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Therapeutic Hypoglycemic Potential of *Pentapetes phoenicea* L. in Experimentally Induced Hyperglycemic Rats

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Abstract: Diabetes mellitus is a metabolic disorder of endocrine system. This dreadful disease is found all over the world and is becoming a serious threat to the mankind health. Alternative to synthetic agents, plants provide a potential source of hypoglycemic drugs. The aim of the present study was to evaluate the hypoglycemic effect of 70% alcoholic extract of *Pentapetes phoenicea* (PPE) on blood glucose level in glucose loaded, normal and experimentally induced diabetic rats. Based on the acute toxicity test, two variable doses (250, 500 mg kg⁻¹ b.wt.) of hydro-alcoholic extract of *P. phoenicea* leaves were compared with glibenclamide for the influence on fasting blood glucose in glucose loaded, normoglycemic and streptozotocin (STZ) (55 mg kg⁻¹, i.p.) induced hyperglycemic rats. All the statistical comparisons were made by one-way analysis of variance (ANOVA) followed by Newman-Keuls Multiple Comparison Test using Graph Pad Prism 4.01 v for windows (Graph Pad Software, San Diego, CA, USA). The difference showing a p level of 0.05 or lower was considered to be statistically significant. The administration of PPE in two doses and glibenclamide (5 mg kg⁻¹) to STZ induced hyperglycemic animals significantly lowered the blood glucose levels with 18.84% (p<0.01) for PPE 250 mg kg⁻¹ and 38.89% (p<0.001) for PPE 500 mg kg⁻¹ in a dose dependant manner. Considering all the results obtained, the study concludes that the hydro-alcoholic extract of *P. phoenicea* leaves produced promising decrease in blood glucose levels in STZ induced hyperglycemic rats which might be related to tannins, terpenoids, sterols and flavonoid contents.

Key words: *Pentapetes phoenicea*, Streptozotocin induced diabetic rats, anti-diabetic, hypoglycemic, OGTT

INTRODUCTION

Diabetes is one of the most prevalent chronic diseases in the world. Hypoglycemic (low glucose) episodes cause blackouts and when severe, they are life-threatening. It has been reported that high glucose may result into stroke, circulatory diseases, renal failure, vision failure, nerve degeneration and stroke. (Heller and Feldman, 2010). It is believed that about 250 million people worldwide have developed this chronic disease and by another next 20 years, this number is expected to increase annually by another 6 million (Hussain *et al.*, 2007).

Compared to insulin dependent diabetes mellitus (IDDM), Non-insulin Dependent Diabetes (NIDDM) is more prevalent, though both are characterized by chronic hyperglycemia associated with disturbances of carbohydrates, fat and protein metabolism that may result from defective insulin action and secretion or both (Bavarva and Narasimhacharya, 2010). Type 2 diabetes mellitus (non-insulin-dependent diabetes mellitus) is also

one of the most common chronic diseases and is associated with co-morbidities such as obesity, hypertension, hyperlipidemia and cardiovascular diseases (Sowers *et al.*, 2001). For a long time, diabetics have been treated by insulin and synthetic drugs which are available at present, but they produced serious side effects. Herbal medicines play a vital role in this part to prevent side effects (Raut and Gaikwad, 2006).

Many of the therapeutic hypoglycemic agents are available for the management of NIDDM (type 2 diabetes) such as acarbose, miglitol and voglibose which inhibit a wide range of glycosidases (Jain and Saraf, 2010). However, these non-specific enzyme inhibitors are known to produce serious side effects (Kim *et al.*, 2004).

The current escalation in the global prevalence of obesity and insulin resistance and the increased risk for coronary artery disease can explain the increased interest in type 2 diabetes (Reaven, 2005). In the search of novel treatments, attention should be given to many traditional herbal medicines for diabetes which have been employed

by various groups throughout the world. With, the growing number of people opting for alternative and herbal medicine with no or less side effects has led for the search of more effective and safer hypoglycemic plant drugs.

Pentapetes phoenicea Linn. (Sterculiaceae), is an annual erect herb. It is commonly known as Dopa-hariya in Hindi. Traditionally, the boiled water of leaves has been reported to be used for the treatment of glandular inflammation, cold and cough (Rai and Lalramghinglova, 2010). The hydroalcoholic extract of leaves showed antidiabetic activity *in vitro* (Sharma *et al.*, 2013). A review of the literature did not throw any light on the scientific pharmacological activity of the plant. Therefore based upon previous work reported, the present work to assess the *in-vivo* hypoglycemic activity of crude hydroalcoholic extract and establish scientific basis for the activity selected was undertaken.

