse the

Table 4. Effects of *P. daemia* extracts on non-protein compounds and glycosylated haemoglobin levels in normal and alloxan induced diabetic mice.

Treatment	Insulin (lu/L)	Urea (mg/dl)	Creatinine (mg/dl)	Hb A _{1c} (%)
Normal control	0.656 ± 0.36	12.20 ± 2.25	0.61 ± 0.03	3.71 ± 0.27
Diabetic control	0.155 ± 0.24**	30.45 ± 1.35*	1.27 ± 0.75*	9.49 ± 0.20**
PDME (250 mg/kg b.w.)	0.545 ± 0.04 ^{aa}	15.65 ± 3.23 ^a	0.79 ± 0.11	3.88 ± 0.10 ^a
PDAE (250 mg/kg b.w.)	0.492 ± 0.02 ^a	16.92 ± 1.02	0.92 ± 0.02	5.29 ± 0.07 ^a
Glibenclamide (10 mg/kg b.w.)	0.578 ± 0.32 ^{aa}	14.55 ± 3.01 ^a	0.62 ± 0.02	3.76 ± 0.20 ^{aa}

Each value is SEM ± 5 individual observations; * P < 0.05, ** P<0.01, *** P<0.001 compared normal control versus - diabetic mice; a -P < 0.05, aa - P<0.01 compared -diabetic mice versus drug treated.

trend of insulin and glycosylated haemoglobin levels against alloxan induced diabetic aberrations was achieved with the optimum dose 250 mg/kg body weight both (aqueous and methanol) extracts of *P. daemia*. Among the two extracts treated, the methanol extract showed significant changes in insulin and glycosylated haemoglobin.

The level of urea and creatine in normal, diabetic and treated animals are shown in Table 4. The normal function of the kidney was assessed as blood urea level. The urea level in diabetic was found to be 30.45 mg/dl, it was altered from treated animals of 15.85 mg/dl against 12.20 mg/dl (control). The level of creatine in groups II and V showed significant variation when compared to control. Among the two extracts treated, the methanol extract showed significant changes in urea and creatine.

DISCUSSION

Diabetes mellitus is ranked five among the leading causes of death and is considered third when its fatal complications are taken into account. Medicinal plants with a potential of decreasing the blood sugar have been tested in experimental animal models and their effects confirmed. Many unknown and lesser known plants are used in folk and tribal medicinal practices in India. The medicinal values of these plants are not much known to the scientific world.

The present study was carried out to evaluate the antidiabetic activity of methanol and aqueous extracts of *P. daemia* on alloxan induced diabetes in rats. Alloxan is known to induce free radical production and cause tissue injury, and the pancreas is especially susceptible to the action of alloxan induced free radical damage (Akah et al., 2011). Accordingly, significantly high levels (P<0.001) of FBG were observed in alloxan control group rats and remained high throughout the experimental period. Alloxan induced diabetic rats treated with the extract showed a significant reduction in blood sugar levels compared to alloxan control group. This decrease in blood sugar levels may be attributed to stimulation of the residual pancreatic mechanism or to a probable increase in

the peripheral utilization of glucose (Akah et al., 2011). Normoglycemic studies, however, revealed *P. daemia* to have no effect on euglycemia. This implies that the extract is probably acting through any of the extra pancreatic mechanisms rather than stimulating insulin secretion from β cells and results in antihyperglycemic action rather than hypoglycemic effect, that is, does not affect normal blood sugar level, which may be beneficial in case of mis-dosing.

Protein can universally bind non-enzymatically with glucose or other sugars present in the vicinity. The degree of glycation is directly proportional to the concentration of the sugar present in the surrounding medium. Therefore, estimation of glycosylated hemoglobin (HbA_{1c}) gives an accurate reflection of mean plasma glucose concentration over this period and correlates best with the degree of the glycemia (Danze et al., 1987). A change in HbA_{1c} of 1% would reflect a blood glucose alteration of about 30 mg%. A significant decrease with leaf extract (P<0.01) was observed in the treated rats as compared to alloxan-induced diabetic rats. On treatment with roots, the decrease was moderate. This is indicative of a better glycemic control for a longer period by the leaf sample.

A significant reduction in the body weight was observed in the alloxan-induced diabetic rats. The decrease in the weight in diabetes is due to continuous excretion of glucose and decrease in peripheral uptake of glucose and glycogen synthesis (DeFronzo et al., 1992). The decrease in weight was arrested on administration of alcoholic leaf extract to a greater extent as compared to root extract. All the aforementioned observations suggest that the test drug that is, alcoholic leaf extract can be a promising antidiabetic.

