

PRELIMINARY PHYTOCHEMISTRY AND ANTIDIABETIC ACTIVITY OF *PORTULACA GRANDIFLORA* HOOK PLANT EXTRACT ON STREPTOZOTOCIN-INDUCED DIABETES IN RATS

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ABSTRACT

Objective: The present study was aimed to evaluate the antidiabetic activity of ethanolic extract of the whole aerial part of *Portulaca grandiflora* Hook on streptozotocin (STZ)-induced diabetic rats.

Methods: Experimental diabetes was induced by a single dose of intraperitoneal injection of STZ (150 mg/kg). Adult male Wistar albino rats were divided into five groups; normal control, diabetic control, diabetic glibenclamide (5 mg/kg), diabetic *P. grandiflora* H. extract (200 mg/kg), and diabetic *P. grandiflora* H. extract (400 mg/kg) for 21 days and analyzed for body weight (BW) and blood glucose.

Results: The STZ-treated diabetic control rats showed a significant increase in blood glucose with a concomitant decrease in BW. Oral administration of *P. grandiflora* H. extract (200 and 400 mg/kg) and glibenclamide (5 mg/kg) for 21 days showed a significant reduction in blood glucose levels and elevation in the bodyweight studies as compared to control and glibenclamide-treated rats.

Conclusion: The results of the present study showed that a potent antidiabetic activity was present in the aerial part of plant *P. grandiflora* H. extract.

Keywords: Diabetes mellitus, Ethanolic extract, Glibenclamide, Blood glucose, Streptozotocin.

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INTRODUCTION

One of the major causes of morbidity and mortality in the world is diabetes mellitus, the most common metabolic disorder causing changes in carbohydrate, fat, and protein metabolism due to defects in insulin secretion or action or both and is characterized by loss of glucose homeostasis [1]. The estimation of the World Health Organization is that one of the serious life-threatening ailments would be diabetes within the next century [2]. Diabetes mellitus is the sixth-leading cause of death in the world [3]. Many different plants and plant extracts have been described to show beneficial effects on diabetics. Most of these plants have been argued to possess hypoglycemic properties. A search for a new class of compounds is being researched and essential to overcome diabetic side effects [4]. Conventionally, several plants have been served in various herbal preparations in the management of diabetes and only a few of them have been proven scientifically [5]. In today's scenario, most of the diabetics on standard anti-diabetic drugs such as sulfonylureas, biguanides therapy suffer from adverse effects of nausea, vomiting, abdominal pain, diarrhea, and headache. To overcome such effects of drugs, the development of a novel safer and potent anti-diabetic herbal formulation is essential [6].

Botanical name: *Portulaca grandiflora* Hook

English name: Eleven o'clock, Moss-rose, Rose moss

Tamil name: Pasalai keerai

Sanskrit name: Paciri, Paviri Telugu: Ganga paavilikoora

Malayalam name: Koluppa

Distribution: Pantropical, common in tropical India and Africa, U.S.

Virgin Islands and Asia

Parts used: Aerial part.

P. grandiflora Hook is a small, diffuse, annual, and erect herb found throughout the tropical parts of India (Fig. 1).

Chemical composition

Alkaloids, glycosides, mucilage, tannins, proteins, flavonoids, saponins, carbohydrates and triterpenoids are the chemical constituents present in *Portulaca grandiflora* Hook.

It is useful in the treatment of asthma, cough, urinary discharges, inflammations, and ulcers. *P. grandiflora* Hook has been reported to possess antioxidant activity [7]. Literature surveys have yielded scanty information on the pharmacological properties of diabetes management. The rationale and novelty in study is that as no or evidence or systematic study was carried out on the aerial extracts of *P. grandiflora* Hook for its *in vivo* anti-diabetic activity using an animal model supporting the anti-diabetic properties of this plant, the present study was to unlock the antidiabetic activity of *P. grandiflora* Hook *in vitro* and *in vivo* streptozotocin (STZ) model. Moreover, due to the feasibility and availability of the plant, the development of the plant extract to a new herbal formulation may pave a way for the development of a novel anti-diabetic formulation that may be economical and readily formulated.

