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Research

In Vitro and *In Vivo* Evaluation of anti-diabetic Potential of Pisonia grandis

A. Kavidha*[1] and G. Nagaraja Perumal[2]

[1]*Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan, India*
[*2]*J.D.T Islam College of Pharmacy, Kerala, India*

*Author for Correspondence: A. Kavidha
Email: kavidhaarumugam369@gmail.com

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Abstract

The general interest in plants stems from their fundamental role in the commencement of life and their ability to produce various chemicals that act on the body, aiding in the treatment of diseases. The extraction of leaves from Pisonia grandis was conducted using various solvents with the Soxhlet apparatus. All extracts showed positive results in both in vitro and in vivo pharmacological studies. Our research findings indicate that for 50% inhibition of alpha-amylase activity, the concentrations were as follows: Ethyl acetate extract at 91 µg/ml, water extract at 80 µg/ml, petroleum ether extract at 75 µg/ml, and Acarbose (positive control) at 61 µg/ml. Our final report shows that the ethanolic extract has the highest anti-diabetic activity compared to the other extracts due to the presence of more anti-diabetic bioactive components. We conclude that this work will be useful in discovering novel entities for the treatment of diabetes mellitus.

Keywords: diseases, *Pisonia grandis,* Soxhlet apparatus, alfa amylase anti-diabetic activity, discovering

INTRODUCTION

In contemporary drug discovery and development, natural products play a crucial role in the early stages of lead discovery. This involves identifying active natural molecules through various bioassays, which could themselves or through their initial analogues be ideal drug candidates. Herbs and herb-based treatments have been used by various societies worldwide for the treatment of numerous diseases for a long time. Over the years, a variety of medicinal plants have become popular for treating various human and animal diseases. The toxicity profile of modern medicine, which can lead to severe pathological conditions, demands great clinical attention. Recently, researchers have been isolating active phytoconstituents from therapeutically promising plants, as these active constituents effectively target specific sites and are considered equipotent to synthetic drugs.

Lifestyle modification is the most practical intervention for preventing diabetes in high-risk groups in India. However, controlling diabetes with diet, weight control, and physical activity has been challenging and often insufficient for most patients. The continuous increase in the incidence of type 2 diabetes has significant economic implications. Patients with type 1 diabetes produce no insulin and must receive insulin by injection. Type 2 diabetics receive injections if their disease cannot be controlled by diet, exercise, and oral medication.

Insulin is produced by beta cells of the pancreas. Human insulin is a polypeptide with a molecular weight of around 6000 Da, consisting of two amino acid chains, A and B, linked by two disulfide (-S-S-) bonds. Glucose enters the cell via the GLUT-2 transporter. Inside the cell, glucose metabolism generates ATP, causing the ATP-sensitive K+ channel to close. This closure leads to cell membrane depolarization, allowing calcium ions to enter the cell via another calcium channel. Increased intracellular calcium activates calcium-dependent phospholipid protein kinase. Proper body functions depend on precise control of blood glucose concentration. The normal fasting level of glucose in the blood is 70-90 mg/100 ml. Hyperglycemia occurs if blood glucose concentration is too high (over 120 mg/100 ml), while hypoglycemia occurs if blood glucose concentration is too low (under 70 mg/100 ml). Hypoglycemia is characterized by general weakness, trembling, drowsiness, headache, profuse sweating, rapid heartbeat, and possible loss of consciousness.

Oral hypoglycemic agents are useful in treating patients with Type 2 diabetes who cannot be managed by diet alone. Patients who develop diabetes after age 40 and have had diabetes for less than five years are most likely to respond well to these agents. Patients with long-standing disease may require a combination of hypoglycemic drugs with or without insulin to control their hyperglycemia. Insulin is added due to the progressive decline in β cells that occurs because of the disease or ageing. Oral hypoglycemic agents should not be given to patients with Type 1 diabetes.

