Asian Journal of Pharmaceutical and Clinical Research
Vol. 4, Issue 1, 2011 ISSN - 0974-2441

Research Article

PRELIMINARY ANTI-DIABETIC STUDIES ON AQUEOUS ROOT EXTRACT OF *PSEUDARTHRIA VISCIDA* LINN.

K. RAJENDRAN*, ANNIE SHIRWAIKAR[1], K.K. SRINIVASAN[2]

***Department of Pharmacognosy, College of Pharmcay, Al- Jabal Al- Gharbi University, Al-Zawia, Libya.**
[1]Department of Pharmacognosy, [2] Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal- 576 104, India. E-mail: rajejesus@gmail.com

ABSTRACT

Pseudarthria viscida (L.) Wight and Arnott. (Leguminosae) commonly known as Salaparani and its roots are commonly used by the tribes of Madhya Pradesh. A preliminary study was undertaken to evaluate the antidiabetic effect of the aqueous root extract by oral glucose tolerance test (OGTT), normoglycaemic and antihyperglycaemic activity in streptozotocin (STZ)-nicotinamide induced non insulin-dependent diabetes mellitus rats. Graded doses (250 and 500mg/kg) of the aqueous root extract suspended in gum acacia were administered to normal and experimental diabetic rats. Effect on glucose tolerance test showed a significant fall in the blood glucose level of extract treated animals after 1 hr, indicating its hypoglyaemic activity. Continuous blood glucose lowering activity was observed till 4 hr of administration in normoglycaemic and diabetic rats. The results were compared with standard drug glibenclamide.

Key words: *Pseudarthria viscida;* Root; Antidiabetic activity; Streptozotocin; Nicotinamide; Rats.

INTRODUCTION

Diabetes is recognized as one of the leading causes of morbidity and mortality in the world. About 2.5-3% of the world's population suffers from this disease, a proportion which, in some countries, can reach 7% or more. Hyperglycemia leads to metabolic disorders and various complications [1]. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries [2]. Besides the use of insulin for the treatment of insulin dependent diabetes mellitus (IDDM), other approaches for the control of hyperglycemia include the use of amylin analogues which regulate gastric emptying and inhibitors of intestinal alpha glucosidases like acarbose, miglitol and voglibiose which delay postprandial hyperglycemia. The sulfonylureas, effective only in the presence of a low insulin reserve, act by closing the ATP sensitive potassium channels in the beta cells and depolarizing them leading to calcium influx that initiates a series of reactions which result in insulin release. Metformin, a biguanide oral antibiotic limits intestinal glucose absorption. These drugs have certain drawbacks like causing hypoglycemia at higher doses, liver problems, lactic acidosis and diarrhea [3]. 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. Many investigations of oral anti-hyperglycemic agents of plant origin used in traditional medicine have been conducted and many of the plants have shown positive activity [4]. With this in view, the present study was undertaken on an indigenous antidiabetic medicinal plant *Pseudarthria viscida* (L.) Wight and Arnott (Leguminosae). The plant is a perennial, viscid, pubescent, semi-erect and diffuse undershrub. The roots of this plant have been reported for its use in the treatment of diabetes [5]. Humaira Yousuf Shawl *et al.* report the antidiabetic use of *Pseudarthria visicda* (PV) by the tribals of Madhya Pradesh [6]. However, there is no scientific evidence to support these claims. This study thus aims to experimentally assess the anitdiabetic effect of the aqueous root extract of *P. viscida* by oral glucose tolerance test (OGTT), normoglycaemic and antihyperglycaemic activity in streptozotocin (STZ)-nicotinamide induced non insulin-dependent diabetes mellitus rats.

MATERIALS AND METHODS

Plant material

The roots of *P. viscida* were collected from Erode, Tamil Nadu, India during the month of August, 2006. The botanical identity of the plant was confirmed by Dr. P. Jayaraman, Botanist, Medicinal Plant Research Unit, Chennai, Tamil Nadu. A voucher specimen (PP 546) has been deposited at the Museum of the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal.

Preparation of PV aqueous root extract

The aqueous extract was prepared by cold maceration of 500 g of the shade dried, coarse powder in 600 ml of drinking water for 5 days. The extract was filtered, concentrated, dried *in vacuo* (yield 36 g) and the residue stored in a refrigerator at 2–8 ºC for use in subsequent experiments.

Phytochemical screening

Preliminary phytochemical screening[7] of the aqueous extract revealed the presence of phenolic substances, tannins and carbohydrates.

