Antidiabetic activity of *Pseudarthria viscida* aqueous root extract in neonatal streptozotocin-induced NIDDM rats

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**Abstract:** The antidiabetic activity of the aqueous root extract of *Pseudarthria viscida* (L.) Wight & Arn., Fabaceae, was investigated in normal and neonatal streptozotocin (n2-STZ)-induced non insulin-dependent diabetes mellitus (NIDDM) rats and compared with glibenclamide as a reference standard. Two different doses (250 and 500 mg/kg) of the extract were administered to normal and diabetic rats for 21 days. Fasting blood glucose levels, serum lipid profiles and changes in body weight were evaluated in normal and diabetic rats while serum insulin, glycated hemoglobin, urea, creatinine, magnesium, protein, albumin and glycogen, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), lactate dehydrogenase (LDH) levels in kidney and liver were evaluated additionally in diabetic rats. Treatment with extract at both dose levels was found to exhibit antidiabetic activity, with the higher dose showing more significant activity.

**Keywords:** diabetes mellitus neonatal *Pseudarthria viscida* rats streptozotocin

**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 (Ayse et al., 2004). 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 (Rahman & Zaman, 1989). *Pseudarthria viscida* (L.) Wight & Arn., Fabaceae, is a controversial plant commonly known as Salaparni in Sanskrit and is an essential component of many famous Ayurvedic formulations like Dashamoola, Mahanarayana taila and Dhatkara taila (Deepa et al., 2004). The plant is a perennial, viscid, pubescent, semi-erect and diffuse undershrub. The roots are used as an astringent, sweetener, bitter, emollient, digestive, anthelmintic, anti-inflammatory, diuretic, cardiotonic, aphrodisiac, febrifuge, rejuvenating and as a tonic. The roots of this plant have been reported for its use in the treatment of diabetes (Warrier et al., 1996). Humaira Yousuf Shawl et al. (2004) reported the use of *Pseudarthria viscida* (PV) by the tribals of Madhya Pradesh for the treatment of diabetes. There are few reports on phytochemical and pharmacological studies on the root extract of PV. Some workers have reported the presence of tannins, flavanoids and proteins in the root of PV (Deepa & Narmatha, 2002). The ethanolic extract of the entire plant was evaluated for antiastacariesia activity (Kaleyasa, 1975). The various extracts of the root were studied for angiotensin-converting enzyme inhibition (Hansen et al., 1995) and antifungal activity (Deepa et al., 2004). Recently, ethanolic extract of its root were proven for *in vitro* antioxidant and cytotoxicity activities (Vijayabaskaran et al., 2010 a,b).

We reported earlier preliminary antidiabetic short term studies on alcoholic and aqueous root extracts of *Pseudarthria viscida* (Rajendran et al., 2010; Ranjendran et al., 2011) and Masirkar et al. (2008) have studied antidiabetic effect of ethanolic extract of PV. However, there is no report on how these extracts exhibit their antidiabetic activity on experimental diabetic animals. In the present study as an extension of our previous report, attempts have been made to evaluate the antidiabetic effect and mechanisms of action of the aqueous root extract of *Pseudarthria viscida* as long term studies in normal and neonatal streptozotocin (n2-STZ)-induced non insulin-dependent diabetes mellitus rats.
Materials and Methods

Plant material

The roots of *Pseudarthria viscida* (L.) Wight & Arn., Fabaceae, were collected from Erode, Tamil Nadu, India during the month of August, 2005. 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 2.5 kg of the shade dried, coarse powder in 600 mL of distilled water for seven days. The extract was filtered, concentrated, dried *in vacuo* (yield 180 g) and the residue stored in a refrigerator at 2-8 ºC for use in subsequent experiments.

Phytochemical screening

Preliminary phytochemical screening (Kokate, 1994; Harborne, 1998) of the aqueous extract revealed the presence of phenolic substances, tannins and carbohydrates.

Animals

Healthy adult male and female 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) as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute toxicity

Nulliparous and nonpregnant two 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 five days prior to dosing. The acute toxicity of PV aqueous extract was evaluated by the methodology described in the OECD (2001) guidelines for testing of chemicals. The animals were fasted for 4 h prior to dosing. The fasted body weight of each animal was determined and the dose to be administered was calculated according to its body weight. The aqueous extract was administered by oral gavage. The experimental procedure was performed in accordance with the Main Test of the OECD. Animals were observed individually during the first 30 min after dosing, every 4 h during the first 12 h and thereafter daily for fourteen days.

Induction of non-insulin-dependent diabetes mellitus (NIDDM)

The neonatal n2-STZ induced diabetic model developed according to the description of Bonner Weir et al. (1981) has been modified by Portha et al. (1989). Wistar rat pups of either sex, aged 48±2 h, were injected (100 mg/kg body weight, *i.p.*.) with streptozotocin in citrate buffer (pH 4.5). The control pups received an equivalent volume of citrate buffer alone. After 10-12 weeks, animals weighing more than 150 g and exhibiting fasting blood glucose levels of more than 140 mg/dL were considered as neonatal-STZ (n2-STZ)-induced diabetic rats resembling type II diabetes in humans (Urmila et al., 2003).