MATERIALS AND METHODS

Alpha-Amylase Inhibition Assay

The alpha-amylase assay was performed using the following method: Briefly, the plant extract at different concentrations (50 µg/ml – 200 µg/ml) (diluted in a phosphate buffer) was added to a porcine pancreatic enzyme solution in a 96-well plate. After 10 minutes of incubation at 37°C, the reaction was initiated by adding 20 µl of starch solution and further incubated for 30 minutes at 37°C. The reaction was then stopped by adding 10 µl of 1M HCl to each well, followed by 75 µl of iodine reagent. A blank containing phosphate buffer (pH 6.9) instead of the extract and positive control (acarbose, 64 µg/ml) was prepared. No enzyme control and no starch control were included for each test. The absorbance was measured at 580 nm and the percentage inhibitory activity was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(1 - \text{Absorbance of the untreated (Control)} \times 100}{\text{Absorbance of the test well)}}$$

In vivo* anti-diabetic activity of *Pisonia grandis

Wistar albino rats (150-200 grams) of both sexes were procured from the College of Veterinary and Animal Science, Mannuthy, Thrissur-Kerala, India. Before the experiment, the rats were housed in clean polypropylene cages (6 rats per cage) for a period of 7 days under standard conditions: temperature (25-30°C), relative humidity (45-55%), and a 12/12 hour dark/light cycle.

Chemicals

Streptozotocin from Loba Chemie, standard Glibenclamide (Daonil) from Aventis Pharma, ethanol (analytical grade), 5% dextrose solution, and Glucose Estimation Kit from Gluco Dr Super Sensor.

Induction of Diabetes in Animals

A single dose (100 mg/kg b.w., i.p.) of streptozotocin dissolved in sodium citrate buffer was used to induce diabetes in rats after overnight fasting. One hour after streptozotocin administration, the animals were given feed ad libitum and a 5% dextrose solution was provided in feeding bottles for one day to counter early hypoglycemic stages. The animals were stabilized for a week, and those with blood glucose levels exceeding 200 mg/dl were selected for the study.

Experimental Design

Eleven groups of rats, with six rats in each group, received the following treatment schedule for 14 days:

- **Group I:** Normal control (normal saline 10 ml/kg, p.o.)
- **Group II:** Streptozotocin-treated control (100 mg/kg, i.p.)
- **Group III:** Streptozotocin (100 mg/kg, i.p.) + Standard drug Glibenclamide (5 mg/kg, p.o.)
- **Group IV:** Streptozotocin (100 mg/kg, i.p.) + Pisonia grandis (PG) Pet. Ether extract (200 mg/kg, p.o.)
- **Group V:** Streptozotocin (100 mg/kg, i.p.) + PG Pet. Ether extract (400 mg/kg, p.o.)
- **Group VI:** Streptozotocin (100 mg/kg, i.p.) + PG Ethyl acetate extract (400 mg/kg, p.o.)
- **Group VII:** Streptozotocin (100 mg/kg, i.p.) + PG Ethyl acetate extract (200 mg/kg, p.o.)
- **Group VIII:** Streptozotocin (100 mg/kg, i.p.) + PG Ethanolic extract (400 mg/kg, p.o.)
- **Group IX:** Streptotozocin (100 mg/kg, i.p.) + PG Ethanolic extract. (200 mg/kg, p.o.)

- **Group X:** Streptotozocin (100 mg/kg, i.p.) + PG Water extract (400 mg/kg, p.o.)
- **Group XI:** Streptotozocin (100 mg/kg, i.p.) + PG Water extract (200 mg/kg, p.o.)

Administration of Treatments

Plant leaf extract, standard drug, and normal saline were administered using an oral feeding needle. The standard drug, Glibenclamide (5 mg/kg, p.o.), and the extracts were given for 14 consecutive days.

Collection of Blood Samples

Fasting blood samples were drawn from the retro-orbital puncture of rats at weekly intervals, specifically on days 1, 7, 14, and 21.

Estimation of Biochemical Parameters: Serum Blood Glucose

On days 1, 7, 14, and 21, fasting blood samples were collected. The serum was separated and analyzed for glucose levels.

Serum Blood Glucose

The serum blood glucose test measures the amount of glucose in the blood sample obtained from the animals. This test is typically performed to check for elevated blood glucose levels, which can indicate diabetes or insulin resistance.

