

Full Length Research Paper

# Antidiabetic, cytotoxic activities and phytochemical screening of *Peltophorum pterocarpum* (DC.) K. Heyne root

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The present investigation was designed to determine the antidiabetic activity of the methanol: ethyl acetate (1:9) extract of root of *Peltophorum pterocarpum* on alloxan and glucose induced diabetic mouse model. The extract was given intraperitoneally at a dose of 200 and 300 mg/kg body weight and the hypoglycemic effects was compared with standard drug vincristine sulphate, a standard drug. The extract reduced blood glucose level in both diabetic model and at 300 mg/kg dose, the test sample showed better activity. The extract was also subjected to brine shrimp lethality bioassay and phytochemical screening test. LC<sub>50</sub> value of the extract was 28.25 µg/ml and for standard drug vincristine sulphate it was 14.55 µg/ml. Preliminary phytochemical screening of the extract revealed the presence of different types of compounds including flavonoids and steroids. Our results suggest that root of *P. pterocarpum* demonstrated promising antidiabetic activity with low cytotoxicity that substantiated its ethnomedicinal use and may provide new molecules for the treatment of diabetes.

**Key words:** Antidiabetic, alloxan, brine shrimp, phytochemical, *Peltophorum pterocarpum*.

## INTRODUCTION

Diabetes is a chronic metabolic disorder that is characterized by either the insufficient production or the lack of response to a key regulatory hormone of the body's metabolism, insulin. Diabetes is divided into two major categories: Type 1 diabetes [insulin dependent diabetes mellitus (IDDM)] and Type 2 diabetes [non-insulin dependent diabetes mellitus (NIDDM)]. Type 1 diabetes, the cause is an absolute deficiency of insulin secretion and the cause of Type 2 diabetes is a combination of resistance to insulin action and an inadequate compensatory insulin-secretory response

(American Diabetes Association, 2005). The overall prevalence of diabetes is approximately 10% of the population, of which 90% is Type 2. There is estimated 246 million people worldwide suffering from diabetes (Anonymous, 2006). In the United States, diabetes is the sixth leading cause of death (Bethesda, 1995). It is predicted that by 2030, India, China and the United States will have the largest number of people with diabetes (Wild et al., 2004). The increasing worldwide incidence of diabetes mellitus in adults constitutes a significant impact on the health, quality of life, and life expectancy of patients, as well as on the global public health care system. The long term manifestation of this disease can result in the development of vascular disorders such as retinopathy, nephropathy, neuropathy, and angiopathy (Pirart, 1978). Sedentary lifestyle, degree of obesity, changes in food consumption, aging, and other concomitant medical conditions have been implicated in this increasing prevalence in the past two decades (Shastri, 1980; Clark, 1998). The present treatment of diabetes is focused on controlling and lowering blood glucose to a normal level. In conventional

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**Abbreviations:** IDDM, Insulin dependent diabetes mellitus; NIDDM, non-insulin dependent diabetes mellitus; WHO, World Health Organization; ICDDR, International Centre for Diarrhoeal Disease Research, Bangladesh; DMSO, dimethyl sulfoxide; OGTT, oral glucose tolerance test; BGL, blood glucose level; FBG, fasting blood glucose.

