

Pharmacological evaluation of anti-Diabetic activity of Pungampoo Chooranam on Streptozotocin induced diabetic rats.

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Abstract: The Traditional Siddha system of medicine has a vast documentation of herbs in its ancient literature for the treatment Neerizhivu/Madhumegham (Diabetes mellitus). Many of these formulations are clinically effective but they lack in scientific evidence which is the chief present day challenge faced by the traditional system of medicine. Pungampoo Chooranam is one such formulation which has been indicated for the treatment of diabetes mellitus in the Siddha Classical literature Boga Munivar Vaithiyam – 700. The present study was aimed to screen the oral antihyperglycemic activity of Pungampoo Chooranam (PC) (200 and 400 mg/kg) against streptozotocin (45 mg/kg i.p.) induced diabetes mellitus in rat models. The animals were grouped into four groups of 6 animals each. Group I (Control group)-received normal saline, Group II rats were administered with 45 mg/kg,i.p of STZ, Group III received 45 mg/kg,i.p of STZ + 200mg/kg of Pungampoo Chooranam and group IV received 45 mg/kg,i.p of STZ + 400mg/kg of Pungampoo Chooranam. The Trial drug Pungampoo Chooranam (PC) was administered for 28 consecutive days, and the effect of the investigational drug was studied on Body weight Changes, blood glucose levels, HbA1C levels, Insulin levels, Blood urea and creatinine levels at regular intervals. The study results were statistically analysed by Welch's ANOVA and paired t test. The study results revealed that the Siddha drug Pungampoo Chooranam had significant antidiabetic effects.

Keywords: Siddha medicine, Diabetes mellitus, Pungampoo Chooranam, Pongamia glabra

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I. Introduction

Diabetes mellitus is a chronic metabolic endocrine disorder that occurs due to either insulin deficiency or malfunction, resulting in disturbed intermediary metabolism and manifestations. [1]. Diabetes is known to be the fifth disease that leads to mortality in 21st century [2]. According to a report by the World Health Organization (WHO) there will be an estimated 300 million diabetics by 2025, worldwide with 57 million diabetics in India. Diabetes mellitus affects all organs and systems with its main impact on eyes, kidneys, skin and nerves resulting in diabetic complications. Present diabetic Population in India is estimated approximately as 32 million of which Type 2 Diabetes caused by insulin resistance constitutes 90% of Diabetics [3]. Before the dawn of conventional medicine, the people of South India used traditional system of medicine to battle this multifaceted disorder that was indicated as Madhumeagam in Siddha literature. Presently, it is the need of the hour that we must rediscover these ancient herbal remedies by filling the gap of lack of scientific explanations [4].

The two major concerns in the usage of presently available synthetic anti-diabetic drugs are the side effects caused and the drug resistance on prolonged usage. Further, the development of an adverse event is one of the major complications in the treatment of any systemic disorder. Hence, many of the research institutes and pharmaceutical companies are emerging out with interest into new hypoglycaemic and potentially anti diabetic agents towards drug development from natural molecules with good therapeutic potential and less adverse events [5]. Plant products they contain many bioactive substances with therapeutic potential and throughout the recent past several authors have reported the antidiabetic potential of traditionally used Indian medicinal plants using experimental animals [6-10] Therefore this study was conducted with an aim to evaluate the antidiabetic potential of Siddha classical formulation Pungampoo chooranam in experimental rats.

II. Materials And Methods

2.1. Study Drug preparation

The herbal ingredients that were used for the preparation of trial drug Pungampoo chooranam consist of Pungampoo (Pongamia glabra flowers). The Herbal drugs were procured from traditional reputed shops of Chennai and authenticated by botanist. The shade dried flowers of Pungam tree (Pongamia glabra flowers) were roasted slowly by adding little bit of cow's ghee, and then powdered and sieved using cloth.

2.2. Animals

Healthy adult Wistar albino male rats weighing between 220-240 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit. A 12 light / dark cycle were maintained .Room temperature was maintained between $22 \pm 2^{\circ}$ C and relative humidity was 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water ad libitum. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama University, Chennai, Tamil Nadu, India (IAEC: SU/CLATR/IEAC/VII/051/2016)

2.3 Experimental Methodology

The animals were grouped into four groups of 6 animals each. Group I (Control group) received normal saline, Group II (Diabetic control) rats administered with 45 mg/kg,i.p of STZ, Group III animals received 45 mg/kg, i.p of STZ and treated with 200mg/kg of Pungampoo Chooranam (PC). Group IV received 45 mg/kg, i.p of STZ and treated with 400mg/kg of Pungampoo Chooranam (PC). Streptozotocin (STZ), at a dose of 45 mg/kg body weight was dissolved in citrate buffer, injected intraperitoneally to induce diabetes. The animals will be fasted for 16hrs before prior to STZ injection, and after the injection 5% sucrose will be supplemented for 24hrs in order to prevent the animals from fatal hypoglycemia. One week after STZ injection, blood glucose level was checked using glucometer. The animals with a blood glucose level of more than 300 mg/dl were considered diabetic and included in the study[11-13].