METHODS**Plant material and authentication of plant**

The entire plant of *P. grandiflora* Hook was collected from a local place in Chennai, Tamil Nadu (India), in January 2018. It was authenticated by Prof. V. Jayaraman, Director, Professor (Rtd), Presidency College, Chennai - 5. The collected aerial part of the plant was shade dried and then crushed to a coarse powder with a mechanical grinder. The powder was kept in an airtight container which was further used for extraction.

Preparation of plant extract

A weighed quantity (50 g) of the powder was subjected to a hot continuous extraction method in a Soxhlet apparatus with ethanol

(500 mL) as a solvent for 2 days until the solvent in thimble became colorless. The extracts obtained were dried at room temperature and the yield was stored in an airtight container.

Drugs and chemicals

Sodium chloride, D-glucose, ethanol, sodium citrate tribasic dehydrate, citric acid monohydrate, STZ, and metformin were all purchased from K B Enterprises, Chennai.

Experimental animals and sampling

Either sex of Wistar albino rats (150–200 g) was used. The animals were housed in the well-ventilated animal house which was maintained at a constant temperature and relative humidity of 55–60% with 12 h light and dark cycle. The animals were housed in spacious polypropylene cages and paddy husk was utilized as bedding material. Sufficient food and water were provided to the animals *ad libitum* except during fasting. The bed material was changed twice a week. All animal cages used in the study had proper identification, i.e., labels. Each animal in the cage was marked on the head and/or tail with picric acid for their appropriate identification.

EXPERIMENTAL PROCEDURE

Phytochemical screening [8,9]

The ethanolic extract obtained was subjected to qualitative tests for the identification of different constituents such as tannins, alkaloids, saponins, glycosides, terpenes, phenolics, flavonoids, carbohydrates, proteins, and steroids. This was done using simple and standard qualitative methods described by Trease and Evans [10-13].

In vitro anti-diabetic effect: Glucose diffusion assay

Plant extracts were mixed glucose and placed in the sealed dialysis membrane and kept in the orbital shaker bath at 37°C, at 150 rpm, and the movement of glucose across the membrane into the external solution was measured at periodic intervals using commercial glucose oxidase – peroxidase (GOD-POD) kit.

Requirements

Dialysis membrane, 0.15 M sodium chloride solution, D- (+) - Glucose - (25 Mm in sodium chloride solution), Orbit shaker, and GOD-POD kit.

Procedure

Dialysis membrane containing 2 ml of 25 mM glucose solution was mixed with 1 ml of plant extract (1%) and was placed in the centrifuge tube containing 45 ml 0.15 M NaCl and then kept in orbit shaker bath at 37°C, at 150 rpm. The movement of glucose into the external solution was monitored at 0, 1, 2, 3, 4, and 5 h using the GOD-POD kit. The diffusion of glucose into the external solution was monitored by measuring the glucose concentration in the external solution every hour.



Fig. 1: *Portulaca grandiflora* Hook

Toxicity studies

Acute toxicity was designed as per the OECD guidelines (423) [14]. Three healthy adult Wistar albino rats weighing between 150 and 250 g were selected for the study. For all the three animals food, but not water was withheld overnight before dosing. Being a traditional herbal medicine, the mortality was unlikely at the highest starting dose level (2000 mg/kg body weight [BW]). Hence, a limit test at one dose level of 2000 mg/kg BW was conducted in all three animals. The animals were observed individually after dosing once during the first 30 min, periodically for the initial 24 h, with special attention given during the first 4 h, and daily thereafter for a total of 14 days. The following clinical observations were done and recorded.

In vivo anti-diabetic effect

P. grandiflora Hook was studied for the antidiabetic activity in diabetic Wistar rats. Diabetes was induced by intraperitoneal injection of STZ 50 mg/kg BW. The antidiabetic effect of plant extract was compared with standard drug glibenclamide.

