Antidiabetic Activity of *Pandanus odoratissimus* Root Extract

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ABSTRACT

The aqueous ethanolic extract of *Pandanus odoratissimus* (Pandanaceae) root was tested for its effect on blood glucose levels in normal and diabetic rats. Hypoglycemia was observed in basal conditions when tested at an oral dose of 75, 150 and 300 mg/kg body weight. The ethanolic extract has displayed a significant dose-dependent antihyperglycemic activity in oral glucose tolerance test and also found to reduce the increased blood glucose in alloxan-induced diabetic rats (37% at 150 mg/kg and 51% at 300 mg/kg body weight). Chronic administration (10 days) of the ethanolic extract of root significantly reduced the blood glucose in alloxan-induced diabetic rats. The extract was also found to reduce the increased blood urea, inhibit the body weight reduction and leucopenia induced by alloxan administration. The ethanolic extract was found to effectively scavenge the DPPH and lipid peroxide free radicals *in vitro* with an IC_{50} value of 10 and 8 µg/ml, respectively. The preliminary phytochemical examination reveals the presence of flavanoids and tannins, which may be attributed to observed antioxidant and significant antihyperglycemic properties.

Keywords: *Pandanus odoratissimus*, alloxan, glucose tolerance, diabetes, roots, antihyperglycemia, antioxidant activity.

INTRODUCTION

Diabetes mellitus is chronic metabolic disorder characterized by abnormalities in carbohydrate, fat, protein metabolism and appropriate hyperglycemia resulting from defects in insulin secretion or peripheral insulin resistance[1]. In India, diabetes has been known for ancient ages and sweetness of the diabetic urine was mentioned by Sushruta in Ayurveda and use of plant extract based on traditional knowledge is common practice. Available ethanobotanical information reports about 800 plants which may possess antidiabetic potential[2]. However, most orally active hypoglycemic remedies extracted from plant material are not scientifically evaluated, incompletely characterized. Although insulin therapy and oral hypoglycemic agents is the mainstay of treatment of diabetes and are effective in controlling hyperglycemia, they have prominent side effects and failed to significantly alter the course of diabetic complications[3]. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of active research.

Pandanus odoratissimus Linn. (Pandanaceae) is a much branched shrub or a small tree with many aerial stilt roots. This plant is popularly known as 'Screw pine Tree' and locally known as 'Mogili'. The plant distributed through out India, commonly found along the seacoast, river banks, ponds and near to water streams[4,5]. In traditional system of medicine the roots are claimed to be useful in treating diabetes[6,7]. The leaves and flowers of *P. odoratissimus* are reported to be useful in leucoderma, tumours, leprosy and skin diseases[8] In India and Burma the male flowers are valued for fragrance and used as a hair decoration. Physcion, cirsilineol, n-triacontanol, β-sitosterol, camphosterol, daucosterol and palmitic acid, stearic acid in rhizomes has been reported[9]. To the best of our knowledge no report is available on antidiabetic effects of *P. odoratissimus* roots. The present study was undertaken to verify the claim and evaluate the antidiabetic property of *P. odoratissimus* roots with the aim of developing a natural antidiabetic drug.

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MATERIALS AND METHODS

Plant material

P. odoratissimus roots were collected fresh from Bethavolue Village, Nalgonda district, Andhra Pradesh, India. The botanical identification of plant was performed by Prof. Ramakrishna, Head, Department of Botany, P.G College of Sciences, Osmania University, Hyderabad. Voucher specimen (PNO-308-9) was deposited in Department of Pharmacognosy and Phytochemistry of G. Pulla Reddy College of Pharmacy, Hyderabad, India. The roots were shade dried and grounded by electric mill and passed through mesh # 60.

Preparation of extract

The dried root powder (850 g) was extracted at room temperature (25-30^{0}C) with 80% aqueous ethyl alcohol for 7 days with occasional shaking followed by re-maceration with the same solvent for 5 more days. The macerates were combined, filtered, and distilled off in reduced pressure. The resulting concentrate was vacuum dried at 40°C to yield the

dry extract (yield:3.03 w/w). The dry extract was kept in a vacuum desiccator until use. The resultant organic extract was tested qualitatively for the presence of various phytoconstituents using standard procedures[10,11].

