

# Investigation of *Pajanelia longifolia* Leaf Extract for Antidiabetic Activity

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## ABSTRACT

Diabetes mellitus (DM) is a medical condition characterized by heightened levels of blood sugar, stemming from either inadequate production of insulin or an impaired response to insulin at the cellular level, whether due to a relative or absolute deficiency of the hormone. Traditional herbal remedies have a significant role in the treatment of DM. *Pajanelia longifolia*, known by the general population as Pajanelia or Tender wild jack has been a component of traditional herbal medicine in India and other Asian countries for the treatment of various ailments. The current research assessed the antidiabetic activity of *Pajanelia longifolia* leaf extract in streptozotocin (35 mg/kg, oral route) induced diabetic rats. The study utilized an *in vivo* streptozotocin induced diabetic model and an *in vitro* rat hemidiaphragm model for assessment of antidiabetic activity. Acute toxicity studies conducted on liposomes of *Pajanelia longifolia* leaf extract (2000 mg/kg body weight) proved the drug to be non-toxic. The effects of various doses (100, 200 and 400 mg/kg p.o.) of extract were assessed. Glimepiride (2 mg/kg) and insulin (0.25 IU/ml) were used as the standard drugs for *in vivo* and *in vitro* studies, respectively. The decrease in blood glucose levels and the elevation of muscle and liver glycogen content in diabetic rats induced by streptozotocin and the increased glucose uptake and glycogen content observed in *in vitro* rat hemidiaphragm technique proved that *Pajanelia longifolia* leaf extract has significant antidiabetic activity.

**Keywords:** *Pajanelia longifolia*, Antidiabetic, Streptozotocin, Glimepiride, Rat hemidiaphragm.

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## INTRODUCTION

Diabetic mellitus (DM) is a medical condition distinguished by irregularities in carbohydrate, protein, and lipid metabolism. DM is primarily attributed to a deficiency of insulin, often accompanied by insulin resistance.<sup>1</sup> This condition affects individuals globally, leading to diverse complications due to the metabolic disturbance caused by diabetes.<sup>2</sup> DM is categorically classified based on its aetiology and clinical manifestations into insulin-dependent diabetes mellitus (IDDM, Type I, T1DM), non-insulin-dependent diabetes mellitus (NIDDM, Type II, T2DM), and gestational diabetes mellitus. T1DM is an autoimmune disorder where the pancreas is unable to produce insulin.<sup>3</sup> Type 2 diabetes mellitus is the most common chronic metabolic disorder caused due to both ambient and genetic issues. Various pathogenic factors contribute to distinct characteristics that influence increased levels of plasma glucose in type 2 DM. These features encompass insulin resistance and disrupted insulin production resulting from impaired  $\beta$ -cell function.<sup>4</sup> Parenteral insulin and oral antidiabetic medications are used to treat diabetes mellitus. Currently available oral hypoglycaemic agents have major side effects, hence a novel antidiabetic agent with high therapeutic effectiveness and minimal side effects is required.<sup>5</sup> Haematological coma, liver and renal function changes, and other major adverse effects are caused by synthetic hypoglycaemic drugs currently used to treat DM.

Additionally, they should not be used during pregnancy. Therefore, therapeutic plants with fewer adverse effects are increasingly being prioritised globally. The World Health Organization (WHO) advises using medicinal herbs to treat DM.<sup>6</sup> The chemistry of the human body is frequently strange to synthetic medications, which stand aside from the precise engineering of nature. Synthetic medications frequently function in the body as poisons and irritants, disturbing the harmony of various systems and resulting in dangerous side effects. In contrast, a wise attitude to one's well-being is the routine and responsible use of herbs to safeguard and promote health.<sup>7</sup> The world's oldest consistently used healthcare items by human beings are medicines made from plants. WHO estimates that 80% of the global population continues to predominantly rely on herbal medicines for their therapeutic needs.<sup>8</sup> The relevance of herbal medicines is contingent upon their production aligning with modern scientific standards and undergoing rigorous testing based on criteria that ensure quality, safety, and efficacy. Only through such processes can herbal medicines be appropriately compared with modern counterparts, instilling the necessary confidence among prescribing doctors. *Pajanelia longifolia* a deciduous or evergreen tree that belongs to the family Bignoniaceae has been documented as one of the ancient medicinal plants in Charaka Samhita. It has been traditionally employed to address various health conditions, including stomach

