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Anti-diabetic effect of methanolic leaf extract of *Pongamia pinnata* on streptozotocin induced diabetic rats

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PEER REVIEW

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Comments

The research article is well presented with sufficient results and discussion. The authors discussed with current problem for diabetes mellitus. Nowadays most of the countries face more problems against diabetes mellitus. This research may be helpful to these problems.

Details on Page 116

ABSTRACT

Objective: To study the anti-diabetic effect of methanolic leaf extract of *Pongamia pinnata* (*P. pinnata*) on streptozotocin induced diabetic rats.

Methods: Anti-diabetic activity of *P. pinnata* leaf extract at dosage of 500 mg/kg and 1 g/kg body weight was evaluated.

Results: The levels of glucose, triglycerides, total cholesterol and serum glutamic pyruvic transaminase were significantly increased in streptozotocin induced diabetic rats when compared to that of the normal rats. After supplemented with plant extract, significant lower blood glucose level was recorded.

Conclusions: The methanolic leaf extract of *P. pinnata* has been potent anti-diabetic effect in male albino rats.

KEYWORDS

Pongamia pinnata, Diabetes mellitus, Medicinal plant, Cholesterol

1. Introduction

Diabetes mellitus is a chronic and endocrine disorder caused by inherited and/or acquired deficiency in the production of insulin by the pancreas, or by the ineffectiveness of the insulin production. Type 2 diabetes mellitus is a heterogeneous disorder characterized by a progressive decline in insulin action, followed by the pancreatic beta cell dysfunction[1]. Complications such as renal failure, coronary artery disorder, cerebrovascular disease, neurological complications,

blindness, dyslipidemia, obesity, limb amputation and failure of various organs and eventually premature death are associated with chronic hyperglycemia[2]. It has been suggested that diabetes is the third leading cause death due to high level of morbidity and mortality in the developing countries. About more than 200 million people worldwide have diabetes mellitus and 300 million will acquire this disease by 2025[3]. It has been estimated that Indian people are more genetically susceptible to diabetes accounting about 40 million and would reach up to 74 million by 2025[4]. In ancient times, medicinal

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plants and herbs were used as remedy for serious health complications. Herbal drugs have lesser or no side effects and are less expensive as compared to synthetic drugs. Medicinal plants and their bioactive constituents are used for the treatment of diabetes throughout the world. Many indigenous Indian medicinal plants have been found to be useful for managing diabetes^[5,6]. After recommendation made by World Health Organization on medicinal plants for anti-diabetic drugs, many researchers focused on traditional medicinal plants for more effective and safer hypoglycemic agents. Many useful plants and herbs introduced in pharmacological and clinical trials have been confirmed their blood sugar lowering effect. So it is essential to know about the pharmacological evaluation of various plants used in the traditional system of medicine^[7]. *Pongamia pinnata* (L.) Pierre (Fabaceae) (*P. pinnata*), popularly known as “Karanja” (in Hindi), “Pongam” (in Tamil) and “Indian beech” (in English), is native to India and widely distributed along Southeast Asia to the West Pacific and North Australia. It is a medium-sized tree with a short crooked trunk and a broad crown of spreading or drooping branches. It is naturally distributed along the coasts and river banks in India and Myanmar^[8]. For centuries, *P. pinnata* is used as a folklore medicinal plant, particularly in Ayurvedha and Siddha systems of Indian medicine for the treatment of abscess, bronchitis, diarrhea, itches, piles, skin diseases, tumors, painful rheumatic joints, ulcers, whooping cough and quench dipsia in diabetes^[9,10]. A number of plant species are well known to have hypoglycaemic^[11], hypolipidemic^[12] or both activities^[13]. Despite the presence of effective antidiabetic medicines in the pharmaceutical market, screening for bioactive substance from natural plants is still attractive because they contain substances that are effective and safe in diabetes mellitus. In the present study, traditional medicinal plant has been selected for the hypoglycemic effect.