Induction of diabetes in rats

After a week of acclimatization, the rats were subjected to overnight fasting. Diabetes was induced by intraperitoneal injection of STZ, freshly dissolved in citrate buffer pH 4.5 (two parts of 0.1 M sodium citrate with three parts of 0.1 M citric acid) [15]. The animals were allowed to drink water 5% of glucose solution overnight to overcome the drug-induced hypoglycemia due to the massive release of insulin from β -cells. After 3 days, blood glucose levels were measured and the animals with a blood concentration of more than 250 mg/dl were considered as diabetic and taken for the experiment. Administration of the plant extract was started on the 4th day after STZ injection and this was considered as the 1st day of treatment, which was continued for 21 days.

Experimental design [16]

The fasting glucose and BW of all animals were recorded at the beginning of the study. The blood glucose was checked by one - touch glucometer [17] throughout the study. In the experiments, 30 rats were divided into groups of six rats each.

- Group 1: Normal control rats received distilled water
- Group 2: STZ-induced diabetic rats received (from 1st day) distilled water and served as diabetic control for 1–21 days
- Group 3: STZ-induced diabetic rats received standard drug metformin (5 mg/kg) for 1–2 days
- Group 4: STZ-induced diabetic rats received the plant extract (200 μ g/kg BW) for 1–21 days
- Group 5: STZ-induced diabetic rats received the plant extract (400 μ g/kg BW) for 1–21 days.

For all rats, BW was measured before the induction of diabetes and on 4th, 7th, 14th, and 21st days of the treatment. Blood glucose level was measured on 1st, 7th, 14th, and 21st days using the tail tip cutting method [18]. At the end of the experiment on the 21st day, sufficient blood was collected by retro-orbital bleeding from all the animals under anesthesia for estimation of biochemical parameters (blood glucose and BW).

Statistical analysis

All the parameters were analyzed by ANOVA [19] followed by Dunnett's test. The results were expressed as mean \pm SD. The statistical analysis was performed using Graph and Prism, version 6.0 software.

RESULTS

Phytochemical screening

The phytochemical analysis of an ethanolic extract of *P. grandiflora* H. showed the presence of alkaloids, flavonoids, tannins, protein, carbohydrates, mucilage, phytosterols, saponins, glycosides, and terpenoids.

In vitro anti-diabetic effect of ethanolic extract of *P. grandiflora* Hook

Results relating to the diffusion effects of *P. grandiflora* Hook and glucose by diffusion assay are shown in Table 1. The inhibition level of glucose movement by the plant extract at various intervals of time was assayed and compared with the control in the absence of plant extract. There was a significant decrease in the glucose movement across the membrane for ethanolic extract when compared to the control.

Acute toxicity study

Animals were observed for behavioral signs of toxicity such as motor activity and tremor, and no significant toxic signs were observed during 14 days.

In vivo anti-diabetic effect

Results relating to the effects of *P. grandiflora* Hook whole plant extracts (200 µg/kg and 400 µg/kg) and metformin (5 mg/kg) to the diabetic rats are shown in Table 2.

The mean BW showed a decrease in Group 2, Group 3, Group 4, and Group 5 on 21st day on comparing with 1st day. Vehicle control animals were found to be almost stable in their BW, but diabetic-induced rats showed a significant reduction in BW ranging from 150.65±1.0 g (1st day) to 131.3±2.0 g (21st day). Group 2 (diabetic rats) showed a significant reduction in BW $p < 0.05$ when compared to the groups. The administration of *P. grandiflora* Hook whole plant extract and metformin (5 mg/kg) to the diabetic rats restored the changes in the BW from 151.7±1.49 g (1st day) to 136.5±3.15 g (21st day) for 200 µg/kg of dose and 150.42±2.14 g (1st day) to 148.8±0.9 g (21st day) for 400 µg/kg of dose of *P. grandiflora* Hook whole plant extract and 152.06±0.00 g (1st day) to 139.05±1.07 g (21st day) for glibenclamide (5 mg/kg)-treated animals.