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Table 1: Preliminary phytochemical analysis

Tests	Phytoconstituent (+ Present, - Absent)
Alkaloids	
Dragendorff's test	+
Hager's test	+
Wagner's test	+
Mayer's test	+
Carbohydrates	
Molisch's test	+
Benedict's test	+
Fehling's test	+
Flavonoids	
Shinoda's test	+
Phenols	
Ferric chloride test	+
Proteins	
Biuret test	+
Milton's test	+
Saponins	-
Starch	-
Steroids	
Liebermann-Burchard's test	+
Salkowski's test	+
Tannins	+
Triterpenoids	
Liebermann-Burchard's test	-

Table 2: Effect of *Pajanelia longifolia* leaf extracts on body weight in STZ induced diabetic rats.

Groups	Body weight (gms)		% change in body weight
	Before Treatment	After Treatment	
I	180.5±3.334	232.5±7.042	-22.3±5.188
II	194.3±4.958	178.0±3.967	9.15±4.4625
III	175.2±3.400	195.7±3.353	-10.47±3.376
IV	197.7±4.731	214.2±4.362	-7.703±4.546
V	219.0±12.88	237.0±11.61	-7.594±12.245
VI	191.0±5.151	206±5.608	-7.506±5.379

Values are presented as mean± standard error of the mean

disorders, wound healing, arthritis, obesity, urinary disorders, neutralizing the local effects of hemotoxic venoms, jaundice, liver ulcers and skin diseases like eczema.<sup>9,10</sup> This study aimed to explore the antidiabetic properties of the leaf extract of *Pajanelia longifolia* leaf extract in streptozotocin induced diabetic rats.

## METHODOLOGY

### Collection of botanical specimens and extraction process

The leaves of *Pajanelia longifolia* were collected and authenticated by a botanist. The leaves were dried in the shade to render them free from moisture. The dried leaves were powdered and alcoholic extract was obtained by maceration. The obtained extract was concentrated, evaporated under reduced pressure and controlled

temperature, and subsequently stored in a desiccator until further use.

$$\% \text{ yield} = \frac{\text{Amount of product obtained (gm)}}{\text{Total amount of powder used (gm)}} \times 100$$

### Phytochemical analysis for qualitative assessment

Qualitative phytochemical analysis of alcoholic leaf extract of *Pajanelia longifolia* was conducted to identify its active constituents. The tests to detect the presence of alkaloids, carbohydrates, starch, flavonoids, steroids, proteins, saponins, tannins, triterpenoids and phenols were carried out by standard methods.<sup>11,12</sup>

### Selection of animals

Albino Wistar rats of both sexes, aged 4-6 weeks and weighing r between 180-200 g were sourced from the Central Animal House at NUCARE, NGSM Institute of Pharmaceutical Sciences, NITTE (DU), Mangaluru for the study. The rats were appropriately grouped and then sheltered in distinct cages. The cages were kept under standard lab conditions at a temperature of 25 ± 2°C with appropriate dark and light cycle of 12 hours. Free access to standard food and water ad libitum was permitted for the animals. Investigations were done in accordance with the guidelines of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), New Delhi, India. Research work was permitted and approved by the Institutional Animal Ethics Committee (NGSMIPS/IAEC/AUG-2022/318).

### Toxicity studies

#### Acute oral toxicity study

Adult female albino rats with a weight range of approximately 150-200 g were used in acute oral toxicity studies following the OECD 425 guidelines. Prior to dosing, animals were subjected to fasting while having access to food ad libitum. The body weight of each animal after fasting body weight was measured and recorded before determining the dosage based on its body weight.