Animals

Male Wistar rats (160-180 g) were used for the study. Animals were fed with standard diet (National Institute of Nutrition, Hyderabad) and water *ad libitum* during quarantine period. Animals described as fasted had been allowed free access to water. The animal experimentation was carried out according to the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA) guidelines and Institutional Animal Ethics Committee approved all the procedures for investigation. The aqueous ethanolic extract of *P. odoratissimus* root (POE) was administered at different doses as a fine suspension using 0.5% (w/v) aqueous carboxy methyl cellulose (CMC).

Acute toxicity

To determine the acute toxicity, a single oral administration of the POE at different doses (0.5, 1, 2, 3.0 g/kg body weight) was administered to different groups of mice[12]. Each group consists of six animals. Control group received the vehicle. The animals were observed continuously for the initial period of 2 h, intermittently for the next 6 h and then 24 h and 48 h following oral administration of different doses of drug administration for death and abnormality in behavioral changes.

Effect of *P. odoratissimus* extract on blood glucose levels of normal fasted rats

Fasted rats were divided into 4 groups of 6 rats per group. The first group of animals are given 0.5% w/v aqueous CMC through oral route and served as control. Group II-IV received CMC suspension of POE at dose of 75, 150 and 300 mg/kg, respectively. Blood samples were collected from retro orbital under light ether anesthesia just prior to and at 1, 2 and 3h following oral administration of extract. Plasma was separated and blood glucose levels were assessed for biological changes. Glucose levels were estimated by using commercially available glucose kit (Autospan, Span diagnostics, India) based on glucose-oxidase method[13] and absorbance was measured at 505 nm.

Effect of *P. odoratissimus* extract on glucose tolerance in rats

Fasted rats were divided into 4 groups of 6 rats in each group. Control rats were given 0.5% aqueous CMC (group I). CMC suspension of POE (75, 150, and 300 mg/kg) was administered orally to II, III and IV group of rats. The rats of all the groups were given glucose (2 g/kg, orally) 30 min after administration of the extract. Blood samples were collected from the retro orbital just prior to glucose administration and at 30, 90 and 120 min after glucose loading[14]. The blood glucose levels were measured immediately by glucose-oxidase method[13].

Effect of the *P. odoratissimus* extract on alloxan induced diabetic rats

Male Wistar rats were made diabetic by a single intra peritoneal injection of 120 mg/kg body weight of alloxan monohydrate in sterile normal saline. Five days later blood samples were drawn and glucose levels were determined to confirm the development of diabetes (> 250 mg /dl). The diabetic rats were divided into 3 groups, each containing 6 animals. Control rats (group I) were given 0.5% w/v aqueous CMC orally, while POE at dose of 150 and 300 mg/kg body weight were given to the II and III groups. Blood samples were collected just prior to and at 1, 3 and 5 h after extract administration and plasma glucose levels were measuredwere measured immediately[13].

The action of POE was also tested for longer duration of treatment. The male Wistar rats were divided into 4 groups of 10 rats each. Group I served as diabetic control group, received vehicle 0.5% aqueous CMC. The rats of group II and III received two different concentrations of POE (150 and 300 mg/kg) for 5 days. Thereafter all rats of the diabetes control and treated groups (groups I, II and III) were injected with alloxan monohydrate (120 mg/kg, *i.p.*). Group IV animals served as the normal and received vehicle[15].

The administration of test extract was continued for 10 more days after alloxan treatment. Blood samples collected through the retro orbital puncture just prior to and on days 5 and 10 after the alloxan injection. The following parameters were measured:

1. Blood sugar 2. Blood urea 3. Body weight and 4. Total leukocyte count.

Antioxidant studies

The free radical scavenging activity of POE was measured by using stable DPPH (2,2-diphenyl-1- picryl hydrazyl) as described by Aquino et al[16]. DPPH in its radical form has an absorption peak at 517 nm, which disappears on reduction by an antioxidant compound. 1 ml of POE in different concentrations (10-100 µg) was added to 2 ml of freshly prepared methanolic solution of DPPH (90 µM) and the volume was made up to 4 ml with methanol. Absorbance was measured at 517nm, after 1 h. Curcumin served as a standard. The percentage inhibition of DPPH in the reaction medium was calculated by comparing with control.