RESULTS AND DISCUSSION

In vitro antidiabetic activity

The results revealed that the 50% inhibition concentrations were as follows: Ethyl acetate extract at 91 µg/ml, water extract at 80 µg/ml, petroleum ether extract at 75 µg/ml, and Acarbose (positive control) at 61 µg/ml. All groups demonstrated alpha-amylase inhibition properties. However, the minimum percentage inhibition was observed in the ethyl acetate extract, resembling the percentage inhibition of the positive control. Therefore, the ethanolic extract of PG contains active constituents with anti-diabetic properties.

Table 1: α-Amylase Inhibition concentrations

Concentration (µg/ml)	Percentage Inhibition (%)				
	Ethyl acetate Extract	Water Extract F1	Pet. ether F2	Ethanolic Extract F3	F4	Acarbose (Positive control)
0	0	0	0	0	0
25	29	34	30	26	25
50	33	41	36	40	45
75	42	48	50	43	53
100	53	56	56	52	56
125	56	60	61	66	65

***In vivo* anti-diabetic activity**

A significant decrease in the body weight of diabetic control rats was observed compared to normal standard values. Diabetic rats treated with the extract did not show any significant changes in body weight compared to the diabetic control group. Diabetic rats treated with Pisonia grandis petroleum ether extract and ethyl acetate extract showed mild changes in body weight compared to the diabetic control group.

Blood glucose levels significantly increased in diabetic rats. In efficacy studies, a slight decrease in blood glucose levels was observed in diabetic rats treated with Pisonia grandis petroleum ether and ethyl acetate extracts compared to diabetic control rats. A significant decrease in blood glucose levels was observed in diabetic rats treated with petroleum ether extract compared to the diabetic control group. Blood glucose levels were also found to be significantly decreased in diabetic rats treated with ethyl acetate, with levels approximately equal to those in the Glibenclamide (standard) treated group.

These results demonstrate that petroleum ether and ethyl acetate extracts provide efficacious oral therapy with reduced dose and dosing frequency, improving patient compliance for the management of diabetes. A significant decrease in body weight was observed in diabetic control rats compared to the normal control group. Diabetic rats treated with the pure compound did not show any significant changes in body weight compared to the diabetic control group. Blood glucose levels significantly increased in diabetic rats. Efficacy studies observed a slight decrease in blood glucose levels in diabetic rats treated with the pure compound and water extract compared

to diabetic control rats. A significant decrease in blood glucose levels was observed in diabetic rats treated with ethanolic extract compared to the diabetic and standard drug-treated groups.

These results demonstrate that Pisonia grandis extracts offer efficacious oral therapy with reduced dose incidence for the management of diabetes and hence improve patient compliance. Streptozotocin-induced diabetes mellitus, oral glucose tolerance, and hypoglycemic studies were performed, and the obtained data is tabulated below. Our final report indicates that the ethanolic extract has more anti-diabetic activity compared to other extracts due to the presence of more anti-diabetic bioactive components.

Table 2: Oral glucose tolerance test at different time intervals

Treatment	Dose mg/kg	Blood Glucose Level (mg/dl) in hr						
		0	0.5	1	1.5	2	2.5	3
Control CMC)	0.5 %	68.67±0.2	140.8±0.5	169.5±0.3	165.2±0.4	156.5±0.3	148.7±0.3	135.8±0.3
Glibenclamide	0.2	68.33±0.3	102.7±0.3	104.8±0.3	99.83±0.4	95.83±0.4	91.67±0.4	83.67±0.3
EA	400	68.83±0.3	109.0±0.	119.3±0.4	113.0±0.3	103.7±0.6	99.83±0.4	94.67±0.3
EA	200	68.67±0.2	121.3±0.	129.5±0.2	127.3±0.5	119.8±0.3	113.7±0.4	106.3±0.3
Pet. E	400	68.33±0.3	103.5±0.3	109.3±0.3	105.7±0.6	99.33±0.4	93.67±0.5	86.33±0.3
Pet. E	200	68.17±0.3	117.0±0.2	112.2±0.3	108.5±0.2	103.3±0.2	99.83±0.4	96.50±0.3
ET	400	68.00±0.3	99.3±0.3	98.17±0.4	95.33±0.4	92.17±0.4	86.83±0.5	81.50±0.4
ET	200	68.17±0.3	100.0±0.1	115 ±0.3	108.5±0.2	103.3±0.2	99.83±0.4	96.50±0.3
Water	400	68.67±0.2	115.8±0.5	139.5±0.34	135.2±0.4	136.5±0.3	128.7±0.3	105.8±0.3
Water	200	68.67±0.2	119.8±0.5	149.5±0.3	145.2±0.4	156.5±0.3	138.7±0.3	115.8±0.3