#### Limit test conducted at a dose of 2000 mg/kg body weight

The test drug was orally administered to one animal at a dose of 2000 mg/kg body weight. Subsequently, four more animals were sequentially dosed, bringing the total number of animals tested to five. The animals were initially monitored within the initial 30 minutes post-dosing, with subsequent regular intervals observed throughout the initial 24 hours with a specific focus on the first 4 hours. This was followed by daily observations for a duration of 14 days. Following the limit test, the maximum tolerated dose was determined, and 1/10th of that dose was selected and designated as X, representing a medium dose level. The low dose was set as half of X, while the high dose was established as double of X.<sup>13-15</sup>

### Pharmacological evaluation

The antidiabetic activity was evaluated on experimental animals by following models:

(1) Streptozotocin induced DM

(2) Isolated diaphragm of rat

#### Streptozotocin induced DM<sup>16,17</sup>

The animals were segregated into six groups, with each group comprising six animals weighing between 180-250g. DM was induced in animals that had been fasted overnight

Table 3: Effect of *Pajanelia longifolia* leaf extract on blood glucose level

Groups	Dose (mg/kg)	Blood Glucose Level (mg/dl)	
		Day 0	Day 28
I	-	111.5±8.257*	112.2±6.052**
II	-	203.3±10.58	312.2±7.181
III	2	246.8±8.803	105.5±3.914**
IV	100	236.7±6.048	150.5±2.335**
V	200	262.5±17.86	104.3±5.270**
VI	400	244.7±33.62	92.00±4.320**

Values are presented as mean± standard error of the mean

Indicates significance when compared to the diabetic group,  $p < 0.05$  is considered significant.

\*\* Indicates significance when compared to the diabetic group,  $p < 0.01$  is considered significant.

through a single intraperitoneal injection of freshly prepared streptozotocin. The injection consisted of a dose of 35 mg/kg body weight in a 0.1M cold citrate buffer with a pH of 4.5. To counteract drug-induced hypoglycaemia, the animals were given a 5% glucose solution to drink. Animals in the control group received a placebo injection of citrate buffer. 1st group was assigned as control which received a suspension of 1ml carboxymethyl cellulose p.o., the 2nd group was taken as diabetic control, 3rd group of diabetic animals was treated with glimepiride 2mg/kg/day. The experimental groups 4th, 5th and 6th groups received the test drug at doses of 100mg, 200mg and 400mg/kg respectively for 28 days. Blood was withdrawn from the retroorbital sinus using capillaries.

#### Determination of blood glucose level by (GOD-POD) method

Serum plasma glucose level was determined by the GOD/POD method using the reagent kit. 10µl of serum, standard and distilled water (blank) with 1000µl of the enzyme was added into three Eppendorf tubes and mixed respectively. Tubes were kept at 37°C for 10 mins. The colour obtained from serum and standard was read at 505 nm against reagent blank.<sup>18</sup>

#### Estimation of glycogen content

Minced tissue was extracted by boiling in 3ml of 30% KOH for 20-30 mins or until the tissue was dissolved. Glycogen

Table 4: Effect of leaf extract of *Pajanelia longifolia* on glycogen content in tissue

Groups	Dose (mg/kg)	Liver Glycogen	Muscle Glycogen
		(mg/g)	(mg/g)
I	-	43.93±1.264**	6.933±0.3232**
II	-	9.900±0.6066	2.000±0.2366
III	2	41.55±1.180**	6.300±0.2517**
IV	100	22.87±1.185**	2.783±0.2386
V	200	24.00±0.884**	2.717±0.2315
VI	400	35.22±1.173**	2.817±0.1956

Values are presented as mean± standard error of the mean from six rats in each group.

Indicates significance when compared to the diabetic group,  $p < 0.05$  is considered significant.

\*\* Indicates significance when compared to the diabetic group,  $p < 0.01$  is considered significant.

was precipitated by adding 0.5ml of saturated Na<sub>2</sub>SO<sub>4</sub> and 3.5ml of ethanol, heating again until the mixture started boiling. The mixture after cooling was centrifuged at 3000rpm and the solution was discarded. The sediment was dissolved in 2 ml of water and reprecipitated with 2.5 ml of 95% ethanol. The collected sediment was hydrolyzed for 2 to 2.5 hrs in 6ml of 0.6M HCl and H<sub>2</sub>SO<sub>4</sub> for 30 mins in 2ml of 5M acid, in a boiling water bath. After cooling the hydrolysate, a single drop of phenol red indicator was introduced, and the mixture was cautiously neutralized with NaOH until the indicator transitioned from pink to orange and eventually to a yellow colour. Subsequently, the solution was diluted to a volume of 5 ml with distilled water, and the glucose content was determined using the glucose oxidase method.