Animals

Healthy adult male Wistar Albino rats between 2 to 3 months of age and weighing between 150–250 g were used for the study. Housed individually in polypropylene cages, maintained under standard conditions (12 h light and 12 h dark cycle, 25±30 ºC, 35–60% humidity), the animals were fed with standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee of Kasturba Medical College, Manipal, India (IAEC/KMC/01/2005-2006) as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute toxicity

Nulliparous and nonpregnant 2 month old female rats were used for the toxicity studies. The animals were marked to permit individual identification and kept in their cages for at least 5 days prior to dosing. The acute toxicity of *P. viscida* aqueous extract was evaluated by the methodology described in the OECD (2001) guidelines for the testing of chemicals [8].

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test was performed in overnight fasted (18hr) normal animals [9]. Rats divided into four groups (n=6) were administered 2% gum acacia solution, aqueous extract (250mg/kg), aqueous extract (500mg/kg) and glibenclamide (0.25mg/kg) respectively. Glucose (3g/kg) was fed 30min after the administration of extracts. Blood was withdrawn from the retro orbital sinus under ether inhalation (to minimize the distress) at 0, 60, 90, 120 and 180min of extract administration.

Table -1: Effect of aqueous root extract of *P. viscida* on the glucose tolerance test

GROUP (n=6)	TREATMENT	FASTING BLOOD GLUCOSE LEVELS (mg/dl) (MEAN± S.E.M)				
		0 min	60 min	90 min	120 min	180 min
I	Control	82.66 ± 3.16	126.00 ± 5.29	110.00 ± 4.51	102.33 ± 2.40	89.66 ± 3.17
II	PV (250 mg/kg)	75.50 ±3.18	108.16 ±4.21[b]	97.33 ±3.94[b]	89.50 ±2.89[b]	77.00 ±1.89
III	PV (500 mg/kg)	79.83 ±2.44	107.83 ±1.99[a]	96.66 ±2.11[b]	82.66 ±2.94[b]	75.16 ±1.66[b]
IV	Glibenclamide (0.25 mg/kg)	78.66 ±1.54	99.16 ± 1.38[b]	83.83 ± 1.66[b]	72.83 ±1.54[a]	67.16 ± 1.49[a]

Values are mean± S.E of 6 animals in each group; [a]p<0.01 vs control; [b]p<0.05 vs control; [a*]p<0.01 vs Glibenclamide and [b*]p<0.05 vs Glibenclamide.

Table- 2: Hypoglycemic effect of aqueous root extract of *P. viscida* in normal rats

GROUP (n=6)	TREATMENT	FASTING BLOOD GLUCOSE LEVELS (mg/dl) (Mean± S.E.M)				
		0 hr	1 hr	2 hr	3 hr	4 hr
I	Control	87.83 ± 3.15	87.50 ±3.04	86.66 ±3.27	86.50 ±3.22	86.16 ±2.86
II	PV (250 mg/kg)	76.83 ±2.49	71.83 ±2.46	60.83 ±2.14[b]	57.83 ±2.34[a]	49.83 ±2.01[a]
III	PV (500 mg/kg)	79.16 ±2.21	73.16 ±2.30	59.16 ±2.46[a]	54.16 ±2.31[a]	46.16 ±2.12[a,b*]
IV	Glibenclamide (0.25 mg/kg)	82.16 ±2.41	72.83 ±1.86	68.16 ±1.97[b]	60.16 ±2.30[a]	54.83 ±2.22[a]

Values are mean± S.E of 6 animals in each group; [a]p<0.01 vs control; [b]p<0.05 vs control; [a*]p<0.01 vs Glibenclamide and [b*]p<0.05 vs Glibenclamide.

Table -3: Anti hyperglycemic effect of aqueous root extract of *P. viscida* in diabetic rats

GROUP (n=6)	TREATMENT	FASTING BLOOD GLUCOSE LEVELS (mg/dl) (Mean± S.E.M)				
		0 hr	1 hr	2 hr	3 hr	4 hr
I	Control	292.66 ± 9.16	291.33 ± 8.67	292.00 ± 8.02	291.66 ± 8.32	290.50 ± 8.03
II	PV (250 mg/kg)	292.00 ±6.50	280.16 ±6.47	274.33 ±6.55	266.50 ±6.49[c]	259.33 ±6.54[c]
III	PV (500 mg/kg)	288.50 ±7.19	272.00 ±7.49	267.66 ±7.21[c]	259.00 ±7.51[b]	251.33 ±7.77[a]
IV	Glibenclamide (0.25 mg/kg)	292.16 ±7.85	275.00 ±8.58[c]	262.16 ±5.74[b]	256.83 ±5.23[a]	253.16 ±4.76[a]

Values are mean± S.E of 6 animals in each group; [a]p<0.01 vs control; [b]p<0.05 vs control; [a*]p<0.01 vs Glibenclamide and [b*]p<0.05 vs Glibenclamide.