Rat liver homogenate was used for determining the extent of lipid peroxidation. The lipid peroxide formed was measured by thiobarbituric acid reacting substance (TBARS) formation as described by Ohkawa et al[17]. Reaction mixture (4 ml) containing 0.5 ml of rat liver homogenate in Tris-HCl buffer (40 mM, p^H-7), 3.2 ml of different concentrations of test substances(10-100 µg/ml) and 100 µl of each of 0.15 M KCl, 15 mM of ferrous sulphate and 6 mM ascorbic acid was added and incubated at 37^{0}C for 1 h. To the incubation mixture 1ml of 10% TCA (Trichloro acetic acid) was added and a sample was centrifuged at 3000 rpm for 20 min at 4^{0}C. 1 ml of 0.8% TBA (thibarbituric acid) was added to the supernatant solution and the mixture is heated at 100^{0}C for 20 min in water bath. After cooling the colored TBA –MDA complex was extracted with 2 ml butanol and absorbance of pink chromophore was measured at 532 nm. Butyl hydroxyl toluene served as a standard. The percentage inhibition of lipid peroxidation was determined by comparing the results of test compound with those of control not treated with extract.

Statistical analysis

All values were expressed as mean ± SEM. Results were analyzed statistically by using analysis of variance (ANOVA) followed by Dunnett's test. Values of $P<0.05$ were considered significant.

RESULTS

In oral acute toxicity test, no mortality and abnormal behavioural changes were observed in mice up to a dose of 3 g/kg body weight. Further the antihyperglycemic activity was carried out at an oral dose of 75, 150 and 300 mg/kg.

Administration of POE was found to reduce blood sugar levels significantly in normal rats; however the reduction was marginal and not severe. The maximum reduction in blood glucose was noted at 3 h after the administration of extract. The reduction was 13.09% at a maximum dose of 300 mg/kg (Table 1).

The effect of POE on glucose tolerance is given in Table 2. A sharp increase in glucose concentration was observed in control rats at 60 min after glucose load. The significant ($P<0.001$) glucose tolerance was observed with all test dose levels at 60 min after glucose loading (90 min after drug dosing), however the tolerance was decreased at 2 h. The maximum glucose tolerance was noted for rats administered with 300 mg/kg of extract. The decreased glucose levels in comparison to control rats are 24.77, 41.66 and 54.31% of 75, 150 and 300 mg/kg of extract, respectively at 60 min after glucose charge.

Administration of *P. odoratissimus* root extract was found to reduce blood glucose levels in alloxan induced diabetes rats

Table 1: Effect of *P. odoratissimus* root extract on blood glucose levels in fasted normal rats

Group	Treatment (Dose/ kg body weight)	Plasma glucose levels (mg/dl)			
		Initial	1 h	2 h	3 h
I	Control -untreated	79.68 ± 0.98	76.77 ± 1.05	77.86 ± 1.02	77.64 ± 0.85
II	Ethanolic ext.; 75 mg	77.12 ± 0.74	73.18 ± 0.85* (4.67)	72.62 ± 0.92** (6.73)	74.72 ± 0.92 (3.76)
III	Ethanolic ext.; 150 mg	81.60 ± 0.98	74.53 ± 0.65 (3.21)	69.51 ± 0.81** (10.72)	68.70 ±0.60** (11.51)
IV	Ethanolic ext.; 300 mg	80.66 ± 0.91	70.73 ± 0.94** (7.86)	71.69 ± 0.84* (7.92)	67.47 ± 0.86** (13.09)

[a] Values are mean ± S.E.M.; *n*=6, **P* < 0.05, ***P* < 0.01 vs Control. Figures in parenthesis indicate the percentage decrease in plasma glucose levels

Table 2: Effect of P. odoratissimus root extract on oral glucose tolerance test in rats [a]

