

## Research Article

## ANTIDIABETIC ACTIVITY OF *PILOCARPUS MICROPHYLLUS* EXTRACT ON STREPTOZOTOCIN-INDUCED DIABETIC MICE

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### ABSTRACT

The present study was designed to investigate the hypoglycemic and hypolipidemic properties of a methanolic extract of *Pilocarpus microphyllus* (*P. microphyllus*) which is widely used as a traditional treatment for diabetes mellitus. The methanolic extract of leaves of *P. microphyllus* administered orally at a dose of 100 mg/kg, for 15 days to streptozotocin-induced diabetic mice. Hypoglycemic effects, oral glucose tolerance test, change in body weight and lipid profile assessed in methanolic extract treated diabetic mice, were compared with normal animal, diabetic control and standard drug treated mice. Histological observation during 15 days treatment was also done. Methanolic extract of leaves of *P. microphyllus* produced a significant reduction in fasting blood glucose level in streptozotocin-induced diabetic mice. Significant differences were also observed in urine glucose level, oral glucose tolerance test, serum lipid profile and change in body weight by methanolic extract treated diabetic mice, when compared with diabetic control, normal control and standard drug treated mice. Histopathological studies of the pancreas of these animals showed comparable regeneration by extract which were earlier necrosed by streptozotocin. Methanolic extract of *P. microphyllus* exhibit significant anti-hyperglycemic and hypolipidemic activities in streptozotocin-induced mice. They also showed improvement in body weight, OGGT, urine glucose as well as regeneration of pancreatic islets of langerhans so might be value in treatment of diabetes.

**Keywords:** Diabetes, Hypoglycemic effect, *Pilocarpus microphyllus*, Streptozotocin, Methanolic extract.

### INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder resulting from insulin deficiency, characterized by hyperglycemia, altered metabolism of carbohydrates, protein, lipid and an increased risk of vascular complication<sup>1</sup>. In present situation diabetes is possibly the world's largest growing metabolic disorder throughout both the developed and developing countries. Hence all newly diagnosed patients with type II diabetes mellitus should have an initial trial of dietary and exercise modifications. However, many patients require pharmacological treatment without an optional trial of nutrition and physical activity. Monotherapy with a sulfonylurea or metformin can be used as first line pharmacotherapy. However, if fasting blood glucose is >250 mg/dl or random blood glucose is >400 mg/dl, insulin should be used as initial therapy. Although insulin and oral hypoglycemic agents are the major players in the treatment of the diabetes mellitus, they have prominent side effects and fail to significantly alter the course of diabetes<sup>2,3,4,5</sup>. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethnobotanical information reports about 800 plants that may possess anti-diabetic potential. The herbal medicines are apparently effective, produce fewer side effects and are relatively inexpensive as compared to oral anti-diabetic agents. A wide array of plant derived active principles representing numerous chemical compounds has demonstrated activity consistent with their possible use in the treatment of diabetes mellitus.

Among these are alkaloids, glycosides, gum, polysaccharides, peptidoglycans, hypoglycans, bioflavonoids, cardenoloids, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids and inorganic ions.

*Pilocarpus microphyllus* (*P. microphyllus*) is a shrubby tree which belongs to the Rutaceae family and cultivated in the garden. It is found mainly in the Brazilian states of Para, Maranhao, and grows to the height of 6 to 10 feet. The plant *P. microphyllus* contains alkaloids; pilocarpine, isopilocarpine, pilocarpin and other constituents are joborine, pilosine, tannic acid, jaboric acid, pilocarpic acid, volatile oil, coumarins, flavonoids, hydrocarbons, and triterpenes<sup>6,7</sup>. Traditionally *P. microphyllus* used for glaucoma, diabetes, emetic, epilepsy, cardiac depression, asthma, inflammation, diarrhoea, wound-healing, alopecia, bright's disease, deafness, flu, gastrostis, gonorrhoea, hepatitis, itch, ischuria, jaundice, nausea, nephrosis, neurosis, pain, pneumonia, rheumatism, syphilis, and best antidote to atropine<sup>8</sup>. The literature survey reveals that some work has been done on *P. microphyllus*. The anti-glaucoma activity and main constituent of this plant being utilized for anti-glaucoma activity have a scientific justification; still some of the activities are without scientific backing. The present work was an attempt to evaluate antidiabetic activity of this plant and to generate scientifically justified data to support the activity.