The fasting blood glucose levels were estimated by glucose oxidase–peroxidase reactive strips (Accu-chek, Roche Diagnostics, USA). The percentage decrease in the glucose concentrations were calculated with the formula = [(Gh-Gf)/Gf] X 100. Where Gh = the highest blood glucose concentration during the study; Gf- fasting blood glucose concentration.

Normoglycaemic study

For normoglycaemic study, rats were divided into four groups (n=6) and were administered 2% gum acacia solution, aqueous extract (250mg/kg), aqueous extract (500mg/kg) and glibenclamide (0.25mg/kg) respectively. Blood samples were withdrawn from the retro orbital sinus under ether inhalation at 0, 1, 2, 3 and 4 hr of extract administration [10]. The fasting blood glucose levels were estimated by glucose oxidase–peroxidase reactive strips.

Antihyperglycaemic activity

Type II diabetes was induced in overnight fasted animals by a single intraperitoneal injection of 60 mg/kg STZ (Sigma Aldrich, Germany), 15 min after the i.p. administration of 120 mg/kg nicotinamide (Qualigens Fine Chemicals, division of Glaxo, Mumbai, India). Hyperglycemia was confirmed by the elevated glucose level in the blood, determined at 72hr and then on day 7 after injection. The animals exhibiting fasting blood glucose level of 200-350 mg/dl were used for the antidiabetic study [11].

For antihyperglycaemic study, the animals exhibiting fasting blood glucose levels between 200-350 mg/dl were used. The diabetic rats were divided into four groups (n=6) and were administered 2% gum acacia solution, aqueous extract (250 mg/kg), aqueous extract (500 mg/kg) and glibenclamide (0.25 mg/kg) respectively. Blood samples were withdrawn from the retro orbital sinus under ether inhalation at 0, 1, 2, 3 and 4 hr of extract administration [10]. The fasting blood glucose levels were estimated by using glucose oxidase-peroxidase reactive strips.

Statistical analysis

Data was expressed as mean ± S.E.M. The significance of the difference between the means of the test groups and control group was established by oneway ANOVA followed by *post hoc* Levene's test for variance using SPSS, version 10. The values were considered significant when $p < 0.05$.

RESULTS

Acute toxicity studies revealed the non-toxic nature of the aqueous extract of PV up to a dose level of 2000 mg/kg body weight in rats.

There was no lethality or toxic reaction found at any of the doses selected until the end of the study. Induction of diabetes in the experimental rats was confirmed by the presence of high blood glucose levels. The difference between experimental and control rats were statistically significant ($p<0.05$). In OGTT, the aqueous extract of PV caused significant decrease in blood glucose level at both dose levels at 60 and 90 min as compared to the control (Table 1). The aqueous extract caused significant decrease in the hyperglycaemic peak (19.8% & 33.3%) at the dose levels of 250 and 500 mg/kg body wt respectively in relation to the control group and glibenclamide (43.5%). In normoglycaemic studies the mean blood glucose level decreased, 4 hr after administration of the aqueous extract at the dose level of 250 and 500 mg/kg body wt from 46.16 and 49.83 mg/dL to 49.83 and 46. mg/dL respectively (Table 2). The fasting blood glucose level of diabetic rats significantly ($p<0.05$) reduced

from 299.0 and 316.50 mg/dL to 251.83 and 269.50 mg/dL, 4 hr after administration of the aqueous extract at the dose level of 250 and 500 mg/kg body wt respectively which is comparable to that of the effect of glibenclamide (0.25 mg/kg) (Table 3).

DISCUSSION

The present paper studies the antidiabetic effect of the aqueous root extract of *Pseudarthria viscida* on glucose loaded, normal and streptozotocin-nicotinamide induced type II diabetic rats. Over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues are the fundamental base of hyperglycemia in diabetes mellitus [12]. In our study the difference observed between the initial and final fasting blood glucose levels of different groups under investigation revealed a significant elevation in blood glucose in the diabetic control group as compared to normal animals. Administration of the aqueous extract of PV caused statistically significant decrease in the blood glucose levels of normal and diabetic rats as compared to the normal control and diabetic control groups respectively. Insulin primarily controls the glycolytic pathway by regulating the cell entry of glucose and its phosphorylation for further metabolism [13]. A number of plants have been observed to exert hypoglycemic activity through insulin-release stimulatory effects[14].

In our studies, preliminary phytochemical investigation revealed the presence of phenolic substances, tannins and carbohydrates. The presence of leucopelargonidin in the roots of *P. viscida* has been earlier reported by Prasad and Nambiasan [15]. Cherian *et al*. isolated the leucopelargonidin derivatives from the bark of *Ficus bengalensis* and demonstrated its significant *in vitro* insulin secretion from β-cells [16]. Epicatechin, a tannin isolated from the ethanol extract of *Pterocarpus marsupium* bark has also been shown to possess significant anti-diabetic effect by enhancing insulin release and by the conversion of proinsulin to insulin *in vitro* [17]. Phenolic constituents such as marsupin and pterostilbene significantly lowered blood glucose level in STZ diabetic rats and the effect was comparable to metformin [18].