2. Materials and methods

2.1. Plant material

A total of six species of Indian traditional plants (*Cassia ariculata*, *P. pinnata*, *Andrographis paniculata*, *Mimosa pudica*, *Coccinia grandis*, and *Solanum surattense*) were collected in and around Vellore District, Tamil Nadu, India. After preliminary screening of the six plants, the crude extract of *P. pinnata* was found to have strong

anti-diabetic activity against streptozotocin (STZ) induced diabetic rats. The fresh leaves of *P. pinnata* were collected from Adhiparasakthi Agriculture College, G.B Nagar, kalavai Tamilnadu, India. The fresh leaves were cleaned and shade dried under room temperature. The plant specimen was authenticated and voucher specimen (No. APAC1343) was deposited in Adhiparasakthi Agriculture College.

2.2. Extract preparation

Shade dried leaves were grinded into fine powder in electrical blender. Extraction was done with 100 g of powdered leaf with 500 mL of methanol by Soxhlet apparatus. Then methanolic extract was concentrated under vacuum to get solid yield of 10%. Extract was stored at 4 °C until further use. The plant extracts were tested for anti-diabetic effect in the albino rats at the selected optimum dosage of 500 mg/kg body weight and 1 g/kg body weight and administered orally in aqueous solution.

2.3. Animals

Adult male albino rats of Wistar strain weighing around 180–190 g were purchased from Tamilnadu Veterinary and Animal Sciences University, Chennai, India. The animals were kept in polypropylene cages (three in each cage) at an ambient temperature of (25±2) °C and 55%–65% relative humidity. A (12±1) hour light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions. They were fed with commercially available rat chow (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. The experiments were designed and conducted in accordance with the institutional guidelines.

2.4. STZ-induced diabetic animal

Freshly prepared solution of STZ (Sigma, USA), 35 mg/kg body weight in 0.1 mol/L of cold citrate buffer pH 4.5 was introduced into the overnight fasted animals by a single intra peritoneal injection^[11]. The control rat was injected with saline. The animals were considered diabetic if the blood glucose level values were more than 250 mg/dL on the third day after STZ injection.

2.5. Experimental design

Rats administered with saline for 21 d. Rats

administered with STZ (55 mg/kg body weight) intraperitoneally. Oral administration of *P. pinnata* leaf extract (500 mg/kg body weight in gum of acacia) in STZ-induced rats from Day 8 to Day 21. Oral administration of *P. pinnata* leaf extract (1 g/kg body weight in gum of acacia) in STZ-induced rats from Day 8 to Day 21.

2.6. Estimation of insulin

Plasma insulin was estimated using RIA assay kit (for rats) supplied by Linco Research Inc. (Stat Diagnostics, Mumbai).

2.7. Preparation of serum, plasma and tissue homogenate

After the experimental period, animals were scarified by cervical decapitation. Blood was collected and centrifuged for serum separation. For plasma, blood was collected with anticoagulant and centrifuged at 2000 r/min for 20 min. The liver tissue was dissected out, weighed, and homogenized (10% w/v) in Tris-HCL buffer (0.1 mol/L; pH 7.4) and centrifuged at 3000 r/min for 20 min at 4 °C. The resulting supernatant was used for the estimation of blood glucose^[14], insulin, serum glutamic pyruvic transaminase (SGPT), cholesterol^[15], high density lipoprotein (HDL) and triglyceride^[16].

2.8. Histopathological studies

Histopathological investigation was carried out after completion of treatment. Both control and experimental rats were scarified, liver tissues were isolated. On glass slides 10% formalin was fixed over the sliced piece of liver and tissues for 3 d and observed under microscope (10×).

2.9. Statistical analysis

The values were expressed in mean±standard deviation. The statistical analysis was carried out by using One-way ANOVA in standard statistical software package of social science (SPSS).