## MATERIALS AND METHODS

### Animals

Swiss albino mice of either sex weight 25-30 g were used for the study. They were housed in polypropylene cages and fed with a standard diet and water *ad libitum*. The animals were exposed to an alternating 12 h light and dark cycle. All the experimental procedures and protocols involving animals were reviewed by the Institutional Animal Ethics Committee (Registration number: 1279/ac/09/CPCSEA) and were in accordance with the guidelines of CPCSEA.

### Collection and authentication of plant material

The plant was purchased from Himalaya Herb Stores, Saharanpur, Uttar Pradesh, India. The plant was identified and authenticated by Dr. H.B. Singh, Scientist F and Head, Raw Materials Herbarium and Museum at National Institute of Science Communication and Information Resources, New Delhi.

### Preparation of the Extract

The powdered *P. microphyllus* leaf material (72.74 g) was extracted separately using petroleum ether, methanol by Soxhlet and aqueous extract by cold maceration method. The filtrate was evaporated using rotary vacuum evaporator under reduced pressure  $\leq 10$  mmHg<sup>9</sup>.

### Preliminary phytochemical screening

Preliminary phytochemical screening were done to find the presence of the active chemical constituents such as alkaloids, flavonoids, tannins, phenolic compounds, saponins, fixed oils and fats<sup>10,11</sup>.

### Induction of experimental diabetes

Diabetes was induced in mice by intraperitoneal (i.p.) injection of streptozotocin (STZ) at a dose of 60 mg/kg body weight, dissolved in 0.1M cold citrate buffer (pH = 4.5). Diabetes was confirmed by the determination of fasting blood glucose concentration on the third day post administration of streptozotocin<sup>12</sup>.

### Oral glucose tolerance test (OGTT)

The oral glucose tolerance test was performed in overnight fasted normal animals. Mice divided into three groups ( $n = 6$ ) were administered distilled water 10 ml/kg, methanolic extract of leaves of *P. microphyllus* (MELPM) 100 ml/kg p.o and glibenclamide (GBC) 10 mg/kg, respectively. Glucose (2.5 g/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from tail- vein just prior to the drug administration (normal fasting) and at 0, 30, 60 and 120 min of glucose loading. Blood glucose level was measured immediately by using glucose oxidase - peroxidase reactive strips and a glucometer<sup>13</sup>.

### Evaluation of antidiabetic activity

Mice were divided into four groups of six mice ( $n = 6$ ) each. Groups 1 and 2 served as control and diabetic

untreated control respectively. Group 3 served as standard and was treated with 10 mg/kg/day glibenclamide. Group 4 was treated with the methanolic extract of leaves of *P. microphyllus* 100 ml/kg p.o. for 15 days<sup>14</sup>. Blood glucose and urine glucose levels were measured on day 1, 4, 7, 10 and 15 of the study. Finally on day 15, blood was collected to perform various parameters.

### Statistical analysis

The results are expressed as mean $\pm$ S.E.M. Statistical difference was tested by using one-way analysis of variance (ANOVA) followed by post hoc Dunnett's multiple comparison test. A difference in the mean P value  $<0.05$  was considered as significant.

## RESULTS

### Percentage yield of crude extracts

The powdered drug (72.74 g) was extracted with petroleum ether (60-80°C), methanol and aqueous by using Soxhlet extraction apparatus. The extractive yields of different solvent were 5.88 %, 22.11 % and 6.83 % respectively.

### Phytochemical tests

The phytochemical tests revealed the presence of alkaloids, flavonoids, glycosides, terpenoids, carbohydrates, tannins and steroids in methanolic extract.

### Oral glucose tolerance test

Administration of glucose (2.5 g/kg,) produces significant change in blood glucose level of normal mice. Treatment with MELPM (100 mg/kg, p.o.) and GLB (10 mg/kg, p.o.) significantly reduced serum glucose level at normal fasting, at 0 minute, 30 minute, 60 minute and 120 minute compared to normal control group (Table 1).