In our study, administration of the aqueous extract of PV caused significant decrease in the blood glucose levels of normal and diabetic rats. Hence, the significant antihyperglycemic activity exerted by the aqueous root extract of *Pseudarthria viscida* in our study may be attributed to the presence of tannins, leucopelargonidin derivatives and phenolic substances. Studies are in progress to elucidate the molecular and cellular mechanism of the extract. Longer duration studies of *Pseudarthria viscida* on chronic models may contribute toward the development of a potent antidiabetic drug.

ACKNOWLEDGEMENT

The authors are thankful to Manipal Academy of Higher Education, Manipal for providing the facilities to carry out the study and to the Manipal Foundation for awarding the Faculty Study Fellowship Grant.

REFERENCES

1. Ayse Can, Nuriye Akev, Nurten Ozsoy, Sehnaz Bolkent, Bahriye Pelin Arda, Refiye Yanardag, et al. Effect of *Aloe vera* Leaf Gel and Pulp Extracts on the Liver in Type-II Diabetic Rat Models. Biol Pharma Bull 2004; 27: 694-698.
2. Sharma SR, Dwivedi SK, Swarup D. Hypoglycemic, antihyperglycemic and hypolipidemic activities of *Caesalpinia bonducella.* J Ethnopharmacol 1997; 58: 39–44.
3. Czyzyk A, Tawecki J, Sadowski J. Effect of biguanides on intestinal absorption of glucose. Diabetes 1969; 17: 492-498.
4. Rahman AU, Zaman K. Medicinal plants with hypoglycaemic activity. J Ethnopharmacol 1989; 26: 1-15.
5. Warrier PK, Nambiar VPK, and Raman Kutty C. *Indian Medicinal Plants*, vol. IV, Orient Longman, Hyderabad, India, 1996, pp. 366.
6. Humaira-Yousuf-Shaw L, Laxmi Tripathi, and Bhattacharya S, Antidiabetic plants used by tribals in Madhya Pradesh, Natural Product Radiance 2004; 3; 427-428.
7. Harborne JB. Methods of extraction and isolation. In: *Phytochemical Methods*, Chapman & Hall, London, 1998, pp. 60-66.
8. OECD. *Guideline for Testing of Chemicals – Acute Oral Toxicity- Up and Down Procedure.* Organisation for Economic Co-operation and Development. 2001; Available at http://www.epa.gov/oppfead/harmonyization/docs/E425guideline.pdf
9. Bonner-Weir S. Morphological evidence of pancreatic polarity of beta cells within islets of Langerhans, Diabetes 1988; 37: 616–621.
10. Akowuah GA, Sadikun A, and Mariam A. Flavonoid Identification and Hypoglycaemic Studies of the Butanol Fraction From *Gynura procumbens.* Pharma Biol 2002; 40: 405-410.
11. Massiello P, Broca P, Gross R, Roye M, Manteghetti M, Hillaire-Buys, et al. 1998. Development of a new model of type II diabetes in adult rats administered with streptozotocin and nicotinamide. Diabetes 1988; 47: 22*5*.
12. Latner A. Clinical biochemistry, *In: Carbohydrate metabolism:Abnormalities of post absorptive blood sugar level*, 2nd ed. Philadelphia: WB Saunders Co., 1958, pp. 48.
13. Marshall CJ. Specificity of receptor tyrosine kinase signaling transient vs. sustained extra cellular signal-regulating kinase activation, Cell 1995; 80: 179–185.
14. Prince SM, and Menon VP. Hypoglycemic and other related actions of *Tinospora cordifolia* in alloxan induced diabetic rats, J Ethnopharmacol 2000; 70: 9–15 (2000).
15. Prasad NBR, and Nambiasan PNK. Journal of Res. Ind. Med. Yoga and Homeo 1976; 11: 104-109.
16. Cherian S, Kumar RV, Augusti KT, and Kidwai JR. Antidiabetic effect of a glycoside of pelargonidin isolated from the bark of *Ficus bengalenesis* Linn., Indian J Biochem Biophy 1992; 29: 380–382 (1992).
17. Sheehan EW, Zemaitis MA, Slatkin DJ, and Schiff PL. A constituent of *Pterocarpus marsupium*, (−)epicatechin, as a potential antidiabetic agent, J Nat Products 1983; 46: 232–234.
18. Manickam M, Ramanathan M, Jahromi MA, Chansouria JP, and Ray AB. Antihyperglycemic activity of phenolics from *Pterocarpus marsupium*, J Nat Products 1997; 60: 609–610 (1997).