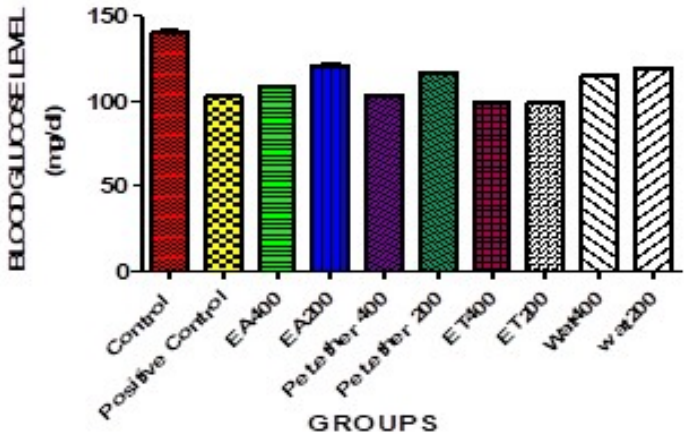

Fig 1: Oral Glucose Tolerance Test at 0.5 hour

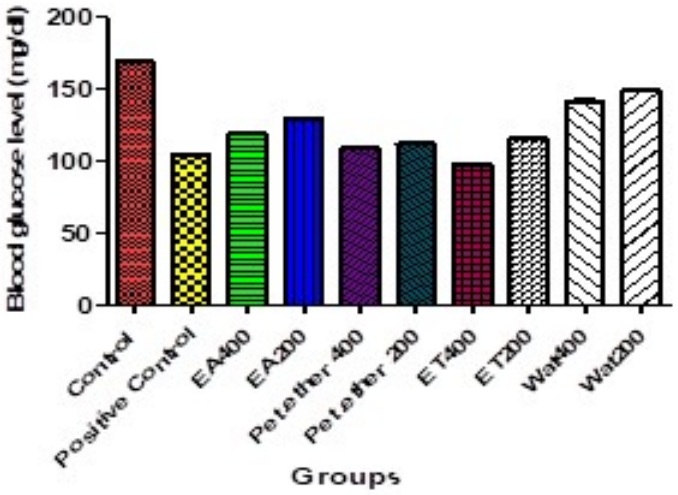

Fig 2: Oral Glucose Tolerance Test at 1 hour

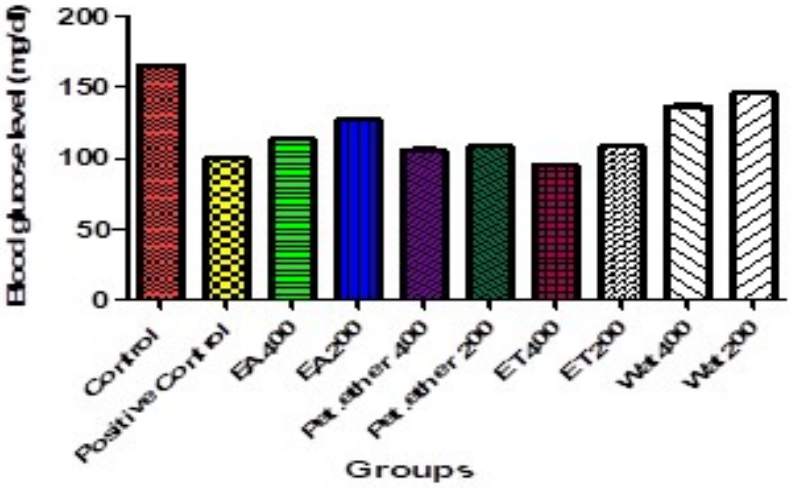

Fig 3: Oral Glucose Tolerance Test at 1.5 hour

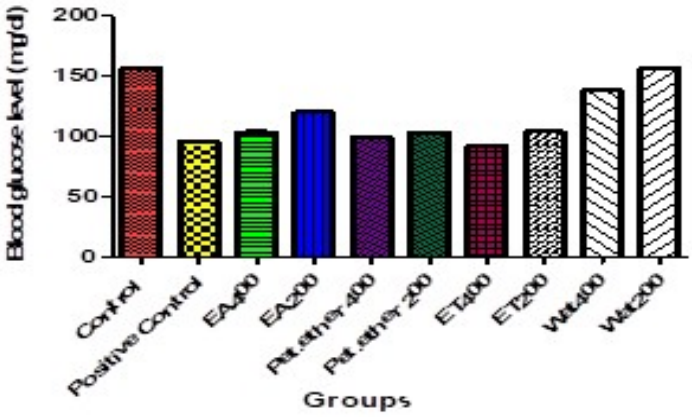

Fig 4: Oral Glucose Tolerance Test at 2 hour

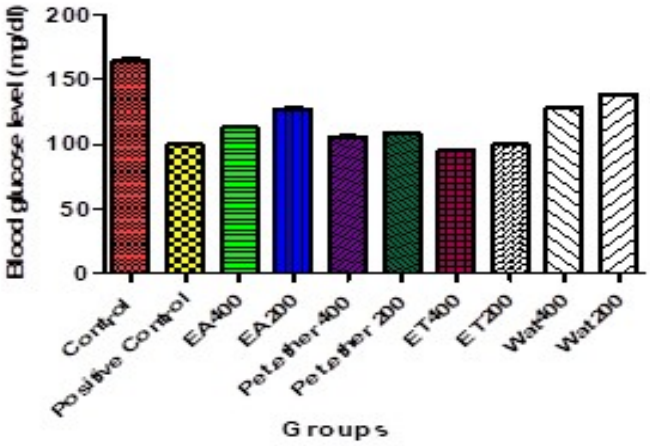

Fig 5: Oral Glucose Tolerance Test at 2.5 hour

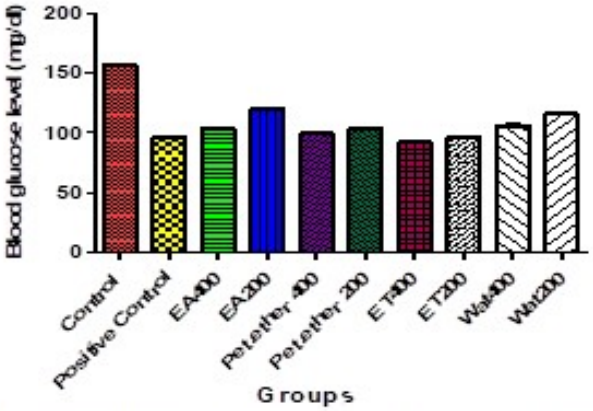

Fig 6: Oral Glucose Tolerance Test at 3.0 hour

Table 3: Streptozotocin Induced Diabetes Mellitus

Treatment	Dose mg/kg	Blood glucose level (mg/dl) day				
		0	3	7	14	21
Control CMC)	0.5 %	79.83±0.307	300.3±0.333	282.5±0.562	268.2±0.477	298.0±0.365
Glibenclamide	0.2	72.33±0.210	270.5±0.223	127.0±0.365	114.2±0.307	118.0±0.258

EA	400	83.17±0.401	290.5±0.341	142.3±0.954	128.7±0.421	121.0±0.258
EA	200	86.17±0.477	294.5±0.223	233.0±0.683	172.2±0.600	198.7±0.421
Pet. E	400	76.50±0.428	272.5±0.562	131.2±0.477	119.3±0.494	112.2±0.703
Pet. E	200	82.83±0.307	286.3±0.210	186.2±0.401	159.0±0.683	179.5±0.341
ET	400	69.50±0.428	263.5±0.341	126.0±0.307	116.3±0.333	114.2±1.014
ET	200	67.50±0.328	267.5±0.241	226.0±0.407	216.3±0.433	194.2±1.024
Water	400	86.17±0.477	284.5±0.23	134.0±0.685	174.3±0.62	188.7±0.422
wat	200	76.50±0.428	292.5±0.563	231.2±0.477	211.3±0.495	212.2±0.703