2.4 Sample Collection

At the end of the study, the animals were fasted for overnight with free access to water. Animals were sacrificed with excess anesthesia. Blood samples were collected from retroorbital and cardiac puncture and stored in clot activator coated test tubes for serum biochemical analysis. Pancreas sample were harvested and carefully investigated for gross lesions[14].

2.5. Induction of Diabetes

Streptozotocin (STZ), at a dose of 45 mg/kg body weight was dissolved in citrate buffer, injected intraperitoneally to induce diabetes. The animals will be fasted for 16hrs before prior to STZ injection, and after the injection 5% sucrose will be supplemented for 24hrs in order to prevent the animals from fatal hypoglycemia. One week after STZ injection, blood glucose level was checked using glucometer. The animals with a blood glucose level of more than 300 mg/dl were considered diabetic and included in the study.

2.6. Body Weight and Glucose estimation

The fasting blood glucose was measured on 0th, 14th and 28th day by glucose estimation strip. Body weight of the animals was measured before start of the study and also at the end of the study. Also serum insulin, HbA1C and renal parameters (urea and creatinine) were determined[15]. OGTT was performed in Normal control and Groups that were administered with STZ+PC 200mg/kg and STZ+PC 400mg. For OGTT glucose (1.5 g/kg/PO) was administered 90 min after pretreatment with respective drug solutions. Blood samples were withdrawn from retro-orbital plexus under light ether anesthesia at 0, 60, and 120 min of PC administration. The serum obtained after centrifugation was used for the determination of glucose levels [16].

2.8. Sample Collection

At the end of the study, before sacrifice, the animals were fasted for overnight with free access to water. Animals were sacrificed with excess anesthesia. Blood samples were collected from retro orbital and cardiac puncture and stored in clot activator coated test tubes for serum biochemical analysis. Pancreas sample were harvested and carefully investigated for gross lesions[17].

III. Results And Discussion

Traditional medicines are widely used for the treatment of various diabetic presentations. The following are the results obtained from the present study might offer a natural key for the management of diabetes.

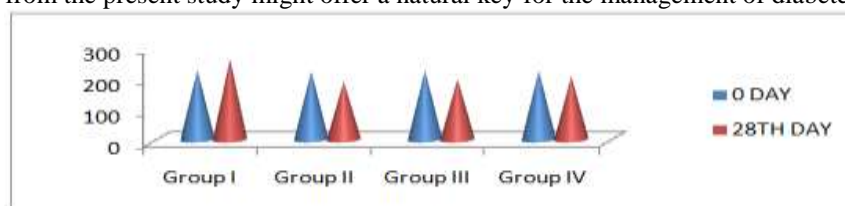


Fig-1. Effect of PC on renal parameters in Body weight in experimental rats

In this study, Streptozotocin which was used to induce diabetes in rats produced a significant reduction in body weight when compared with the normal control rats (P Value<0.0001) when compared to normal rats during the study. Diabetic control rats continued to lose weight till the end of the study while Pungampoo chooranam (PC) treated rats at a dose (200 and 400mg/kg) showed significant improvement in body weight (P Value<0.0001) compared to diabetic control group. Hence the treated group rats reveal that the test drug is able to restore the body weight to a significant level thereby it can prevent the hyperglycemia induced muscle wastage. (Fig-1).

Serum Urea and creatinine are the important parameters to diagnose the renal function. Any significant alteration in serum creatinine concentration more consistently reflect changes in GFR than altered serum urea concentrations[18]. The values in Table-1 below showed that serum Urea (70.16 mg/dl) and creatinine (1.34mg/dl) were significantly elevated in the diabetic group compared to that of the normal rats. Administration of PC at different doses (200 and 400mg/kg) significantly reduced the renal parameters in a dose-dependent manner compared to that of the diabetic rats.

Table-1. Effect of PC on renal parameters in experimental rats

Treatments	Serum Urea		Serum Creatinine	
	mg/dl	S.D	mg/dl	S.D
Normal control (Normal saline)	24.66	2.16024690	0.56	0.18618987
Diabetic control(STZ 45mg/kg,i.p)	70.16	4.62240918	1.34	0.24344746
45 mg/kg,i.p of STZ + 200mg/kg of PC	57.83*	4.30890551	0.94*	0.14610499
45 mg/kg,i.p of STZ + 400mg/kg of PC	43.50*	5.12835256	0.78*	0.15617298

Values are expressed as mean. n=6 . *P < .05 (Welsch’s ANOVA) is considered to be statistically significant.

