

Pancreatic beta cell regenerative effect of *Costus pictus* D Don leaf extracts on streptozotocin induced diabetes on Wistar rats

Anitha Nancy¹, Jeneth Berlin Raj², Manimekalai K.³

¹ Department of Anatomy, ² Department of Physiology, ³ Department of Pharmacology, Mahatma Gandhi Medical College & Research Institute, Sri Balaji Vidyapeeth (Deemed to be University), Puducherry-607402, India

SUMMARY

With the increasing prevalence of diabetes and the economic burden caused by its treatment, people seek alternative medicine. *Costus pictus* D Don, belonging to the family Costaceae, commonly called Insulin plant/spiral ginger, is preferred by many in recent days. The current study was carried out to determine the effect of methanolic leaf extract of *Costus pictus* D Don on the pancreas of diabetic-induced albino Wistar rats.

Methanolic extract from *C. pictus* was prepared by soxhalation. The effect of this extract at the dose of 100 mg and 200 mg/kg bw was studied biochemically on blood glucose and blood insulin levels. Histological and histo-morphological observations were studied on the pancreas after 21 days of treatment. The parameters were compared with diabetic and normal rats. Glucose-lowering effect of the plant extract was observed biochemically in diabetic animals treated with both doses of the extracts. It was observed that the effect was more pronounced with 200 mg/kg BW of the extract. The presence of hyperchromic islet cells, granulated beta cells, increase in diameter of islets and number of beta cells as observed by histological examination and histo-morphometric analysis

revealed the pancreatic beta cell regenerative property of *Costus pictus* D Don.

Key words: Diabetes – *Costus pictus* D Don methanolic leaf extract – Glucose-lowering effect – Pancreatic regeneration

INTRODUCTION

The prevalence of diabetes is increasing year after year sparing no country. Diabetes has been the cause of 1.5 million deaths in the year 2019. Raised blood glucose levels have been associated with 20% of cardiovascular death and nearly 4.6 million deaths from renal diseases (WHO, 2023). There has been a huge economic burden on countries in controlling this non-communicable disease. Uncontrolled diabetes leads to many microvascular and macrovascular complications. The cost of medicines used to control the glycemic status in diabetic patients has been estimated as one of the major contributors to this economic burden (Ramachandran et al., 2022). Adjunct therapy with plants and plant-derived products will reduce the economic burden in treating and controlling diabetes. Around 21,000 potential medical plant spe-

Corresponding author:

Dr. Jeneth Berlin Raj T. Department of Physiology, Mahatma Gandhi Medical College & Research Institute Sri Balaji Vidyapeeth (Deemed to be University), Puducherry-607402, India. Phone: 9994737997. E-mail: jenethberlinraj@gmail.com

Submitted: February 11, 2023. Accepted: March 21, 2023

<https://doi.org/10.52083/KKOB4199>

cies are identified worldwide and have been used by 80% of the world population (Chowdhury et al., 2009). Around 2500 medicinal plants are found in India, which is considered the botanical garden of medical plants. Most of these plants have diverse medical properties, and many of them are used to lower blood glucose levels. This forces many researchers to do extensive research to improve the therapy and minimize the chance of developing chronic complications in diabetes (Seth and Sharma, 2004).

One such medical plant which has been recently studied by researchers for extensive medical properties such as antimicrobial (Raj and Kalaivani, 2018), anthelmintic (Raj and Kalaivani, 2016), hepatoprotective (Nancy et al., 2019), anticancerous (Nandumane et al., 2011), etc., is *Costus pictus* D Don. In this study, we have made an attempt to check for the beta-cell protective effect of *Costus pictus* D Don on albino Wistar rats.

MATERIALS AND METHODS

Plant materials

Leaves from one-year-old *Costus pictus* D Don plants were collected from the local garden of Pondicherry during summer. The plant specimen was identified and authenticated by the Department of Botany, Annamalai University, Chidambaram (No.326). The Voucher specimen of the same is preserved at the department of Pharmacology, Mahatma Gandhi Medical College and Research Institute (MGMCRI), Sri Balaji Vidyapeeth, Pondicherry.

Preparation of plant extract

The leaves of *Costus pictus* D Don were washed and shade-dried for 5-7 days, and then powdered. Since methanolic extract was proved to preserve more phytochemicals (Jothivel et al., 2007), methanolic extract was prepared using Soxhlet apparatus. The final extract was dried with a rotary evaporator and refrigerated in a brown airtight container.

Experimental animals

After obtaining ethical clearance (C3/IAEC/MG/2016), 16 weeks old healthy male adult Wis-

tar rats weighing > 250 g procured from Kings Institute, Chennai, were used in this study. The animals were maintained in a standard cage under controlled temperature (25±2 °C) and light (12:12 light-dark cycle) in MGMCRI central animal house. The animals will be fed with standard rat pellets and hygienic water *ad libitum*.

Drugs and Chemicals

Methanol was procured from Changshu Yangyuan Chemicals; Streptozotocin and Sodium pentobarbital were purchased from Sigma-Aldrich.