therapy, Type 1 diabetes is treated with exogenous insulin and Type 2 with oral hypoglycemic agents such as sulfonylureas, biguanides,  $\alpha$ -glucosidase inhibitors, and glitazones, which are used as monotherapy or in combination to achieve better glycemic regulation. Many of these oral antidiabetic agents have a number of serious adverse effects; thus, managing diabetes without any side effects is still a challenge (Pepato et al., 2005; Saxena and Vikram, 2004). Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of investigation. Traditional medicines from readily available medicinal plants offer great potential for the discovery of new antidiabetic drugs. The hypoglycemic effect of several plants used as antidiabetic remedies has been confirmed, and the mechanisms of hypoglycemic activity of these plants are being studied. Biguanides include the drug metformin, which was originally derived from a medicinal plant, *Galega officinalis*. Metformin reduces plasma glucose via inhibition of hepatic glucose production and increase of muscle glucose uptake. It also reduces plasma triglyceride and LDL-cholesterol levels (Lucy et al., 2002). The effect of *Tinospora cordifolia* W. (Menispermaceae) roots at 2.5 and 5.0 g/kg b. w. was better than that of glibenclamide (Prince et al., 2004). Three new isoquinoline alkaloids, schulzeines A, B, and C, were isolated from the marine sponge *Penares schulzei* inhibit  $\alpha$ -glucosidase. The natural sweetener stevioside, which is found in the plant *Stevia rebaudiana* Bertoni (Asteraceae), has been used in the treatment of diabetes for many years in many parts of the world. Stevioside, with a mechanism for stimulating insulin secretion via direct action on the beta cells of pancreatic islets, is considered to have the potential of becoming a new antidiabetic drug for use in treatment of Type 2 diabetes (Li et al., 2004; Jeppesen et al., 2000).

To date, over 400 traditional plants treatment for diabetes have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy. The World Health Organization (WHO) Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated (Bailey and Day, 1989). So, as a part of the ongoing research works to find out noble antidiabetic agents, here we study the root of the *Peltophorum pterocarpum*.

*P. pterocarpum* is known as Radhachura (Beng.) belongs to the family Fabaceae, native to tropical Southeastern Asia and a popularly ornamental tree grown around the world. *P. pterocarpum* is a deciduous tree usually reaching a height of 15 m. The leaves are bipinnate, 30 to 60 cm long and the flowers are yellow in color. It is a widely-appreciated shade and shelter tree due to its dense spreading crown and wind firm. In traditional medicine it is used as an astringent to cure or relieve intestinal disorders after pain at child birth, sprains, bruises and swelling or as a lotion for eye

roubles, muscular pains and sores (Ganda, 1980). It is also used for gargles and tooth powders. The leaves and barks of this plant were reported to contain phenolic compounds that showed antioxidant activity (Jain et al., 2011). But there is no report on antidiabetic activity of this plant. This study was thus undertaken to evaluate the hypoglycemic effect of methanol: ethyl acetate (1:9) extract of *P. pterocarpum* root.

## MATERIALS AND METHODS

### Plant material

The root of this plant was collected from Rajshahi, Bangladesh, during the month of April 2010 and authenticated by expert Mr. Md. Arshed Hossain, Department of Botany, Rajshahi University. Voucher specimens, collection # 38, dated 10/30/2009 for *P. pterocarpum* kept in the Department of Botany, Rajshahi University, Rajshahi-6205, Bangladesh.

### Preparation of extract

Dried root of *P. pterocarpum* was pulverized in a ground mill. 100 g of powder was extracted three times by sonication for 30 min with methanol:ethyl acetate in the ratio of 1:9 (1000 ml) and then filtered. Here methanol:ethyl acetate in the ratio of 1:9 was used as a solvent to extract medium polar compounds from the plant part which are most often physiologically active. On the other hand, this solvent system does not allow the extraction of highly polar compounds like glycosidic glucose that are physiologically inactive and hence decrease the unwanted compounds in the test extract. Filtrate was concentrated by vacuum evaporator and dried. The extract was then preserved in the refrigerator for the experimental use.

### Animals

Male Swiss Albino mice (20 to 25 g) were collected from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) and housed in polypropylene cages under controlled conditions. The animals were exposed to alternative 12 h light and dark cycle. Animals were allowed free access to drinking water and pellet diet, collected from ICDDR, B; Dhaka. Mice were acclimatized for 7 days. All animal experiments were performed in accordance with NIH guidelines. The study protocol was approved by the animal Ethics Committee of the Institution.

### Drugs

Alloxan was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India and used for the induction of diabetes. The active drug, Metformin hydrochloride was collected from Square Pharmaceuticals Ltd., Pabna plant, Bangladesh. All other chemicals used were of analytical grade.