### Changes in body weight

At the end of 15 days treatment the body weight of diabetic control group decreased whereas treatment with MELPM (100 mg/kg, p.o.) and GLB (10 mg/kg, p.o.) significantly recovered the body weight towards normal level. (Table 2).

### Hypoglycemic Effect of methanolic extract

The results from the study clearly indicated that the methanolic extract exhibited significant hypoglycemic activity in STZ-diabetic rats. The standard drug glibenclamide also indicated a significant decrease of blood glucose levels. (Table 3)

### Urine glucose estimation

After 15 days treatment period, it was observed that the animals treated with methanolic extract and glibenclamide showed a significant decrease diabetes induced urine glucose level. (Table 4)



**Table 1:** Effect of methanolic extract of leaves of *P. microphyllus* on OGTT in normal mice

Group	Blood glucose concentration (mg/dl)				
	Normal fasting	0 min.	30 min.	60 min.	120 min.
I. Glucose (2.5 g/kg)	124.66±5.92	269.83±3.43	373.66±4.08	250.33±4.5	174.5±4.5
II. GBC (10 mg/kg)	104.83±2.31***	181.83±5.07***	237.5±2.73***	165.16±3.31***	147±2.82***
III. MELPM (100 mg/kg)	111.5±3.73***	189.67±7.06***	257.16±6.52***	179.5±7.71***	153.83±5.49***

Values are given as mean±S.E.M. from six rats in each group.

\*\*\* Represents statistical significance vs. control ( $P < 0.001$ ).

**Table 2:** Effect of methanolic extract of leaves of *P. microphyllus* on body weight in STZ-induced diabetic mice.

Group	Body weight (g)	
	Day 1	Day 15
I. Normal control	26.83±0.98	28±1.22
II. Diabetic control	27.91±0.66	24.5±0.5
III. Standard (10mg/kg)	28.91±0.86	27.5±1**
IV. MELPM (100mg/kg)	29.33±0.82	27.42±0.58 *

Values are given as mean±S.E.M. from six rats in each group.

\* Represents statistical significance vs. diabetic control ( $P < 0.05$ ).

\*\* Represents statistical significance vs. diabetic control ( $P < 0.01$ ).

**Table 3:** Effect of methanolic extract of leaves of *P. microphyllus* on fasting blood glucose level in diabetic mice.

Group	Fasting blood glucose concentration (mg/dl)				
	Day 1	Day 4	Day 7	Day 10	Day 15
I. Normal control	120.83 ±12	113.16 ±9.19	107 ±15.53	116.83±16.26	118.83±14.02
II. Diabetic control	312.67 ±34.46	325.17±40.93	326.4 ± 37.48	308.5 ±51.86	305 ±47
III. Standard (10mg/kg)	363.5± 35.55**	215± 30.96***	168.17±40.65***	150.5± 27.04***	129.33±19.21***
IV. MELPM (100mg/kg)	315.67±14.84	252.83±37.70**	182.67±27.88***	163.83± 20.93***	139.83±17.87***

Values are given as mean±S.E.M. from six rats in each group.

\*\* Represents statistical significance vs. control ( $P < 0.01$ ).

\*\*\* Represents statistical significance vs. control ( $P < 0.001$ ).

**Table 4:** Effect of methanolic extract of leaves of *P. microphyllus* on urine glucose test in STZ-induced diabetic mice.

Group	Urine glucose test (No. of +)				
	Day 1	Day 4	Day 7	Day 10	Day 15
I. Normal control	Nil	Nil	Nil	Nil	Nil
II. Diabetic control	+++	+++	+++	+++	++++
III. Standard (10mg/kg)	+++	++	++	+	+
IV. MELPM (100mg/kg)	+++	+++	++	++	+

(Intensity of glucose in urine; + mild, ++ moderate, +++ higher, ++++sever)

**Table 5:** Effect of methanolic extract of leaves of *P. microphyllus* on lipid profile in STZ-induced model.

Serum parameter	STG (mg/dl)	STC (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)	STC/ HDL-c ratio	LDL-c/HDL-c ratio
I. Normal Control	90.67±4.5	82.33±3.79	38.87±1.02	25.33±2.27	18.13±0.9	2.11±0.06	0.67±0.02
II. Diabetic Control	132.67±4.16	157±5.29	18.67±1.52	111.8±6.6	26.53±0.83	8.45±0.89	6.03±0.8
III. MELPM(100mg/kg)	115.67±3.51*	119.67±5.23**	33±2.64**	65.53±6.33**	23.13±0.70*	3.64±0.37**	1.93±0.30**

Values are given as mean±S.E.M. from six rats in each group.