MATERIALS AND METHODS

The leaves of *P. phoenicea* (L.) were collected from the local area of the Kanpur city in the month of September 2011. The taxonomic identification of the plant material was confirmed by Prof. B.K. Misra (Botanist, DAV College, Kanpur). The voucher specimen was kept in the departmental museum for future reference. The leaves of the plant were washed, dried under shade and ground to pass through a 40 mesh sieve.

Chemicals: Streptozotocin (STZ) was obtained from Sigma Aldrich, India. Glibenclamide was obtained as gift sample from Zyclus Cadila, Ahmadabad, India. All other Solvents and chemicals used were of analytical grade and were of SD Fine and Merck brand. Quercetin, rutin and gallic acid were purchased from Hi-media Lab. Mumbai, India. The biochemical kits were obtained from Span Diagnostic Ltd. India.

Preparation of hydroalcoholic extract of *P. phoenicea* (PPE) leaves: The freshly collected leaves were washed thoroughly with de-mineralized water and then shade dried. The shade dried leaves (500 g) were finely powdered and was macerated with petroleum ether to remove the fatty materials. The marc was further extracted three times with fresh 70% aqueous ethanol at room temperature for 3 days and filtered. The crude extract (PPE) was concentrated on a rotavapor (40°C) under reduced pressure and lyophilised to get the dry residue; the yield of PPE was 12.8% w/w. The extract was stored in the dark at 4°C for further studies.

Preliminary phytochemical examination and HPTLC analysis: Preliminary phytochemical examination of PPE

tested for the presence of various chemical constituents. Based on the phytochemical examination, Thin Layer Chromatography (TLC) analysis was performed to identify the phenolic compounds and flavonoids. The sample solution of PPE was prepared at a concentration of 10 mg mL⁻¹ and the TLC plate was eluted in Toulene: Ethyl acetate: Formic acid (7:5:1) as solvent system, using various standards. The plate was visualized under short and long wavelength U.V. light chamber. Presence of rutin was confirmed against standard flavonoid and gallic acid against phenolic compound was confirmed with preliminary TLC studies. HPTLC finger print profile of hydro-alcoholic extract of *P. phoenicea* (10 µL) was developed. The HPTLC analysis was carried out on precoated Silica gel 60-F₂₅₄ plate (Merck, India) with the help of Camag Linomat IV applicator. The plate was eluted with Chloroform:Methanol (9:1) as mobile phase. After development, the plate was dried and densitometrically scanned on a TLC scanner III at 366 nm using Wincat software (CAMAG, Switzerland) and peak area was recorded.

Animals: Sprague-Dawley rats of either sex weighing between 140-180 g were used in this study. Animals were housed under standard conditions of temperature (25±2°C), relative humidity 45-55% and 12:12 h light/dark cycle (lights on 7:00 h) and fed with commercial rodent pellets (Dayal, India) and tap water *ad libitum*. The animals were allowed to adapt to the laboratory conditions for 1 week before the study. Food was withdrawn 18-24 h before the experiment although water *ad libitum* was allowed and divided into different experimental groups each of five rats. All procedures were performed in accordance with Institutional Animal Ethics Committee (1589/PO/a/12/CPCSEA) of University Institute of Pharmacy, C.S.J.M. University, Kanpur for animal care and use.

Pharmacological evaluation: Acute Oral toxicity study Sprague-Dawley rats weighing (150-180 g body weight) were randomly divided into six groups of five animals per group and the test was performed according to OECD guideline No. 420 (Organization for Economic Co-operation and Development). After sighting study, a starting dose of 200 mg kg⁻¹ (p.o) of the test sample (PPE) was administered to various groups containing 5 animals in each group. The animals were monitored for 14 days for any mortality and change in general behavior. No deaths were observed by the end of study. The extract was found to be safe up to a dose of 2000 mg kg⁻¹ b.wt. and from the results obtained a dose of 500 mg kg⁻¹ b.wt. was selected as maximum dose for further experimentation.

Hypoglycemic activity in glucose overloaded hyperglycemic rats: Hypoglycemic study was carried out in glucose overloaded hyperglycemic rats. Overnight fasted animals were divided into various groups (n = 5). Glibenclamide (5 mg kg⁻¹) was used as reference standard. The negative group received only vehicle. The test group was treated with 250 and 500 mg kg⁻¹ of PPE extract of the plant suspended in 1% Tween 80. Zero hour blood sugar level was assessed from overnight fasted animals. After 30 min of drug treatment, animals were fed with glucose (4 g kg⁻¹) and blood glucose level was assessed after 30, 60, 90 and 120 min after glucose challenge. Blood glucose concentration was determined by glucose oxidase enzymatic method using a commercial glucometer and test strips (Accu-chek Active test meter) (Jarald *et al.*, 2008).