Table 4: Hypoglycemic Test

Treatment	Dose mg/kg	Blood glucose level (mg/dl)		
		0 hr	0.5 hr	1 hr
Control CMC)	0.5 %	68.50±0.223	68.33±0.333	72.33±0.557
Glibenclamide	0.2	68.33±0.333	50.33±0.557	27.50±0.500
EA	400	68.17±0.307	60.17±0.4014	44.00±0.516
EA	200	68.33±0.421	68.17±0.401	55.50±0.428
Pet. E	400	68.00±0.258	54.00±0.365	33.83±0.654
Pet. E	200	67.83±0.307	62.83±0.401	44.50±0.500
ET	400	67.50±0.428	49.00±0.577	26.17±0.703
ET	200	68.01±0.259	54.04±0.365	33.85±0.655
Water	400	68.50±0.225	54.33±0.333	42.33±0.557
wat	200	68.17±0.308	63.17±0.4014	54.01±0.516

Standard error (n= each group consist of 6 animals)(p<0.05), (p<0.001)**&*

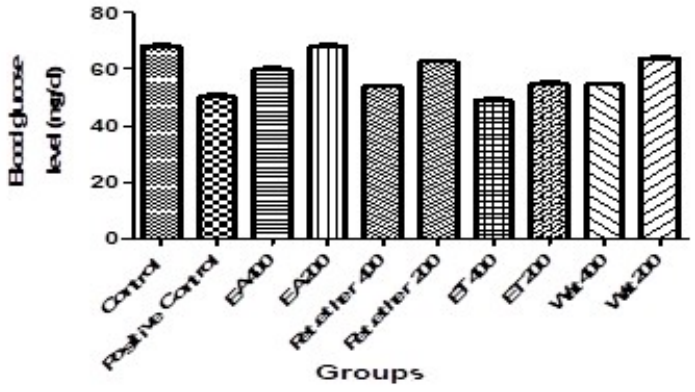

Fig 7: Hypoglcemic effect 30 minutes

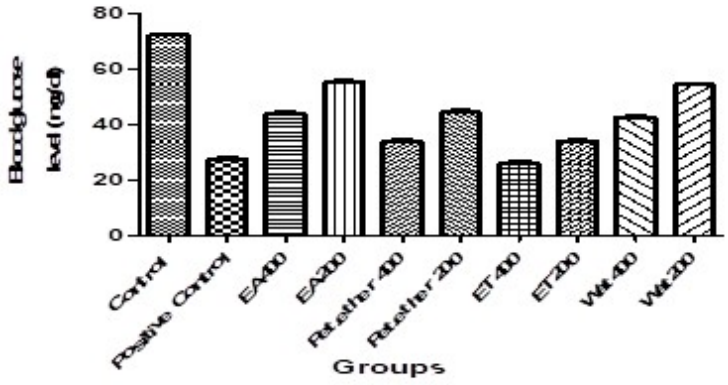

Fig 8: Hypoglcemic effect 1 hour

SUMMARY AND CONCLUSION

The in vitro assays demonstrated that the inhibition of alpha-amylase might be one of the mechanisms by which the extract of this plant controls and prevents postprandial hyperglycemia. This work indicates that Pisonia grandis has a significant long-term antidiabetic effect that can be well-established for treating diabetes.
In conclusion, the aqueous leaf extract of Pisonia grandis has beneficial effects in reducing elevated blood glucose levels and improving the lipid profile of STZ-induced diabetic rats, with no effect on normal rats. This justifies the claims made by Ayurvedic texts. Therefore, the chemical constituents of the plant extract might help in preventing diabetic complications and may serve as an alternative to current antidiabetic drugs. Further studies to substantiate the use of the plant as an antidiabetic treatment are recommended.

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