Group	Treatment (Dose/ kg body weight)	Plasma glucose levels (mg/dl)			
		Initial	30 min	60 min	120 min
I	Control- glucose; 2g	79.40 ± 1.41	108.47 ± 1.17**	193.32 ± 1.32	108.12 ± 1.07
II	Ethanolic ext. 75mg+glucose 2g	87.44 ± 1.31*	95.81 ± 1.25** (11.68)	145.43 ± 1.33** (24.77)	100.70 ± 1.23** (6.86)
III	Ethanolic ext. 150mg+glucose 2g	77.74 ± 1.32	110.13 ± 1.18	112.77 ± 1.30** (41.66)	105.04 ± 1.40 (2.84)
IV	Ethanolic ext. 300mg+glucose 2g	96.96 ± 1.19*	101.61 ± 1.29* (6.32)	88.32 ± 1.43** (54.31)	98.99 ± 1.45** (8.44)

[a] Values are mean ± S.E.M.; *n*=6, **P* < 0.05, ***P* < 0.01 vs Control. Figures in parenthesis indicate the percentage decrease in plasma glucose levels

(Table 3). The fasting blood glucose levels in alloxan induced diabetes rats were 262 – 298 mg/dl. There was no significant difference in blood glucose levels of diabetic control. However the administration of POE has shown a significant dose dependent fall in blood glucose levels. The antihyperglycemic effects were observed from 1 h after drug administration and the activity was found to be maximum at 5 h of drug administration. The decreased blood glucose levels in diabetic rats at 5 h after the administration of root extract is 37.81 and 51.44% of 150 and 300 mg/kg extract.

Sub-chronic administration of *P. odoratissimus* root extract was found to reduce blood glucose levels in alloxan induced diabetic rats. The plasma glucose levels were markedly raised (up to 4.75 times) in the diabetic control as compared with the normal control on day 5 after the alloxan injection. The maximum reduction in blood glucose levels is observed with animals administered with 300 mg/kg on day 5, with percent protection of 37.73. Continuous administration of POE significantly (P<0.001) reduced the blood glucose levels to 197 mg/dl. The extract has produced a dose dependent antihyperglycemic property. Treatment with POE significantly reduced the serum glucose levels by 9.03 and 25.03% respectively of 150 and 300 mg/kg of extract on day 10. The decreased blood glucose level from 340 to 263 mg/dl in diabetic rats is observed indicating the pancreatic tissue gets repaired by itself after a single alloxan injection. The repaired was found to be much faster in animals received *P. odoratissimus* extract (Table 4).

A decrease in rat body weight ($p < 0.001$) was noted in alloxan induced diabetic rat but when the animals were treated with *P. odoratissimus* extract, the decrease in body weight was completely suppressed and recovery of body weight was observed in the animals received POE with the dose of 300 mg/kg body weight (Table 5). In diabetic animals the normal function of the kidney is assessed as blood urea levels and is distributed (30.86 in control versus 69.50 mg/dl in diabetic rats) in diabetic rats 5 days after of alloxan administration.

Table 3: Effect of P. odoratissimus root extract on alloxan- induced diabetic rats [a]

Group	Treatment (Dose/ kg body weight)	Plasma glucose levels (mg/dl)			
		Initial	1 h	3 h	5 h
I	Diabetic control	290.05 ± 5.19	283.68 ± 5.78	293.81± 3.18	298.04 ± 4.10
II	Ethanolic ext.; 150mg	275.50 ± 5.82	223.45 ± 4.88** (21.23)	206.72 ± 4.08** (29.64)	185.34 ± 3.93** (37.81)
III	Ethanolic ext.; 300 mg	262.10 ± 4.79	251.69 ± 5.91* (11.27)	180.06 ± 3.53** (38.71)	149.72 ± 3.57** (51.44)

[a] Values are mean ± S.E.M.; *n*=6, **P* < 0.05, ***P* < 0.01 vs Control. Figures in parenthesis indicate the percentage decrease in plasma glucose levels

Table 4: Effect of chronic administration of *P. odoratissimus* root extract on blood glucose level in rats treated with aloxan