Hypoglycemic activity in normal healthy rats: Animals were divided into various groups. Group I served the control and received only single dose of vehicle (0.5 mL/100 g). Group II was treated with glibenclamide (5.0 mg kg⁻¹) as a hypoglycemic standard drug. Group III and IV were treated with 250 and 500 mg kg⁻¹ b.wt. PPE extract. Blood samples were collected from tail vein at 0 (just before oral administration), 30, 60, 120, 180 min. after the vehicle, standard and test extract administration. Blood glucose concentration was determined by glucose oxidase enzymatic method using a commercial glucometer and test strips (Accu-chek Active test meter).

Induction of Hyperglycemia: The rats were fasted overnight and were then administered with freshly prepared solution of streptozotocin (STZ) dissolved in citrate buffer pH 4.5 at a dose of 55 mg kg⁻¹ intraperitoneal (i.p.), 15 min after i.p. administration of 100 mg kg⁻¹ b.wt. nicotinamide. Normal rats (n = 5) were administered with 1 mL citrate buffer as vehicle. As administration of STZ can induce severe hypoglycemia, due to massive pancreatic insulin release, the rats were given 10% glucose solution after 6 h of STZ administration for the next 24 h to prevent hypoglycemia. After 72 h and then on day 7th of STZ injection, blood glucose level of each animal was assessed. The rats with fasting blood glucose level above 200 mg dL⁻¹ were considered to be hyperglycemic and were selected for further investigation (Kumar *et al.*, 2012).

Hypoglycemic activity in STZ induced Hyperglycemia: The selected hyperglycemic animals were divided into four groups (n = 5) and one more group of normal animals without STZ. Group I served as normal control (without STZ) and received a single dose of vehicle 0.5 mL/100 g of the vehicle, group II served as negative control

(STZ induced) and received single dose of vehicle 0.5 mL/100 g of the vehicle, group III hyperglycemic induced was treated with glibenclamide (5 mg kg⁻¹) as standard hypoglycemic drug. Group IV and V hyperglycemic induced were treated with PPE leaves extract at two dose levels (250 and 500 mg kg⁻¹). Treatment was given for 7 consecutive days (p.o.) by oral gavage. At the 7th day, food was withdrawn for 16 h and fasting blood glucose levels were determined.

Statistical analysis: The results are expressed as Mean±SD and all the statistical comparisons were made by one-way analysis of variance (ANOVA) followed by Newman-Keuls Multiple Comparison Test. The data were analyzed with Graph Pad Prism 4.01 v for windows (Graph Pad Software, San Diego, CA, USA). The difference showing a p-level of 0.05 or lower was considered to be statistically significant.

RESULTS

Preliminary Phytochemical screening and HPTLC

Finger printing analysis: The preliminary phytochemical screening of crude hydro-alcoholic extract showed the presence of tannins, flavonoids, phenols, carbohydrates, sterols, terpenoids. A densitometric HPTLC analysis was performed for the development of characteristic finger print profile for hydro-alcohol extract of leaves. Seven bands in the sample were obtained at R_f 0.02, 0.21, 0.47, 0.52, 0.56, 0.59 and 0.78 which can be used as identifying marker Fig. 1.

Effect of crude hydroalcoholic extract of *P. phoenicea* in glucose overloaded hyperglycemic rats:

To select the optimum dose for hyperglycemic animals two doses were selected 250 and 500 mg kg⁻¹ and evaluated on glucose tolerance of hyperglycemic animals. Table 1 demonstrates the effect of two doses of the crude hydro-alcoholic extract (PPE) on the blood glucose level of rats during the OGTT studies. Initially, there was significant rise in blood glucose levels till 1 h in control animals (Group I) and thereafter declined at the end of 2 h. The plant extract PPE 250 and 500 mg kg⁻¹ produced the hypoglycemic activity 18.86 and 29.02% (p<0.01) respectively at the end of 3 h which was comparable to the standard drug which produced 38.47% reduction.