Experimental design

Animals were divided into seven groups of six rats each. Treatment schedule of test, standard drugs and control was for a period of 21 days by orogastric tube. Group I were administered with gum acacia suspension (2%) and served as normal control; group II normal rats received aqueous extract (250 mg/kg); group III normal rats received aqueous extract (500 mg/kg); group IV diabetic control rats were administered with gum acacia suspension (2%); group V diabetic rats received aqueous extract (250 mg/kg); group VI diabetic rats received aqueous extract (500 mg/kg) and group VII diabetic rats received standard drug glibenclamide (0.25 mg/kg).

The effect of administration of PV aqueous root extract was determined by measuring fasting blood glucose level, serum lipid profile (Trinder, 1969) and changes in body weight in normal and diabetic rats while serum insulin, glycated hemoglobin, urea (Fawcett & Scott, 1960), creatinine, magnesium (Benjamin & Frederick, 1921), protein (Weichselbaum, 1946), albumin, glycogen levels (Nicholas, 1956), glutamate oxaloacetate transaminase (GOT) (EC 2.6.1.1), glutamate pyruvate transaminase (GPT) (EC 2.6.1.2) (King 1965) and lactate dehydrogenase (LDH) (EC 1.1.1.27) (Bergmeyer, 1983) in kidney and liver were evaluated additionally in diabetic rats. Fasting blood glucose was estimated on days 0, 7, 14 and 21 of extract administration. The animals were euthanized by an overdose of intraperitoneal anesthesia and tissue samples were collected for the assessment of biochemical parameters on day 21.

Serum insulin levels were estimated by using a
radio immunoassay kit issued by the Board of Radiation and Isotope Research, Bhaba Atomic Research Centre (BARC), Mumbai, India. Serum lipid profiles, glycated hemoglobin levels (turbidic inhibition immunoassay), urea, creatinine, magnesium, protein and albumin were measured by an autoanalyser in the Department of Biochemistry, Kasturba Medical College, Manipal, India.

Statistical analysis

Data was expressed as mean±SEM. The significance of the difference between the means of the test groups and control group was established by one-way ANOVA followed by post hoc Levene’s test for variance using SPSS, version 10. Values were considered significant when \( p<0.05 \).

Results and Discussion

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 observed at any doses selected until the end of the study. Induction of diabetes in the experimental rats was confirmed by the presence of high blood glucose level. Administration of graded doses of aqueous extract for a 21-day experimental period produced a statistically significant decrease in blood glucose concentration when compared with the diabetic control \( (p<0.05) \). Treatment with the aqueous extract of PV at both dose levels was found to be dose dependant, with the higher dose showing more significant activity.

The effects of the aqueous root extract on fasting blood glucose levels in diabetic animals are presented in Table 1. The difference between the fasting blood glucose level in diabetic and control rats were found to be statistically significant. Significant \( (p<0.05) \) difference was observed in body weight changes (Table 2), serum lipid profiles (Table 3), serum insulin, glycated hemoglobin, magnesium, protein, albumin (Table 4), urea, creatinine, glycogen levels (Table 5), GOT, GPT and LDH in kidney and liver of extract-treated diabetic animals when compared with the diabetic control (Table 6).

Over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues are the fundamental basis of hyperglycemia in diabetes mellitus (Latner, 1958). Administration of the aqueous extract of PV caused statistically significant decrease in the blood glucose levels of normal and n2-STZ induced diabetic rats as compared to the normal control and diabetic control groups respectively.

Table 1. Effect of aqueous root extract of *Pseudarthria viscida* on fasting blood glucose levels in normal and diabetic animals.

<table>
<thead>
<tr>
<th>Group (n=6)</th>
<th>Treatment</th>
<th>Fasting blood glucose levels (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>80.16±0.65 79.83±0.60 79.16±0.40 78.83±0.70</td>
</tr>
<tr>
<td>II</td>
<td>Normal+PV (250 mg/kg)</td>
<td>79.00±0.91 69.16±1.01 63.66±1.04 55.83±1.13</td>
</tr>
<tr>
<td>III</td>
<td>Normal+PV (500 mg/kg)</td>
<td>73.66±0.87 58.33±1.02 53.00±0.92 43.50±0.94</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic control</td>
<td>158.50±5.77 162.33±5.54 165.50±5.71 176.50±5.56</td>
</tr>
<tr>
<td>V</td>
<td>Diabetic+PV (250 mg/kg)</td>
<td>172.66±7.10 158.00±6.62 153.33±6.56 144.16±6.69</td>
</tr>
<tr>
<td>VI</td>
<td>Diabetic+PV (500 mg/kg)</td>
<td>161.00±4.41 140.83±4.79 134.83±4.81 125.66±4.95</td>
</tr>
<tr>
<td>VII</td>
<td>Diabetic+Glibenclamide (0.25 mg/kg)</td>
<td>171.50±5.57 153.66±5.03 146.50±4.76 132.00±3.74</td>
</tr>
</tbody>
</table>

Table 2. Effect of aqueous root extract of *Pseudarthria viscida* on changes in body weight of normal and diabetic animals.