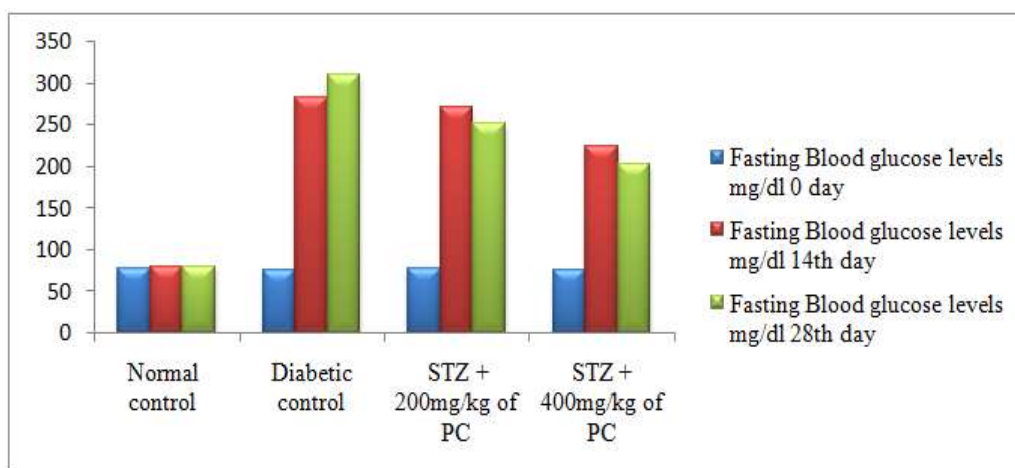


Fig-2. Effect of PC on Fasting Blood glucose levels in experimental rats

It was intended to assess the effect of PC on blood glucose level in STZ-induced severely diabetic rats. The fasting blood glucose level was assessed in all the four groups of experimental rats on day 0day, 14th and 28th day. The study results revealed that the enhanced levels of fasting blood glucose in diabetic control group on day 14 and 28 were brought down significantly (P < 0.001) after 28 days of treatment (fig-2). Besides, diabetic animals showed enhanced levels of HbA1c due to excessive production of glucose in blood, which further reacts with blood hemoglobin and produces HbA1c. PC also caused a significant reduction in HbA1c levels and serum insulin levels (fig 3 & 4) and showed significant improvement in OGTT (Table-2) (P < 0.001). This antidiabetic effect may be due to improvement in the glycemic control mechanisms and insulin secretion from remnant pancreatic β-cells in diabetic rats[19].The reduction in glucose levels may be due to increase in plasma insulin levels or enhanced transport of blood glucose in the peripheral tissue[20]. The dose dependent increase in plasma insulin levels upon administration of PC provides a strong evidence that this formulation has promising antidiabetic activity[21].Previous studies have demonstrated that P. pinnata is rich in flavonoids and related compounds. Seeds and seed oil, flowers and stem bark yield karanjin, pongapin, pongaglabrone, kanugin, desmethoxykanugin and pinnatin[22]. These phytoconstituents may be responsible for all the above antidiabetic effects of the test drug Pungampoo chooranam.