### Preparation of dose

Diabetes was induced with a single dose of freshly prepared alloxan (80 mg/kg b. w., i.p.) in sterile saline water. Two doses (200 and 300 mg/kg b. w.) of extract of *P. pterocarpum* root were used for testing antidiabetic activity and extract was administered

intraperitoneally after dissolving in dimethyl sulfoxide (DMSO) vehicle. Standard drug metformin was injected in the same route at the dose of 150 mg/kg b. w.

### Antidiabetic effect

#### *Effect of extract on alloxan induced diabetic mice*

In the experiment, a total 25 Swiss Albino mice about 20 to 25 g; 4 to 6 weeks were used and divided randomly into five groups (five mice in each group). Treatment was done for 24 h as follows:

Group I: Normal control mice (Vehicle treated).

Group II: Diabetic control (Received alloxan 80 mg/kg b. w, i.p.).

Group III: Diabetic mice given metformin (150 mg/kg b. w, i.p.).

Group IV: Diabetic mice given root extract (200 mg/kg b. w, i.p.).

Group V: Diabetic mice given root extract (300 mg/kg b. w, i.p.).

Group II to V received a single dose of alloxan (80 mg/kg i.p.) after overnight fasting.

Group I received only DMSO as normal control group and Group II was diabetic control group, which did not receive either metformin, or plant extract. Metformin and extract were injected intraperitoneally to the respective groups after 24 h of alloxan injection and blood samples were analyzed for blood glucose content at 0, 2, 6, 12, 16 and 24 h subsequently using a glucometer kit (Accu-Check active, Roche Diagnostic GmbH, Mannheim, Germany). Fasting blood glucose levels of 15 to 17 mmol/L were considered as diabetic and included in the study (Nagappa et al., 2003).

#### *Effect of extract on glucose induced hyperglycemic mice*

For oral glucose tolerance test (OGTT), 25 more mice were divided into five groups (n = 5) from Group I to Group V. Group I and Group II were selected for normal control and diabetic control group respectively. After overnight fasting, a baseline blood glucose level (BGL) was estimated (0 min). Without delay, a glucose solution (2 g/kg b. w.) was administered by gavages from Group II to Group V. At the same time Group III received standard drug and the test groups (Group IV and Group V) received 200 and 300 mg/kg of plant extracts intraperitoneally. Five more readings were taken at 30, 60, 90, 150, 270 min after glucose and test sample administration.

### Statistical analysis

The experimental data were presented as the means  $\pm$  SEM. The differences between the groups were considered as significant at  $*P < 0.05$  by student's T-test and Tukey's test using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

### Brine shrimp lethality bioassay

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds (Meyer et al., 1982; Zhao et al., 1992). Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The dried cyst of the brine shrimp were collected from an aquarium shop (Chittagong, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) with strong aeration for 48 h day/dark cycles to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii at eight concentrations of 3.125, 6.25, 12.5, 25, 50,

100, 200 and 400  $\mu$ g/ml. All doses were calculated by serial dilution technique. The eight test tubes were marked as 1, 2, 3, 4, 5, 6, 7 and 8 for each concentration of the extract. The sample (extract) was prepared by dissolving 4 mg of extract in 50  $\mu$ l DMSO and volume adjusted to 5 ml with sea water (3.8% NaCl in water) to attain concentrations 800  $\mu$ g/ml in the first test tube. Now 2.5 ml sample was transferred from first test tube to second test tube where volume adjusted to 5 ml with sea water and from this test tube 2.5 ml sample was transferred to third test tube and so on. In this case, all test tubes contained 2.5 ml sample with double concentrations of the test doses of the extract. Now, ten nauplii were transferred to each test tube and the final volume was made 5 ml by adding sea water. So, due to double dilution, our expected concentrations were then attained in the respective test tubes.