<table>
<thead>
<tr>
<th>Group (n=6)</th>
<th>Treatment</th>
<th>Body weight in g</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>171.50±1.17 182.00±1.65 194.50±9.2 207.30±1.18</td>
</tr>
<tr>
<td>II</td>
<td>Normal+PV (250 mg/kg)</td>
<td>166.93±1.64 180.76±1.85 195.58±1.43 198.31±2.07</td>
</tr>
<tr>
<td>III</td>
<td>Normal+PV (500 mg/kg)</td>
<td>175.36±1.05 188.06±0.99 199.91±1.66 206.45±1.07</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic control</td>
<td>156.16±1.49 160.00±1.63 163.33±1.35 167.17±1.07</td>
</tr>
<tr>
<td>V</td>
<td>Diabetic+PV (250 mg/kg)</td>
<td>157.50±0.88 162.16±0.94 165.33±1.05 167.16±0.79</td>
</tr>
<tr>
<td>VI</td>
<td>Diabetic+PV (500 mg/kg)</td>
<td>162.33±2.62 168.33±2.62 171.00±2.47 173.00±2.82</td>
</tr>
<tr>
<td>VII</td>
<td>Diabetic+Glibenclamide (0.25 mg/kg)</td>
<td>160.16±1.53 165.33±1.47 171.83±1.68 174.83±1.62</td>
</tr>
</tbody>
</table>

Values are mean±SE of six animals in each group; ‘\( p<0.01 \) vs control; ‘\( p<0.05 \) vs control; ‘‘\( p<0.01 \) vs diabetic control and ‘‘\( p<0.05 \) vs diabetic control.
Chronic administration of the aqueous extract of PV for 21 days in diabetic rats caused significant increase in the serum insulin levels of all groups, indicating that these fractions may probably activate the surviving β-cells of the islets of Langerhans and revert them to the normal state i.e. an insulinogetic effect. The decrease in body weight observed in the diabetic control group in our study may be attributed to protein wasting caused by the unavailability of carbohydrates for utilization as an energy source. A significant increase was observed in the body weight of the diabetic control group compared to the normal control group.
weight of rats treated with the aqueous extract of PV after the completion of 21 days as compared to the standard drug glibenclamide. Hence, it can be postulated that PV does not have any effect on the degradation of depot fat but it probably maintains the body weight in the type II diabetic state by its protective effect in controlling muscle wasting.

The present study showed a marked increase in total cholesterol and a decrease in HDL cholesterol of diabetic rats, which is in agreement with the findings of Sharma et al. (1997). The significant reduction of HDL cholesterol on treatment with the aqueous extract of PV may be attributed to the insulin secretagogue activity. Our finding showed diabetic rats with higher levels of glycated hemoglobin thereby indicating their poor glycemic control. Administration of the aqueous extract of PV decreased the concentration of glycated hemoglobin in n2-STZ diabetic rats, which could be due to an improvement in insulin secretion (Vasudevan & Sreekumari, 1998). A significant decrease was observed in the serum creatinine and urea levels of the aqueous extract of PV treated diabetic groups as compared to the diabetic control. STZ induced decrease in Mg levels was significantly reversed in groups treated with the aqueous extract of PV. An overall reduction in serum total proteins and albumin of diabetic animals was observed in the present study, corroborating earlier reports of Porte & Halter (1981). The reversal of these changes on administration of the aqueous extract proved that insulin deficiency had been grossly corrected. Treatment with aqueous extract caused a significant decrease in the level of LDH. This may be attributed to an increase in glucose utilization through the pentose phosphate pathway, which interferes with the mitochondrial respiratory chain and promotes peripheral glucose utilization by enhancing anaerobic glycolysis (Burtis et al., 2005). Administration of the aqueous extract of PV significantly reduced the elevated levels of GOT and GPT in the kidney and liver in diabetic rats, suggesting a partial reversal of enhanced gluconeogenic activity in the diabetic tissues by revival of insulin secretion to normal levels. This also indicates the protective action of PV in reversing any organ damage due to the induction of experimental diabetes.

Preliminary phytochemical investigation revealed the presence of phenolic substances, tannins, flavonoids and carbohydrates. Cherian et al. (1992) isolated leucopelargonidin derivatives from the bark of *Ficus bengalensis* and demonstrated its significant *in vitro* insulin secretion from β-cells. Literature reveals that leucopelargonidin has been isolated from the roots of *P. viscida* (Prasad & Nambisan, 1976). In our studies, administration of the aqueous extract of PV caused significant increase in the serum insulin levels in chronic diabetic rats. Hence, the significant antihyperglycemic activity exerted by the aqueous root extract of *P. viscida* in our study may be attributed to the presence of the tannin, leucopelargonidin derivatives. Further studies are in progress to isolate and characterize the active constituents of the aqueous extract of the medicinal plant and to elucidate the molecular mechanism at cellular level.

**References**


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