#### Isolated diaphragm of rat

Albino male Wistar rats weighing 100-150gms that had been fasted overnight were decapitated and the diaphragm was quickly removed to avoid damage and separated into 10 halves. In a small conical flask with 2ml Tyrode solution and 2% glucose, the hemi-diaphragm was rinsed in cold Tyrode solution to dissolve any blood clots.

#### For glucose uptake

The hemi-diaphragms were incubated for 30 mins at 37°C in an incubator. A total of eight experiments were carried

Table 5: Effect of *Pajanelia longifolia* leaf extract on glucose uptake by isolated rat hemidiaphragm

Groups	Groups	Glucose uptake (mg/g)
I	Glucose	4.892±0.3793**
II	Glucose+ insulin	9.810±0.1010**
III	Glucose+100 mg/kg extract	9.628±0.1732**
IV	Glucose + 200 mg/kg extract	11.10±0.2449**
V	Glucose +400 mg/kg extract	10.96±0.5067**
VI	Glucose + insulin +100 mg/kg extract + standard	10.28±0.076**
VII	Glucose + insulin + 200 mg/kg extract + standard	11.45±0.1228**
VIII	Glucose +I nsulin + 400 mg/kg extract + standard	10.73±0.5232**

Values are presented as mean± standard error of the mean.

Indicates significance when compared to the diabetic group,  $p < 0.05$  is considered significant.

\*\* Indicates significance when compared to the diabetic group,  $p < 0.01$  is considered significant.

out. The hemidiaphragms were exposed to the following conditions:

Tyrode solution with 2% glucose only - control

Tyrode solution with 2% glucose + insulin (0.25IU/ml)

Tyrode solution with 2% glucose + leaf extract (100mg/ml)

Tyrode solution with 2% glucose + leaf extract (200mg/ml)

Tyrode solution with 2% glucose+ leaf extract (400mg/ml)

Tyrode solution with 2% glucose + insulin + leaf extract (100mg/ml)

Tyrode solution with 2% glucose + insulin + leaf extract (200mg/ml)

Tyrode solution with 2% glucose + insulin + leaf extract (400mg/ml)

The hemidiaphragm was removed, and the incubated medium's glucose content was determined using the glucose-peroxidase (GOD/POD) process. The glucose uptake was determined by calculating the variance between the initial and final glucose content in the incubation medium.

#### Statistical analysis

The acquired data obtained was analyzed through a one-way analysis of variance (ANOVA), followed by post hoc Scheffe's test using SPSS software version 10. A *p*-value below 0.05 was considered indicative of statistical significance.

## RESULTS

### Percentage yield

A total of 500 g of dry, coarsely powdered *Pajanelia longifolia* leaf was subjected to maceration with 95% ethanol. The percentage yield was found to be 22.4 %

$$\text{i.e., percentage yield} = \frac{112}{500} \times 100$$

### Acute toxicity study

The safety assessment of the ethanolic extract from *Pajanelia longifolia* revealed no adverse effects up to an oral dosage of 2000 mg/kg body weight. After 24 hours, the animals exhibited good tolerance with no mortality or signs of toxicity, confirming the safety of the extract. For the study, three dose levels; 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight were chosen.

### Pharmacological evaluation

Basal values in all groups were not significantly different from each other. While the body weight of group I increased by 22.3% in 28 days, a decrease in body weight by 9.15% was noted in group II. However, in groups IV, V and VI the % increase was 7.703%, 7.594% and 7.506% respectively. In these groups, a nearly total decrease in body weight was observed on the 28th day. The weight gain in group VI was comparable with that of group III which showed a 10.47% increase in body weight on the 28th day. On the first day, the blood glucose levels (mg/dl) of groups IV, V and VI were 236.7±6.048, 262.5±17.86 and 244.7±33.62 respectively and that of group III was 246.8±8.803. In the case of group II and group I, the blood glucose levels were 203.3±10.58 and 111.5±8.257 respectively. On the 28th day there was a decrease in the blood glucose level (mg/dl) of groups IV, V and VI were 150.5±2.335, 104.3±5.270, 92.00±4.320 respectively when compared with group II (312.2±7.181) and group I (112.2±6.052) in case of group

III it was 105.5±3.914. In case of the group II the liver and muscle glycogen levels declined to a very low value on the final day. The liver and muscle glycogen on the final day for the group III animals was comparable to group I which is comparable to group VI. The tissue exhibited a dose-dependent rise in glucose uptake when exposed to different concentrations of the leaf extract. Groups III, IV, and V demonstrated lower glucose uptake compared to group II. The impact of the extract of leaf on insulin-enhanced glucose uptake was noticeable in groups VI, VII, and VIII.