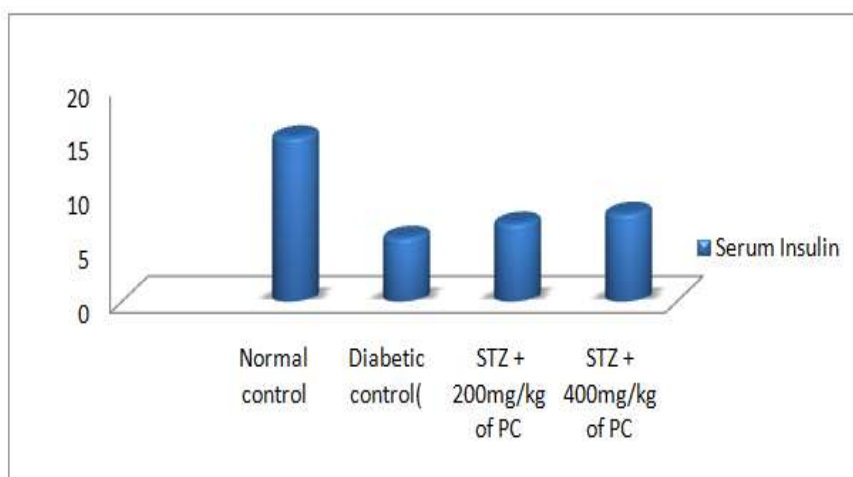


Fig-3. Effect of PC on serum Insulin levels in experimental rats

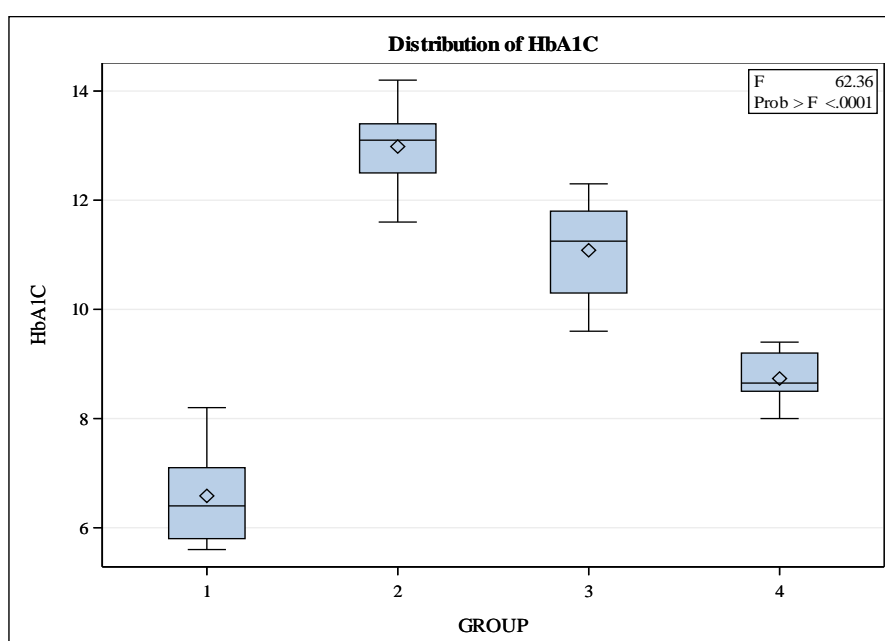


Fig-4. Effect of PC on HbA1C levels in experimental rats

Table-2. Effect of PC on Oral glucose tolerance test (OGTT)

Treatments	0 Min		60 min		120 min	
	Mean	S.D	Mean	S.D	Mean	S.D
Group I (Normal control)	77.66	6.53	79.33	5.50	79.16	4.35
Group II (STZ + 200mg/kg of PC)	142.16*	5.38	135.50*	5.08	126.00*	4.42
Group III(STZ + 400mg/kg of PC)	124.33*	3.61	118.83*	2.71	105.83*	5.23

Values are expressed as mean. n=6 . *P < .05 (Welsch's ANOVA) is considered to be statistically significant Administration of glucose (1.5 g/kg, PO) did not produce any significant change in the serum glucose levels of control group. However Serum glucose levels of diabetic rats treated with PC in the dose of 200 and 400 mg/ kg were significantly decreased 142.6, 135.5, 126 mg/dl and 124.33, 118.83 and 105.83 mg/dl at dose levels of 200 and 400 mg/ kg of PC respectively. Previous study was conducted on acute oral and 28 days repeated toxicity of Pungampoo Chooranam. The study results showed that neither the acute toxicity study of Pungampoo Chooranam at the dose level of 2000mg/kg nor the repeated dose study produced any toxic sign or mortality during study. Also, no significant changes were observed in the haematological and biochemical parameters, relative organ weight, gross necropsy and histopathological examination with Pungampoo Chooranam treatment in repeated 28 days oral toxicity study[23].