A vial containing 50  $\mu$ l DMSO diluted to 5 ml was used as a control. Standard vincristine sulphate was used as positive control. Then matured shrimps were applied to each of all experimental vials. After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of mortality of the brine shrimp nauplii was calculated for each concentration using the following formula:

$$\% \text{Mortality} = \frac{N_t}{N_0} \times 100$$

Where,  $N_t$  = Number of killed nauplii after 24 h of incubation,

$N_0$  = Number of total nauplii transferred, that is 10.

The  $LC_{50}$  (Median lethal concentration) was then determined using Probit analysis.

### Phytochemical screening tests

Phytochemical screening for bioactive principles was done by general chemical test. Phytochemical tests had been performed according to the literature by Nayak and Pereira (2006).

#### *Test for saponins*

300 mg of extract was boiled with 5 ml water for 2 min. The mixture was cooled and mixed vigorously and left for three minutes. The formation of frothing indicated the presence of saponins.

#### *Test for tannins*

To an aliquot of the extract, sodium chloride is added to make to 2% strength. Then it was filtered and mixed with 1% gelatin solution. Precipitation indicated the presence of tannins.

#### *Test for Triterpenes*

300 mg of extract was mixed with 5 ml chloroform and warmed for 30 min. The chloroform solution was then treated with a small volume of concentrated sulphuric acid and mixed properly. The appearance of red color indicated the presence of triterpenes.

#### *Test for alkaloids*

300 mg of extract was digested with 2 M HCl. Acidic filtrate was mixed with amyl alcohol at room temperature, and examined the alcoholic layer for the pink colour which indicated the presence of alkaloids.

#### *Test for flavonoids*

The presence of flavonoids was determined using 1% aluminium

**Table 1.** Antidiabetic effect of root extract of *P. pterocarpum* on alloxan induced diabetic mice.

Time hour	Blood glucose level (mmol/L)				
	Group				
	Normal control	Diabetic control	Metformin (Standard)	Root (200 mg/kg)	Root (300 mg/kg)
0	6.10±0.52	5.04±0.44	6.10±0.53	6.70±0.52	6.50±0.46
30	5.67±0.60	14.36±0.72	13.93±0.68	12.32±0.73	12.47±0.84
60	6.60±0.69	11.31±0.64	7.16±0.45*	9.87±0.34	6.82±0.45*
90	5.76±0.52	6.78±0.68	4.98±0.38*	7.91±0.42	6.44±0.41*
150	6.73±0.37	5.02±0.43	4.38±0.45*	7.08±0.42	4.90±0.42*
270	5.39±0.51	6.72±0.78	5.75±0.45	6.18±0.34	6.90±0.21

Values are mean ± SEM, where \*P<0.05 indicates significant activity comparing with diabetic control group. Each group contains 5 animals.

**Table 2.** Antidiabetic effect of root of *P. pterocarpum* on glucose induced hyperglycemic mice.

Time hour	Blood glucose level (mmol/L)				
	Group				
	Normal control	Diabetic control	Metformin (Standard)	Root (200 mg/kg)	Root (300 mg/kg)
0	6.10±0.52	5.04±0.44	6.10±0.53	6.70±0.52	6.50±0.46
30	5.67±0.60	14.36±0.72	13.93±0.68	12.32±0.73	12.47±0.84
60	6.60±0.69	11.31±0.64	7.16±0.45*	9.87±0.34	6.82±0.45*
90	5.76±0.52	6.78±0.68	4.98±0.38*	7.91±0.42	6.44±0.41*
150	6.73±0.37	5.02±0.43	4.38±0.45*	7.08±0.42	4.90±0.42*
270	5.39±0.51	6.72±0.78	5.75±0.45	6.18±0.34	6.90±0.21

Values are mean ± SEM where \*P<0.05 indicates significant activity comparing with diabetic control group. Each group contains 5 animals.

chloride solution in methanol, concentrated HCl, magnesium turnings, and potassium hydroxide solution.