Effect of crude hydroalcoholic extract on hypoglycemic activity in normal healthy rats:

Table 2 shows the hypoglycemic effect of two graded doses of crude hydro-alcoholic extract of *P. phoenicea* leaves on fasting blood glucose rats. Rats treated with 250 and 500 mg kg⁻¹

Table 1: Effect of crude hydro-alcoholic extract of *P. phoenicea* in glucose overloaded hyperglycemic rats

Groups treatments and doses	Blood Glucose Level (mg dL ⁻¹)				
	0 h	½ h	1 h	2 h	3h
I (Glucose control, 4 g kg ⁻¹)	288.5±4.49	290.2±4.45	375.3±4.45	317.4±5.84	265.7±3.85
II (Extract PPE, 250 mg kg ⁻¹)	282.7±2.08	282.9±4.38 ^a	313.2±4.29	256.1±2.83 ^c	215.6±1.61 ^e
III (Extract PPE, 500 mg kg ⁻¹)	263.9±4.29	269.6±4.89 ^b	299.7±3.97	249.1±3.35 ^c	188.6±3.35 ^e
IV(Glibenclamide 5 mg kg ⁻¹)	272.5±2.42	249.3±2.30 ^f	258.7±2.54	215.7±2.85 ^e	163.5±2.50 ^f

Each value represents mean±SEM of five observations. ^ap<0.05, ^bp<0.01, ^cp<0.001 versus control (ANOVA followed by Newman-Keuls Multiple comparison test)

Table 2: Effect of crude hydro-alcoholic extract of *P. phoenicea* on hypoglycemic activity in normal healthy rats

Groups treatments and doses	Blood Glucose Level (mg dL ⁻¹)				
	0 h	½ h	1 h	2 h	3h
I (Normal control, Vehicle)	86.9±0.78	82.6±2.59	83.2±0.84	81.8±2.53	81.2±1.67
II (Extract PPE, 250 mg kg ⁻¹)	84.5±3.20	79.9±4.11 ^a	73.1±3.85 ^b	66.9±4.11 ^b	59.5±2.81 ^c
III (Extract PPE, 500 mg kg ⁻¹)	86.8±3.08	70.4±3.77 ^a	62.2±3.37 ^a	51.6±3.27 ^a	44.3±3.83 ^a
IV(Glibenclamide 5 mg kg ⁻¹)	83.5±3.66	46.9±2.31 ^c	37.6±3.26 ^c	35.6±3.01 ^c	30.9±1.91 ^c

Each value represents mean±SEM of five observations. ^ap<0.05, ^bp<0.01, ^cp<0.001 versus control (ANOVA followed by Newman-Keuls Multiple comparison test)

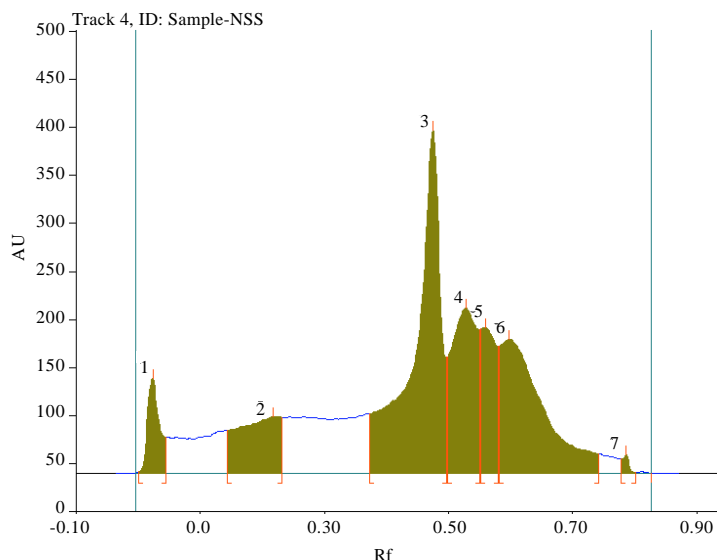


Fig. 1: Qualitative Analysis of hydro-alcoholic extract of leaves, Sample preparation-10 mg mL⁻¹; Application-Linomat 5 Applicator (Camag); Solvent System-Chloroform: Methanol (9:1); TLC plate Development-Presaturated Camag Twin Trough Chamber

showed significant fall of 26.73 and 45.45%, respectively (p<0.001) after 3 h of oral administration, in a dose dependent manner, whereas standard drug produced reduction of 61.95%.

Effect of crude hydroalcoholic extract on hypoglycemic activity in STZ induced hyperglycemia: The basal blood glucose level of glibenclamide and PPE 500 mg kg⁻¹ was statistically different compared to diabetic control group (p<0.001), whereas that of PPE 250 mg kg⁻¹ was less statistically different from control diabetic group (p<0.01). After 7 days, values of blood glucose decreased in all

treatment groups but the diabetic control animals showed a little increase in some set of animals. The administration of plant extracts in two doses and glibenclamide to hyperglycemic animals significantly lowered the blood glucose levels with 18.84% (p<0.01) for PPE 250 mg kg⁻¹ and 38.89% (p<0.001) for PPE 500 mg kg⁻¹ (Fig. 2).