In diabetic mellitus, due to persistent hyperglycemia, the excess blood glucose reacts with haemoglobin in a nonenzymatic process to form glycosylated haemoglobin. Since the glycation rate is directly proportional to blood glucose concentration, level of glycosylated haemoglobin indicates glycemic control in the diabetic state (Monnier and Cerami, 1982). Estimation of haemoglobin is a well established parameter useful in the management and prognosis of the disorder (Chang and Nobel, 1979). In the

present study, administration of methanol and aqueous extracts of *P. daemia* leaves significantly reduced the elevated glycosylated haemoglobin levels in alloxan-diabetic rats further substantiating its potential in long term glycemic control of diabetes mellitus. Urea and uric acid are organic waste products produced during the breakdown of amino acids. Creatinine is generated in the skeletal muscle tissue by the breakdown of creatinine phosphate. Their increased level in serum is an indication kidney disorder. It was found that urea and creatinine levels were normal in all the experimental groups. Many oral hypoglycemic agents are normally metabolized or cleared by the kidneys and so accumulate in uraemic patients thus increasing the risk of hypoglycemia and toxicity (Marlin Cynthia and Rajeshkumar, 2012). Our results on the creatinine and urea are very close to normal range and are significant. The remarkable hypoglycaemic potential of *P. daemia* was quite competent with standard drug.

Further studies are necessary to elucidate details of active phytochemicals and their mechanism of hypoglycaemic action. Isolation and study of active principles are under process.

REFERENCES

- Akah PA, Uzodinma SU, Okolo CE (2011). Antidiabetic activity of aqueous and methanol extract and fractions of *Gongronema latifolium* (Asclepidaceae) leaves in Alloxan Diabetic Rats. J. Appl. Pharm. Sci. 01(09):99-102
- Anderson L, Dinesen B, Jorgensen PN, Poulsen F, Roder ME (1993). Enzyme immune assay for intact human insulin in serum or plasma. Clin. Chem. 39:578-582.
- Anil K, Sunil K, Vipin K (2013). Evaluation of antidiabetic activity of hydroalcoholic extract of *Cestrum nocturnum* leaves in streptozotocin-induced diabetic rats. Adv. Pharmacol. Sci. <http://dx.doi.org/10.1155/2013/150401>.
- Chang AT, Nobel J (1979). Estimation of HbA1c like glycosylated proteins in kidneys of streptozotocin diabetes and controlled rats. Diabetes 28:408-415
- Danze PM, Tarjoman A, Rousseaux J, Fossati P, Dautrevaux M (1987). Evidence for an increased glycation of IgG in diabetic patients. Clin. Chim. Acta. 166:143-53
- Defronzo RA, Bonadonna RC, Ferrannini I (1992). Pathogenesis of type 2 (non-insulin dependent) diabetes mellitus: A balanced overview. Diabetologia 35:389-97
- Grover JK, Yadav S, Vats V (2002). Medicinal plants of India with anti-diabetic potential. J. Ethnopharmacol. 81(1):81-100.
- Hebbbar SS, Harsha VH, Shripathi V, Hedge GR (2010). Ethnomedicine of Dharwad district in Karnataka, India plants use in oral health care. J. Ethnopharmacol. 94:261-266.
- Karunanayake EH, Chandrasekharan NV (1985). An evaluation of a colorimetric procedure for the estimation of glycosylated haemoglobin and establishment of reference values for Sri Lanka. J. Natl. Sci. Counc. Sri Lanka 13:235-258.
- Kumudhavalli MV, Jaykar B (2012). Evaluation of Antidiabetic activity of *Costus igneus*(L) leaves on STZ induced diabetic rats. Der Pharmacia Sinica 3(1):1-4
- Marlin Cynthia J, Rajeshkumar KT (2012). Effect of aqueous root extract of *Aristolochia indica* (Linn) on diabetes induced rats. Asian J. Plant Sci. Res. 2(4):464-467.
- Monnier VK, Cerami A (1982). Non-enzymatic glycosylation and browning in diabetes and aging. Diabetes 31:57-66
- Noor AS, Gunasekaran AS, Manickam, Vijayalakshmi MA (2008). Antidiabetic activity of *Aloe vera* and histology of organs in streptozotocin induced diabetic rats. Curr. Sci. 94:1070-1076.
- Omale J, Ebiloma GU, Agbaji AO (2011). Assessment of biological activities: A Comparison of *Pergularia daemia* and *Jatropha curcas* Leaf Extracts. Br. Biotechnol. J. 1(3):85-100
- Owen JA, Iggo JB, Scangrett FJ, Steward IP (1954). Determination of creatinine in plasma serum, a critical examination. J. Biochem. 58:426-437.
- Prasad SK, Kulshreshtha A, Taj N. Qureshi (2009). Antidiabetic activity of some herbal plants in streptozotocin induced diabetic albino rats. Pak. J. Nutr. 8:551-557.
- Sasaki T, Matsy S, Sorae A (1972). Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose estimation. Rinsho Kagaku 1:346-353.
- Sharma VK, Suresh Kumar, Patel HJ, Shivakumar Hugar (2010). Hypoglycemic activity of *Ficus glomerata* in alloxan induced diabetic rats. Int. J. Pharm. Sci. Rev. Res. 1(2):18-22.
- Varley H (1976). Practical clinical biochemistry, Arnold Heinemann Publication Pvt. Ltd. p. 452.
- Wild SG, Roglic A, Green R, King H (2004). Global prevalence of diabetes. Estimated for the year 2000 and projection for 2030. Diabetes Care 27:1047-1054.