#### Salkowski reaction for steroids

A few crystals of the compounds were dissolved in chloroform and a few drops of concentrated sulfuric acid were added to the solution. Production of brown color indicated the presence of steroids.

## RESULTS

### Antidiabetic effect

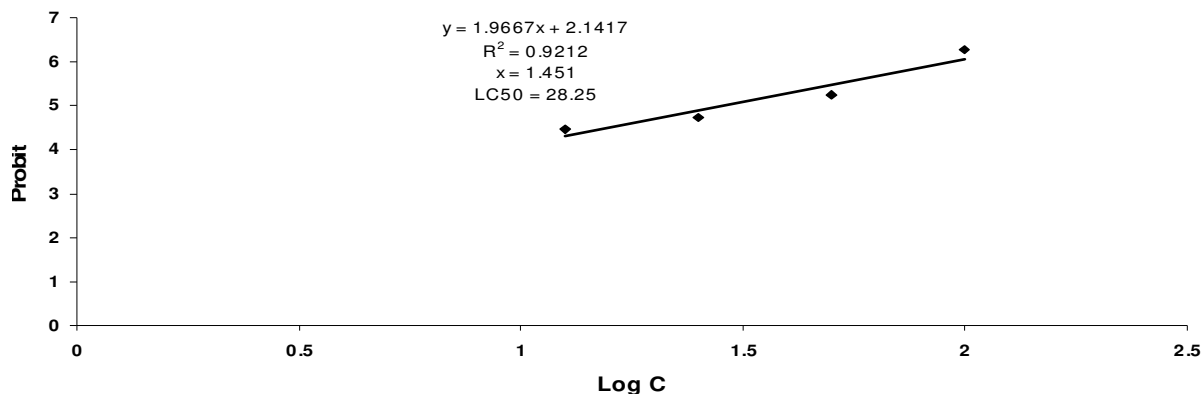
#### Effect of extract on alloxan induced diabetic mice

The effect of single intraperitoneal injection of methanol: ethyl acetate (1:9) extract of *P. pterocarpum* root and metformin on blood glucose levels in diabetic mice are shown in Table 1. Following a 24 h post alloxan injection, all diabetic mice exhibited hyperglycemia, which ranged between 15.86±0.44 and 16.36±0.79 mmol/L while normal control mice showed a normal blood sugar level of about 6 mmol/L. After treatment, the blood glucose levels were decreased both in positive control and test control groups. Maximum reduction of 46.47 and 61.79%

blood glucose levels were observed for extract at 200 and 300 mg/kg b.w. respectively at 12th hour of the 24 h experimental period and the result at 300 mg/kg was comparable with standard drug metformin which showed maximum 66.91% blood glucose level reduction at the dose of 150 mg/kg. So, the extract showed better antihyperglycemic activity at higher dose than 200 mg/kg.

#### Effect of extract on glucose induced diabetic mice

The results for methanol:ethyl acetate (1:9) extract of *P. pterocarpum* root on glucose induced hyperglycemic mice as to lowering fasting blood glucose (FBG) showed significant differences as compared to the diabetic control group. The effects of a single intraperitoneal dose of the extract on the blood glucose level in glucose induced diabetic mice and normal mice are summarized in Table 2. After overnight fasting, administration of glucose (2 gm/kg b.w.) led to approximately 2 to 3 fold elevation of blood glucose levels in the mice. Six readings were then taken at 0, 30, 60, 90, 150 and 270 min after oral glucose solution and intraperitoneal test sample administration. All test samples including extract and metformin reduced BGL significantly, whereas the extract showed better action at higher doses. In this model, the extract showed



**Figure 1.** Determination of  $LC_{50}$  values for extract of leaf of *P. pterocarpum* from linear correlation between log concentrations versus Probit value.