Group	Treatment (Dose/ kg body weight)	Plasma glucose levels (mg/dl)		
		Initial	Day 5	Day 10
I	Diabetic control-alloxan 120mg	72.37 ± 1.16	342.97 ± 1.23	263.88 ± 2.36
II	Ethanolic ext. 150mg+alloxan 120mg	79.42 ± 1.27	296.86 ± 3.03** (15.53)	242.00 ± 5.99* (9.039)
III	Ethanolic ext. 300mg+alloxan 120mg	78.96 ± 1.35	213.75 ± 3.91** (37.73)	197.30 ± 5.23** (25.03)
IV	Normal	76.56 ± 1.0	275.64 ± 1.3	274.72 ± 1.43

[a] Values are mean ± S.E.M.; *n*=6, **P* < 0.05, ***P* < 0.01 vs Control. Figures in parenthesis indicate the percentage decrease in plasma glucose levels

Table 5: Effect of P. odoratissimus root extract on body weight in rats treated with alloxan [a]

Group	Treatment (Dose/ kg body weight)	Body Weight (g)		
		Initial	Day 5	Day 10
I	Diabetic Control – alloxan 120 mg	160 ± 4.47	130 ± 3.65***	120 ± 2.24***
II	Ethanolic ext. 150 mg + alloxan 120 mg	156 ± 4.22	125 ± 2.55	135 ± 3.104
III	Ethanolic ext. 300 mg + alloxan 120 mg	163 ± 2.11	115 ± 3.52*	141 ± 3.07
IV	Normal	176 ± 4.11*	186 ± 3.42	189 ± 4.94**

[a] Values are mean ± S.E.M.; *n*=6, **P* < 0.05, ***P* < 0.01 vs Control.

Animals treated with *P. odoratissimus* extract along with alloxan had lower (P> 0.001) blood urea levels significantly. The maximum reduction in urea level is noted with the animals received the POE at the dose of 300 mg/kg with percent reduction of 32.26 (Table 6).

Effect of *P. odoratissimus* extract on total white blood cells (WBC) count in alloxan treated animals is shown in (Table 7). Total WBC was significantly reduced from 7434 to 5263, five days after administration of alloxan indicating cellular injury brought about by alloxan. Administration of POE significantly prevented alloxan induced cellular damage at both test dose levels, as seen from the number of total WBC.

The free radical DPPH was effectively scavenged by POE.

Fig. 1: Scavenging effects on DPPH radical of *P. odoratissimus* root extract

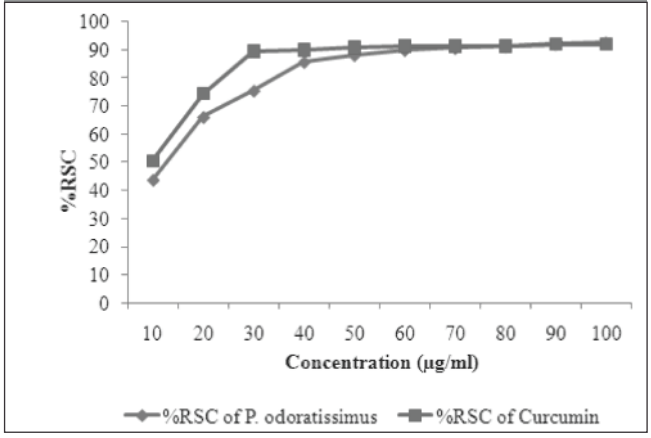
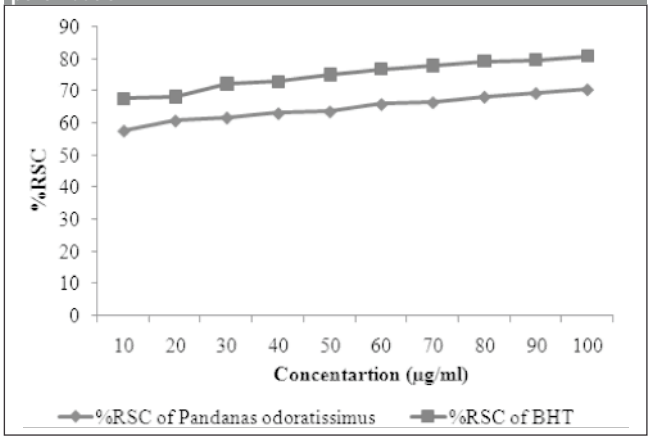