3. Results

Table 1 revealed that the body weight changes in the STZ-induced diabetic animals. After methanolic leaf extract of *P. pinnata* supplemented, group animals body weight were significantly augmented. STZ-induced diabetic

animals were assessed by lipid profile. Oral administration of methanolic leaf extract of *P. pinnata* with the dosage 500 mg/kg and 1 g/kg was carried out in experimental animals. Results revealed that the levels of glucose, triglycerides, total cholesterol and SGPT were significantly increased in STZ-induced diabetic animals when compared to that of normal animals.

Table 1

Effect of methanolic leaf extract of *P. pinnata* on the body weight of rats in different time interval.

Day	Control	Diabetic rats	STZ+ <i>P. pinnata</i> (500 mg/kg BW)	STZ+ <i>P. pinnata</i> (1 g/kg BW)
0	180.5±0.5*	184.0±0.4*	185.0±0.5*	180.4±0.2*
7	184.5±0.8*	155.5±0.4*	160.0±0.3*	162.5±0.1*
14	189.5±0.3*	153.0±0.4*	166.5±0.4*	168.0±0.8*
21	192.0±0.1*	148.0±0.3*	176.0±0.3*	175.0±0.7*

Data are expressed as mean±SD of 6 individual observations. *P<0.001.

STZ-induced diabetic animal showed increase in hyperglycemia, HDL and insulin levels were significantly depleted when compared to that of control rats (Table 2). After supplementation of methanolic leaf extract of *P. pinnata* at the dosage of 500 mg/kg in the STZ-induced diabetic rats, a significant reduction in glucose, triglycerides, total cholesterol, SGPT and significant increase in insulin and HDL were recorded when compared to that of control animals (Table 2).

Table 2

Effect of methanolic leaf extract of *P. pinnata* on the glucose, insulin, triglycerides, cholesterol, SGPT levels.

Parameters	Control	Diabetic rats	STZ+ <i>P. pinnata</i> (500 mg/kg BW)	STZ+ <i>P. pinnata</i> (1 g/kg BW)
Glucose	93.83±0.20*	107.66±0.70*	101.16±0.90*	96.16±0.30*
Insulin	4.96±0.80*	0.58±0.60*	1.52±0.30*	5.18±0.50*
Triglycerides	77.48±0.10*	111.93±0.40*	87.63±0.80*	71.66±0.60*
Cholesterol	38.50±0.50*	69.16±0.20*	51.33±0.40*	38.50±0.30*
SGPT	42.50±0.90*	68.50±0.70*	55.00±0.90*	45.00±0.40*
HDL	45.66±0.90*	27.50±0.30*	33.16±2.00*	45.50±0.90*

Data are expressed as mean±SD of 6 individual observations. *P<0.001.

When the dosage level increased to 1 g/kg, results revealed higher increment when compared to 500 mg/kg. The histological studies were carried out to prove the efficacy of plant extract. Figure 1A shows a normal liver lobular architecture and cell structure. In STZ-induced diabetic rat congestion of portal vessel, sinusoidal dilation and hemorrhagic necrosis of the liver cells were observed (Figure 1B). The liver cells of methanolic leaf extract of *P. pinnata* fed rat revealed restoration of the hepatic tissue architecture (Figure 1C). In the Group-IV treated rat, the liver cells showed a normal lobular architecture. Portal vessel congestion and sinusoidal dilation were not present when compared to that of control animal (Figure 1D).