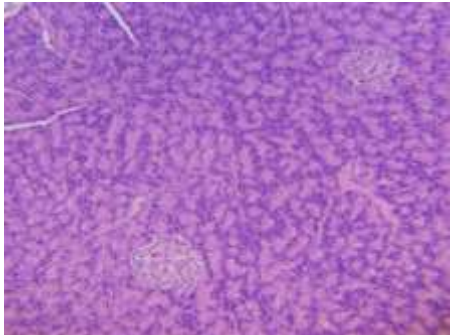
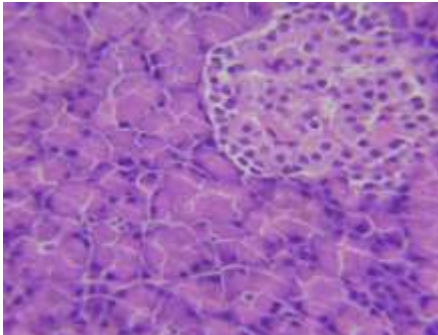
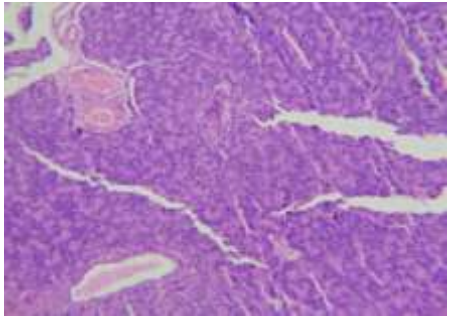
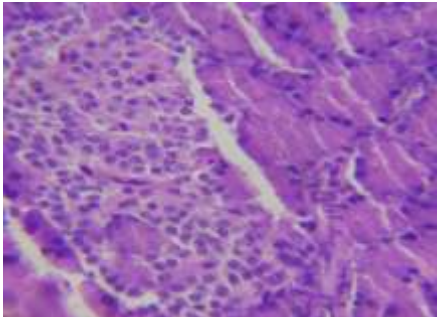
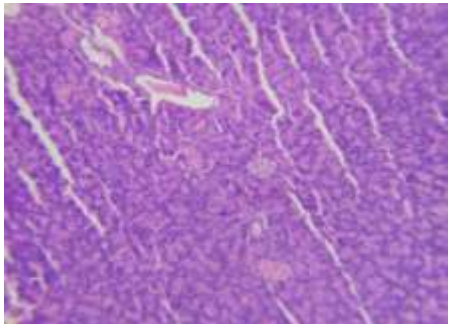
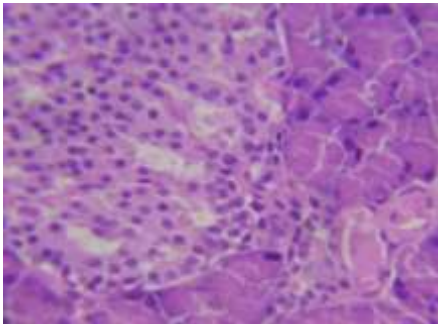
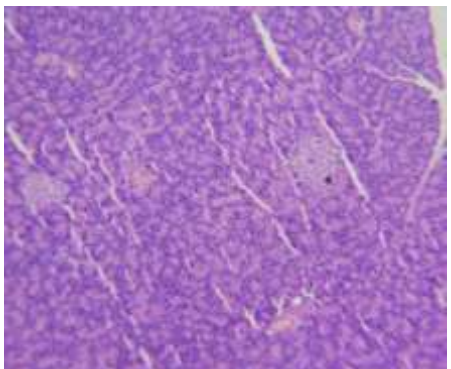
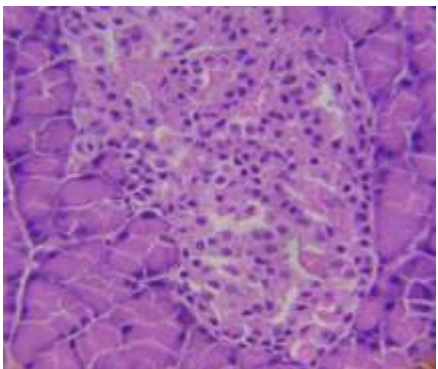
Magnification	Low Power Magnification 10 X	High Power Magnification 40 X
Group I Control Group		
Group II STZ Induced group		
Group III STZ+200MG PC		
Group IV TZ+400MG PC		

Fig-5. Histopathology of Rat Pancreas (H&E) Staining

The histopathological reports of pancreas samples of all the four groups were analysed by (H&E) Staining . The control group rat revealed normal histology of islet of Langerhans. Endocrine portion of acini zone appeared normal with no signs of degeneration. In group II(STZ diabetic induced) rats , zone of fibrosis were observed with marginal loss of beta cells on islet of Langerhans. In Group III , deposition of collagen around inter lobular duct and vascular stroma was observed with apparent change in islets density. Further there is a mild congestion of inter lobular blood vessels with occasional atrophic conditions of islet of Langerhans. In Group IV , almost normal density of beta cells were preserved with regular acini cellular zone and proper arrangement of islet of Langerhans. Gradual restoration of pancreatic endocrine cells were observed in this group(Fig-5).

IV. Conclusion

Thus, our study findings demonstrate the antidiabetic effect of the single herbal formulation Pungampoo chooranam (PC) at the dose levels of 200 and 400 mg/kg. The antidiabetic potential is evidenced by decreased levels of blood glucose, HbA1c, urea, creatinine and an increase in plasma insulin, levels.

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