Effect of ethanolic extract of *P. grandiflora* Hook on whole blood glucose in STZ-induced diabetic rats

Diabetic rats (Group 2) showed a significant increase in blood glucose. This fall in fasting blood glucose level progressively increases until the end of 3rd week. When the reduction glucose level with 200 µg/400 µg was compared, there was a statistically significant reduction in blood glucose levels ($p < 0.05$) with 400 mg of the extract (Table 3).

DISCUSSION

The quality of the plant can be assessed by the phytochemical screening. Colored reactions of the bioactive compounds show the presence or

absence of the compounds. Curative activity against several human problems is produced by the phytoconstituents in medicinal plants. A wide range of active phytoconstituents was found to be present in this present study of ethanolic extract of the plant. Ethanol is one of the good solvents in plant extractions, which include low toxicity, easy evaporation at low heat, preservative action, and inability to cause the extract to complex or dissociate. Hence, the presence of phytochemicals in the plant extracts might serve in the prevention of diabetes mellitus along with protection from free radicals produced in the body systems due to various metabolic activities. Natural products have been used as promising sources of novel agents for the treatment of various disorders due to their less toxic effect. Moreover, the lethal dosage not only designates the toxic level of a particular extract but also helps in determining the effective dosage that can be used for the experiment. There was no lethality or any toxic reactions in the present study, found in the animals at any of the doses selected until the end of the investigation period. The results of the acute toxicological studies revealed that the administration of ethanolic extract of *P. grandiflora* H. by oral route up to 2000 mg/kg BW did not produce any mortality and it was tolerated. Plant extract decreased the concentration of glucose into the external solution by retarding its diffusion through the membrane. Plant extract possesses a dose-dependent effect on glucose diffusion and it showed the highest activity at a concentration of 1500 µg/ml. Glucose diffusion is useful *in vitro* index to predict the effect of plant fibers on the delay in glucose absorption in the gastrointestinal tract. In addition to glucose adsorption, the retardation in glucose diffusion might be attributed to the physical obstacle presented by fiber particles toward glucose molecules and entrapment of glucose within the network formed by fibers. On comparing the doses of 200 µg/kg with 400 µg/kg, there was a significant difference in BW ($p < 0.05$) for a dose of 200 µg/kg. The dose-dependent antidiabetic property of the ethanolic extract of *P. grandiflora* Hook exhibited improvement in BW. In the present Glucose diffusion study, at 5 h, the blood glucose level was increased to a peak in STZ-induced diabetic rats when compared to normal control rats. The study also indicated that the ethanolic extracts of treated groups produced a fall in the blood glucose level at 5 h when compared to diabetic rats. The extracts might be enhancing glucose utilization and glucose metabolism. In the present study, the STZ-induced diabetic rats (Group 2) showed a significant rise in blood glucose to a level when compared to normal control rats (Group 1). On the contrary, diabetic rats treated with ethanolic extracts of and standard drug glibenclamide for 21 days, exhibited a decrease in blood glucose level. It was observed that ethanolic extracts reversed

Table 1: Glucose diffusion assay

Concentration (µg/ml)	Glucose concentration (mg/ml)					
	0 h	1 st h	2 nd h	3 rd h	4 th h	5 th h
100	2.48±0.08	33.69±1.44	44.44±1.69	43.54±0.49	47.37±1.13	66.83±2.23
250	2.79±0.02	32.02±1.75	41.9±2.52	45.07±1.26	45.96±2.07	54.76±0.81
500	2.41±0.01	27.93±1.34	38.2±1.03	42.1±1.12	443.2±2.81	50.63±1.52
1000	2.54±0.04	25.63±0.93	30.7±0.70	34.9±0.91	39.37±0.61	44.83±2.21
1500	2.43±0.17	23.63±0.82	25.63±0.52	27.1±1.39	34.10±4.06	33.37±1.44
Control	3.16±0.34	35.37±1.15	47.3±0.4	53.57±0.49	58.87±1.59	73.57±2.16