DISCUSSION

Medicinal plants play an important role for the development of new drugs. Search for alternative, effective and safe anti-diabetic agents is a major thrust

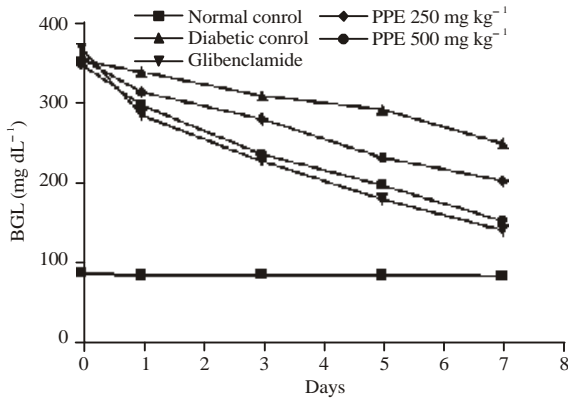


Fig. 2: Effect of crude hydro-alcoholic extract of *P. phoenicea* on Hypoglycemic activity in STZ induced Hyperglycemia. Significance against Diabetic control values ^a $p < 0.01$, ^b $p < 0.001$. One way ANOVA followed by Newman-Keuls Multiple comparison test, Values represents Mean \pm SEM of five observations

area in the mainstream of pharmaceutical research. Diabetes becoming a greater health concern, use of alternative treatments for diabetes has become more popular. The number of herbal remedies being marketed for diabetes has increased with nearly all pharmacies offering herbal treatments (Triller and Snitkoff, 2001). Acute toxicity test of the PPE in rats did not showed any signs of mortality at a maximum dose level of 2000 mg kg⁻¹. Based on this study the dose selected did not exceed 500 mg kg⁻¹. The present study investigated the hypoglycemic effect of crude hydro-alcoholic extract of *P. phoenicea* leaves. The results indicated that the extract at 500 mg kg⁻¹ b.wt. showed a statistically significant difference in potency of reducing hyperglycemia (38.89%) compared with the STZ induced diabetic control group. STZ induced diabetes is characterized by severe loss in body weight (Ravi *et al.*, 2004). The weight gain after treatment with extract in diabetic rats may be attributed due to the ability of the extract to reduce hyperglycemia.

Crude hydro-alcoholic extract showed the presence of flavonoids, saponins, phenolic compounds, triterpenoids and tannins. The versatility in its chemical constituents attracts the plant as a potential source of hyperglycemic activity. It is reported that flavonoid constitute the active biological property of most medicinal plants with hypoglycemic and anti-diabetic properties (Gupta *et al.*, 2012).

In the glucose-loaded hyperglycemic model, the plant extract at a dose level of 500 mg kg⁻¹ and glibenclamide exhibited anti-hyperglycemic activity of 29.02% and 38.47% respectively. From the study it was found that for

the glucose control animals, the secreted insulin required 2-3 h to get the glucose level back to normal. In the crude hydroalcoholic extract (PPE) and reference drug treated groups, the glucose levels did not increased abruptly like the control group, giving an indication about the supportive action of the extract and drug in the glucose utilization. The extract of *P. phoenicea* leaves reduces the blood glucose levels in normal rats and normalizes the high blood glucose levels in diabetic rats. The hypoglycemic effect produced by extract may involve increased insulin release resembling the mechanism of action of sulphonyl ureas (Okine *et al.*, 2005; Miura *et al.*, 2001). The crude hydro-alcoholic extract PPE decreases blood glucose levels both in normal and STZ induced diabetic rats. The results showed hypoglycemic activity of extract.

CONCLUSION

The present study exhibited the anti-diabetic activity of PPE extract for the first time. In our study three different experimental models have been used. In general, for the study of *in vivo* anti-diabetic activity, three basic models are used to determine whether or not the plant possesses anti-diabetic activity. To reveal the hypoglycemic effect normoglycemic rats are used, glucose loaded rats for effect on glucose absorption from the intestines and STZ-induced diabetic rats to evaluate hyperglycemic activity. The crude hydro-alcohol extract showed promising hypoglycemic activity. It could be speculated that the observed hypoglycemic effect of *P. phoenicea* leaves might be related to tannins, terpenoids, sterols and flavonoid contents and can be used to decrease the blood sugar levels. Further studies are in progress to isolate the responsible compound and to determine the exact nature of active principles and mechanism of action.

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