**Table 3.** Brine shrimp cytotoxicity of crude extract of *P. pterocarpum* root.

Conc. ( $\mu\text{g/ml}$ )	Log C	Total	Alive	Death	% Mortality	Probit	$LC_{50}$
12.5	1.09691	10	7	3	30	4.48	
25	1.3979	10	6	4	40	4.75	
50	1.69897	10	4	6	60	5.25	
100	2	10	1	9	90	6.28	28.25
200	2.30103	1	0	10	10	-	
400	2.60206	10	0	10	10	-	

maximum reduction of 42.53 and 60.70% blood glucose level at 200 and 300 mg/kg b.w., respectively at 150 min of the experimental period and maximum 65.04% reduction was observed for standard drug metformin at the dose of 150 mg/kg b.w at the same time period.

### Brine shrimp lethality bioassay

Brine shrimp lethality results of the methanol:ethyl acetate crude extract of *P. pterocarpum* root is shown in Figure 1 and  $LC_{50}$  value calculated is recorded in Table 3. The crude extract showed positive result, indicating that the sample was biologically active. Crude extract resulting in  $LC_{50}$  value of less than 1  $\mu\text{g/ml}$  are considered as significantly active which suggest that the *P. pterocarpum* crude extract, with  $LC_{50}$  value of 28.25  $\mu\text{g/ml}$  has a very low toxicity. Vincristin sulphate served as the positive control for this brine shrimp lethality assay and its  $LC_{50}$  value was 14.55  $\mu\text{g/ml}$ . No mortality was found in the control group, using DMSO and seawater.

### Phytochemical screening test

The phytochemical screening test was carried out using various chemical reactions to identify the presence of different bioactive compounds in the test extract. The result is shown in Table 4. The root extract of *P.*

**Table 4.** Results of general test for different compounds in root extract of *P. pterocarpum*.

Chemical compound	Root extract
Alkaloids	-
Tannins	-
Saponins	-
Flavonoids	+
Triterpenes	-
Steroids	+

(-) = not detected; (+) = detected.

*pterocarpum* contains flavonoids, steroids and negative results were obtained for rest of the tested compounds.

### DISCUSSION

People on all continents have used hundreds to thousands of indigenous plants for treatment of ailments since prehistoric times. According to WHO, about 80% of the world's population presently uses phytotherapy for some aspect of primary health care system. There are many pharmaceutical products which are available in modern medical treatment have a long history of use as herbal remedies including aspirin, opium, digitalis and

quinine (WHO, 2008). A large number of world's population who live in developing countries can not take the benefits of modern pharmaceuticals as those are very expensive. Hence, phytotherapy is still a popular means of primary healthcare for which people bear a little or no cost. In addition to the use in the developing world, phytotherapy is used in the industrialized nations by alternative medicine practitioners such as naturopaths. Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80% show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived (Fabricant and Farnsworth, 2001). Approximately 25% of modern drugs used in the United States have been derived from plant origins (WHO, 2008). So, research on phytotherapy has got great momentum in recent years to find out noble pharmaceuticals.

Our present study revealed that methanol:ethyl acetate (1:9) of root extract of *P. pterocarpum* has significant effect in lowering fasting blood glucose level in alloxan and glucose induced diabetic mice. Metformin showed maximum reduction of blood glucose level at twelve hour and at the same time maximum reduction was obtained for extract in alloxan induced mice. Blood sugar levels were then raised slightly for both extract and metformin treated mice group till observation probably due to loss of their duration of action. In glucose induced diabetic mice, a gradual declination of blood sugar level were observed in all treatment groups throughout the reading period. The extract showed dose dependent antihyperglycemic activity that is better antidiabetic effect was obtained at higher doses. So, the root extract has considerable hypoglycemic activity considering the blood sugar level in standard and diabetic control mice in both diabetic models. In Brine shrimp lethality bioassay, the extract did not show considerable cytotoxicity comparing standard drug vincristine sulphate. Flavonoids and steroids were identified in the preliminary phytochemical analysis using various chemical tests. A deeper insight into the molecular mechanisms of action of these natural compounds could pave the way for developing therapeutic strategies for their antidiabetic activity.

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