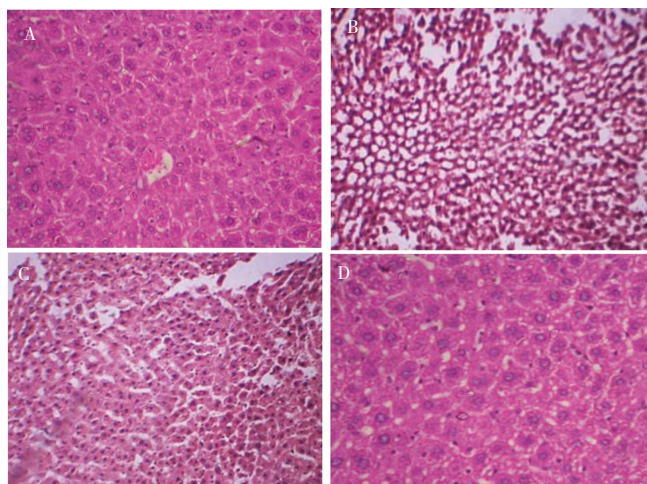


Figure 1. Histopathological studies of liver tissue of diabetic rats treated with methanolic leaf extract of *P. pinnata* for 21 d.

A: Normal control, B: STZ induced diabetic animal, C: STZ+*P. pinnata* (500 mg/kg body weight); D: STZ+*P. pinnata* (1 g/kg body weight).

4. Discussion

STZ-induced hyperglycemia in rodents is considered to be a good model for the preliminary screening of agents active against diabetes and is world widely used[17]. In this model, diabetes arises from destruction of the β -islet cells of the pancreas, causing degranulation or reduction of insulin secretion. In the present study, STZ-induced diabetic rats showed significant increase in plasma glucose and decrease in insulin levels when compared to the normal rats. Lipid profile, which is altered in the serum of diabetic patients[18,19], appears to be a significant factor in the development of premature atherosclerosis and includes an increase in triglyceride and total cholesterol levels. This abnormally high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots, mainly due to impairment of insulin secretion at diabetic state. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state, lipoprotein lipase is not activated due to insulin resistance deficiency, resulting in hypertriglyceridemia and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities[20].

In the present study, both extracts significantly reduced the triglyceride levels in treated diabetic rats when compared to untreated diabetic rats. The methanolic leaf extract was also able to significantly deplete the total cholesterol concentration in treated STZ-induced diabetic rats. These reductions could be beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetics[21]. Increased in body weight

of experimental animal and decrease in blood glucose might be due to improving glycemic control mechanisms and insulin secretions from remnant pancreatic cells in diabetic animals. The present study also revealed that *P. pinnata* leaf extract may reduce the levels of blood glucose, triglycerides, total cholesterol, SGPT and increased levels of insulin and HDL. These observations are consistent with those reported earlier[22,23]. The histopathological studies of diabetic rats showed necrosis of the hepatic cells, degeneration, vacuolation in hepatic cells in comparison to that of control. Similar observations were observed during STZ-induced diabetic rat[24,25]. These damages may be due to oxygen free radicals exerting their cytotoxic effect by peroxidation of membrane phospholipids leading to a change in permeability and loss of membrane integrity. After supplemented with methanolic leaf extract of *P. pinnata* may reduce membrane integrity.

In the present study, it can be concluded that *P. pinnata* has significance anti-diabetic and hypolipidemic effect.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

In traditional medicine system, *P. pinnata* has been used against several diseases such as diabetes mellitus, cardiovascular disease, cerebrovascular disease and hyperlipidemia. *P. pinnata* plant have been used for nanotechnology field. Many systematic scientific studies led to reveal of the active compounds and their efficacy against curing diseases.

Research frontiers

This research article is well documented with usage of *P. pinnata* in traditional medicine thereby leading to the systematic scientific study.

Related reports

The way of presentation is good. Authors have followed very good experimental protocol. The article is well documented with folklore medicinal properties which lead to a systematic study on their efficacy and isolation of active ingredients.

Innovations and breakthroughs

The various medicinal uses against various ailments of the parts of *P. pinnata* were recorded consistently for reference and for further probe.

Applications

This research opened new study area of discovery of new plant based drugs for anti-diabetic. It could be more useful for diabetic patients.

Peer review

The research article is well presented with sufficient results and discussion. The authors discussed with current problem for diabetes mellitus. Nowadays most of the countries face more problems against diabetes mellitus. The research may be helpful to these problems.

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