Fig. 2: Effects of P. odoratissimus root on inhibition of lipid peroxidation



The radical scavenging capacity of test extract and standard curcumin is increased with increasing concentration (Fig.1). The IC_{50} was found to be 13 µg/ml whereas standard curcumin shows 10 µg/ml. POE was found to inhibit *in vitro* tissue lipid peroxidation in a concentration dependent manner ranging from 10-100 µg/ml. Fig. 2 represent, the inhibition of lipid peroxidation activity. IC_{50} value of POE is 8 µg/ml. The butyl hydroxyl toluene (BHT) was used to compare the antioxidant potential, and the IC_{50} of BHT (6 µg/ml) was comparable with that of POE.

DISUSSION

The results of present study indicates that POE was found to

Table 6: Effect P. odoratissimus root extract on blood urea in animal treated with alloxan [a]

Group	Treatment (Dose/ kg body weight)	Blood urea (mg/dl)		
		Initial	Day 5	Day 10
I	Diabetic Control-alloxan 120mg	30.86 ± 0.38	69.50 ± 0.33	42.37 ± 0.46
II	Ethanolic ext. 150mg+alloxan 120mg	26.47 ± 0.29	60.70 ± 0.47** (12.26)	34.96 ± 0.34** (17.48)
III	Ethanolic ext. 300mg+alloxan 120mg	28.158 ± 0.34	68.28 ± 0.38* (1.75)(32.26)	28.70 ± 0.28**
IV	Normal	25.44 ± 0.34	29.56 ± 0.38**	27.536 ± 0.29**

[a] Values are mean ± S.E.M.; *n*=6, **P* < 0.05, ***P* < 0.01 vs Control. Figures in parenthesis indicate the percentage decrease in plasma glucose levels

Table 7: Effect of P. odoratissimus root extract on total leukocyte counts in rats treated with alloxan [a]

Group	Treatment (Dose/ kg body weight)	Total leukocyte counts (mm^3)		
		Initial	Day 5	Day 10
I	Diabetic Control – alloxan 120mg	7434 ± 326	5263 ± 429	5337 ± 413
II	Ethanolic ext. 150 mg +alloxan 120mg	7760 ± 357	6570 ± 399**	7457 ± 453*
III	Ethanolic ext. 300 mg + alloxan 120mg	8940 ± 493*	7269 ± 520**	8340 ± 333**
IV	Normal	8732* ± 406	9048 ± 326**	9160 ± 310**

[a] Values are mean ± S.E.M.; *n*=6, **P* < 0.05, ***P* < 0.01 vs Control. Figures in parenthesis indicate the percentage decrease in plasma glucose levels

reduce the glucose levels in normal, glucose loaded animals and in animals made diabetic with alloxan. Alloxan has been shown to induce free radical production and cause tissue injury[18]. The pancreas is especially susceptible to the action of alloxan induced free radical damage. The present findings indicate that *P. odoratissimus* can act as free radical scavenger *in vitro* in both DPPH and lipid peroxides and ameliorate the destruction of WBC and confirms the possibility that the major function of the extract is on the protection of vital tissues including the pancreas, thereby reducing the causation of diabetes in these animals. Other possible mechanism includes the stimulation of β-cells and subsequent release of insulin and activation of the insulin receptors. Estimation of insulin levels may give more insight into the mechanism of the antidiabetic activity shown by the extract.

The present study also indicates that *P. odoratissimus* can partially inhibit alloxan renal toxicity as seen from the blood urea levels. The preliminary phytochemical investigation results indicate the presence of tannins, steroids, triterpenoids and flavonoids and/or their glycosides in POE. Flavonoids and tannins are well-known polyphenolic natural antioxidants, which may be responsible for the antioxidant role of *P. odoratissimus* and for observed antihyperglycemic properties. The active ingredient in the extract which reduces the blood sugar is not known at present.

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