\*Represents statistical significance vs. diabetic control ( $P < 0.05$ ).

\*\*Represents statistical significance vs. diabetic control ( $P < 0.01$ ).

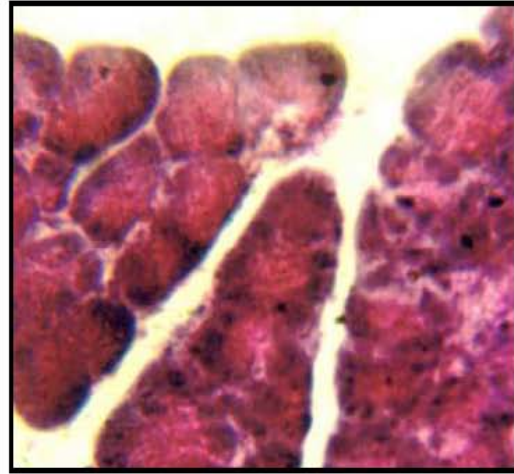




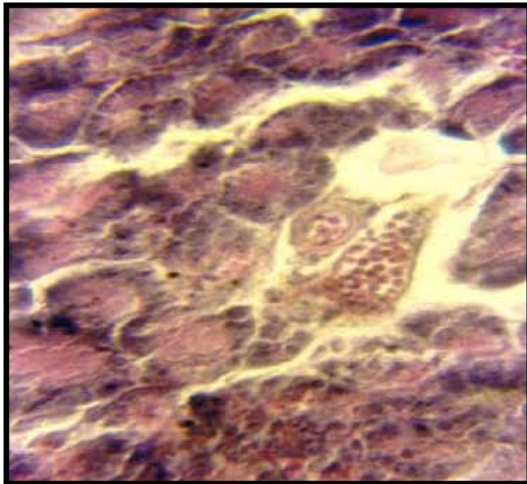
**Figure1:** Effect of methanolic extract of *P. microphyllus* at 100 mg/kg and reference compound on pancreases. (A) Normal islets with secretory granules (B) Clear and normal parenchyma (C) Degenerative and necrotic changes with shrunken islets of langerhans (D) Regeneration of islets of langerhans (E) Restoration of normal cellular size of the islet with hyperplasia



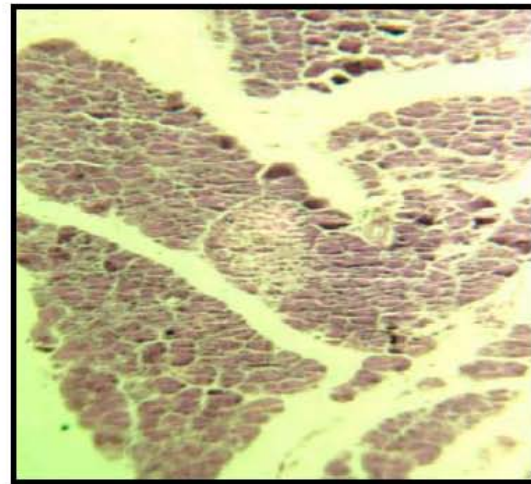
(A)



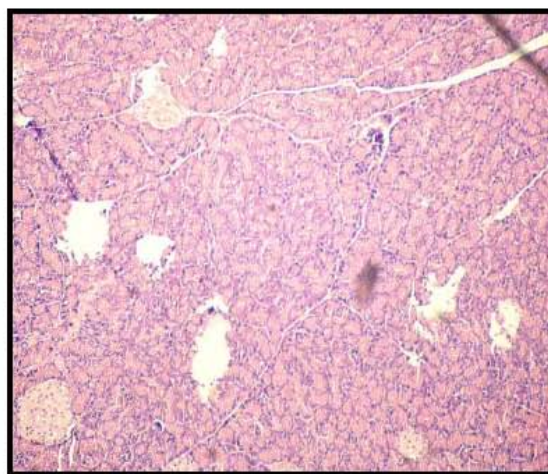
(B)



(C)



(D)



(E)

## Lipid profile

Significant difference were observed in serum lipid profile (STG, STC, VLDL-c, LDL-c, HDL) and STC/HDL-c and LDL-c/HDL-c ratios in *P. microphyllus* methanolic extract treated animals, when compared with diabetic control animals ( $P < 0.01$ ). (Table 5).