## DISCUSSION

The research work was designed to evaluate the antidiabetic properties of ethanolic leaf extract of *Pajanelia longifolia*. Two models were used for the study i.e., *in vitro* rat hemidiaphragm model and *in vivo* streptozotocin induced diabetic model. The parameters that were used for the assessment of antidiabetic activity were body weight, blood glucose levels in the case of the *in vivo* streptozotocin induced diabetic model, liver glycogen and glucose uptake, in the case of *in vitro* rat hemidiaphragm model. In *in vivo* studies glibenclamide was used as a standard and insulin was used as standard in *in vitro* studies. The phytochemical evaluation showed the presence of alkaloids, carbohydrates, flavonoids, steroids, proteins, tannins and phenols (Table 1). Based on acute oral toxicity assessments, the extract demonstrated safety at a dosage of 2000mg/kg, without exhibiting any symptoms of toxicity. Three dose levels of ethanolic extract of *Pajanelia longifolia* leaf extract were taken for the current study, based on the acute toxicity studies, i.e., 100mg/kg, 200mg/kg and 400mg/kg body weight. Streptozotocin induces DM in a diverse range of animal species by inflicting damage to the insulin-secreting cells within the pancreas. The use of lower doses of streptozotocin leads to partial destruction of pancreatic  $\beta$ -cells despite the animals developing a persistent diabetic condition. Thus, in these animals' presence of surviving  $\beta$ -cells and regeneration is possible. In the current study, diabetes was induced using 35 mg/kg body weight of streptozotocin administered intraperitoneally. This administration led to a marked decrease in body weight when compared to that of normal rats (Table 2). *In vivo* studies which involved inducing diabetes with streptozotocin demonstrated a significant marked elevation in blood glucose levels compared to the normal state. Treatment of diabetic rats with doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg on the 28th day resulted in a reduction in blood glucose levels that was dependent on the dosage (refer to Table 3). The liver glycogen levels in diabetic control, when compared to normal levels, decreased to a very low value. However, administration of ethanolic extracts of leaves to groups IV, V, and VI restored the levels of liver glycogen in each case when compared to group II. These findings indicate that *Pajanelia longifolia* leaf extract reduces the level of blood glucose in diabetic rats and promotes the restoration of liver glycogen storage (Table 5). *In vitro* studies demonstrated a substantial increase in glucose uptake in group II as opposed to group I. The glucose uptake in groups III, IV, and V was lower than that of group II. Incubation with liposomes

demonstrated a dose-dependent increase in glucose uptake by the tissue, suggesting that the leaf extract may operate through a mechanism akin to insulin. Additionally, there was a significant increase in glycogen content observed in group II in comparison to group I. Incubation with varying doses of *Pajanelia longifolia* leaf extract led to a dose-dependent rise in glycogen content by the tissue. This augmentation in glycogen content induced by the leaf extract suggests its potential to promote glycogenesis, similar to the action of insulin

## CONCLUSION

The results obtained from both the *in vivo* streptozotocin induced diabetic model and the *in vitro* rat hemidiaphragm model lead to several conclusions. The marked decrease in body weight observed in streptozotocin-induced diabetic rats was nearly completely reversed with the administration of *Pajanelia longifolia* leaf extract. In *in vitro* studies, the leaf extract demonstrated a notable increase in glucose uptake and glycogen storage by the tissue. The antidiabetic efficacy of *Pajanelia longifolia* leaf extract was nearly comparable to that of glimepiride in the *in vivo* model. The findings from *in vitro* studies suggest that the leaf extract may share a similar mechanism of action with insulin, facilitating glucose uptake and promoting glycogenesis. The observed synergistic effect with insulin may be attributed to an increased sensitivity of the tissue to insulin. However, it is crucial to emphasize that further investigations are warranted, including *in silico*, *in vitro*, and clinical studies of the extract and its formulations.

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