Values are expressed as mean±SD (n=6). Values are statistically significant at $p < 0.05$ using one way ANOVA followed by Dunnett's test

Table 2: Effect of ethanolic extract of *P. grandiflora* Hook on body weight in STZ-induced diabetic rats

Group	Treatment	Average body weight in days (g)			
		1 st day	7 th day	14 th day	21 st day
1.	Normal control	147.90±2.90	149.63±2.30	148.5±2.40	147.85±2.9
2.	Diabetic control STZ (50 mg/kg)	150.65±1.00	144.21±2.9	138.05±2.80	131.3±2.00
3.	Glibenclamide (5 mg/kg)	152.06±0.00	147.90±2.30	144.18±1.20	139.05±1.07
4.	<i>P. grandiflora</i> (200 mg/kg)	151.70±1.49	144.50±1.32	141.75±1.90	136.5±3.15
5.	<i>P. grandiflora</i> (400 mg/kg)	150.42±2.14	149.95±1.90	149.45±1.80	148.8±0.90

Values are expressed as mean±SD (n=6). Values are statistically significant at $p < 0.05$ using one way ANOVA followed by Dunnett's test.

P. grandiflora: *Portulaca grandiflora*, STZ: Streptozotocin

Table 3: Effect of ethanolic extract of *P. grandiflora* Hook on WBG in STZ-induced diabetic rats

Group	Treatment	WBG (mg/dL)			
		1 st day	7 th day	14 th day	21 st day
1.	Normal control	79.3±3.02	75.07±0.67	80.05±0.80	92.32±0.44
2.	Diabetic control STZ (50 mg/kg)	395.5±1.46	411.05±1.52	419.02±1.41	423.3±1.30
3.	Glibenclamide (5 mg/kg)	346.62±2.04	275.97±1.42	214.58±0.69	188.1±1.19
4.	<i>P. grandiflora</i> (200 mg/kg)	355.22±7.53	281.81±1.01	218.48±1.44	190.93±1.06
5.	<i>P. grandiflora</i> (400 mg/kg)	411.4±4.97	383.72±1.15	252.03±1.52	185.23±9.58

Values are expressed as mean±SD (n=6). Values are statistically significant at p<0.05 using one way ANOVA followed by Dunnett's test.

P. grandiflora: *Portulaca grandiflora*, STZ: Streptozotocin, WBG: Whole blood glucose

these effects in diabetic animals. These results imply that the ethanolic extract of *P. grandiflora* Hook can reduce the complications of BW and associated cardiovascular risk factors during diabetes.

CONCLUSION

Estimation of glucose was performed for the diagnosis and fall up of diabetes mellitus. In a normal healthy individual, the fasting blood glucose level is between 70 and 100 mg/dL. This level may rise to 500 mg/dL or more in diabetic person which is referred to as hyperglycemia and it mainly occurs due to deficiency of insulin. The continuous treatment for 21 days with the ethanolic extract showed a significant reduction in the blood glucose levels. This plant was found to decrease the level of glucose significantly (p<0.05) in STZ-induced diabetic rats. The lower dose of *P. grandiflora* Hook itself exhibits its activity and the effect was observed to be dose-dependent. The data from the above preliminary phytochemical studies suggest that *P. grandiflora* Hook aerial part has beneficial effects in diabetes mellitus holding the hope of a new generation for anti-hyperglycemic drugs. This activity is useful in further experimental analysis in the future.

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AUTHORS' CONTRIBUTIONS

All the contributors were involved in the concept, laboratory investigations, laboratory report interpretation, examination, evaluation, drafting the final report, and publication. Ms. Logeshwari. B has compiled the data and summarized all the information, Mrs. Devi. M has supervised the manuscript, and Mrs. Komal. S has been involved in drafting the manuscript or revising it critically for important intellectual content.

DECLARATION OF CONFLICTS OF INTEREST

The authors state that they have no conflicts of interest.

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