## Histopathology of pancreas

After termination of experiment the mice from all the groups were anesthetized and dissected out. Pancreas was taken in formaldehyde solution and histological preparations were made. 5 $\mu$  thick sections were cut and stained with haematoxyline and eosin.

## DISCUSSION

The fundamental mechanism underlying hyperglycemia involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues<sup>15</sup>. Persistent hyperglycemia, the common characteristic of diabetes can cause most diabetic complications. In all patients, treatment should aim to lower blood glucose to near-normal levels. In our investigation, the normoglycaemic and oral glucose tolerance test studies revealed that the methanolic extract of leaves of *P. microphyllus* has the capacity to lower blood glucose levels. The diabetic syndrome in rats administered STZ is characterized by stable moderate hyperglycemia, glucose intolerance and altered but significant glucose stimulated insulin secretion<sup>16</sup>. In normoglycaemic study, the data indicates that the MELPM treatment significantly reduced the blood glucose levels ( $***P < 0.001$ ) in the diabetic mice towards the normal level in the 15 days of study period. In oral glucose tolerance test, treatment of MELPM significantly reduced the serum glucose level ( $***P < 0.001$ ) over the period of 120 min compared to normal control group. These data suggested that treatment with MELPM showed tolerance to exogenously administration glucose in the treated group of mice in OGTT.

Urine glucose estimation study observed that the animals treated with methanolic extract and glibenclamide showed a significant decrease diabetes induced urine glucose level toward the normal level. A marked reduction in blood glucose level and urine glucose level towards normal level suggested acquisition and retrieval of normal condition.

Our histological study showed that treatment with *P. microphyllus* caused enhanced islet regeneration in the pancreas and restoration of normal cellular size of the islet with hyperplasia (Fig 1). It is thus apparent that the hypoglycaemic effect may be probably brought about by pancreatic mechanism. The analysis of histological parameters from treated animals further confirmed that the given extract is effective and could act in the management of diabetes.

The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia. In our study, administration of the *P. microphyllus* extract to the STZ induced diabetic mice significantly decreased STG, STC, VLDL-c, LDL-c, STC/HDL-c and LDL-c/HDL-c whereas HDL-c significantly increased towards normal level. The observed hypolipidaemic effect may be because of decreased cholesterologenesis and fatty acid synthesis. Significant lowering of total cholesterol and raise in HDL cholesterol is a very desirable biochemical state for prevention of atherosclerosis and ischaemic conditions<sup>17</sup>.

The characteristic loss of body weight associated with STZ induced diabetes is due to increased muscle wasting in diabetes<sup>18</sup>. The *P. microphyllus* methanolic extract treated animals recovered the body weight significantly towards normal level ( $*P < 0.05$ ). Which may be directly due to the lipid lowering activity of the extract or indirectly to the influence on various lipid regulation systems.

Methanol extract showed the presence of alkaloids, flavonoids, glycosides, terpenoids, carbohydrates, tannins and steroids. The anti-diabetic activity of *P. microphyllus* may be due to the presence of flavonoids. It is reported that flavonoid constitute the active biological principle of most medicinal plants with hypoglycemic and anti-diabetic properties<sup>19</sup>. However the extract should further be subjected to bioactivity guided drug discovery to isolate the lead compound responsible for anti-diabetic and possible mechanism(s) of action.

## CONCLUSION

Methanolic extract of *P. microphyllus* exhibit significant anti-hyperglycemic activities in streptozotocin-induced mice. The methanolic extract of *P. microphyllus* also showed improved in lipid profile, body weight, OGGT, urine glucose as well as regeneration of pancreatic islets of langerhans so might be value in treatment of diabetes. However, further phyto pharmacological investigations are needed to identify the lead molecule and to elucidate its exact mechanism of action for anti-diabetic effect.

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