Experimental design

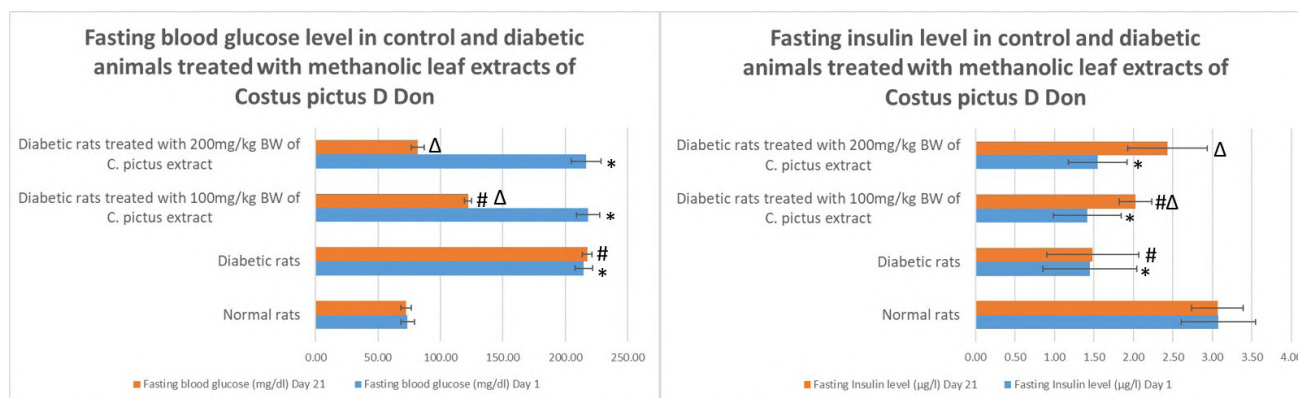
After an accommodation period of one week, 24 adult Wistar rats of either sex were randomized into 4 groups with 6 animals each as described in Table 1.

Table 1. Grouping of animals into study and control group.

Group 1	Normal control
Group 2	Diabetic control
Group 3	Diabetic rats treated with 100 mg/kg BW of <i>Costus pictus</i> D Don methanolic leaf extract orally
Group 4	Diabetic rats treated with 200 mg/kg BW of <i>Costus pictus</i> D Don methanolic leaf extract orally

Induction and confirmation of diabetes

Diabetes was induced by administering a single intra-peritoneal (i.p) injection of 65 mg/kg BW of Streptozotocin (STZ) after an overnight fast. No mortality was observed after inducing diabetes with STZ. The hyperglycemic status was confirmed after 2 weeks by estimating blood glucose level and fasting insulin level by drawing blood from the tail vein. Blood glucose levels were estimated by one touch select simple glucometer by Johnson and Johnson and fasting insulin level by ELISA kit method (Crystal Chem Inc. - Downer Grove, USA) (Raj et al., 2022). Following confirmation of diabetic status, methanolic leaf extract of *Costus pictus* D Don was ingested using a lavage tube for 21 days, and the biochemical tests were repeated.



Data expressed in Mean \pm SD and analyzed for statistical significance ($P < 0.001$) by Tukey multiple comparison test. * - day 0 data compared with control. # - Day 21 data compared with control, Δ - data compared with diabetic rats.

Fig. 1.- Fasting blood glucose and insulin level in control and treatment groups.

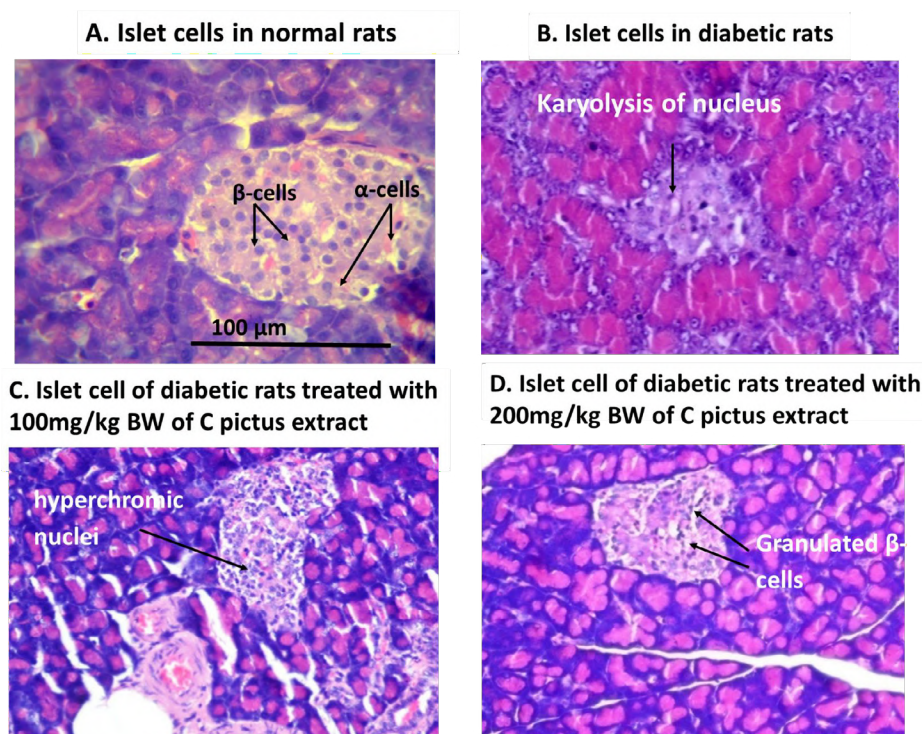


Fig. 2.- Histological changes in the pancreas of animals in control and treatment groups studied. x40.

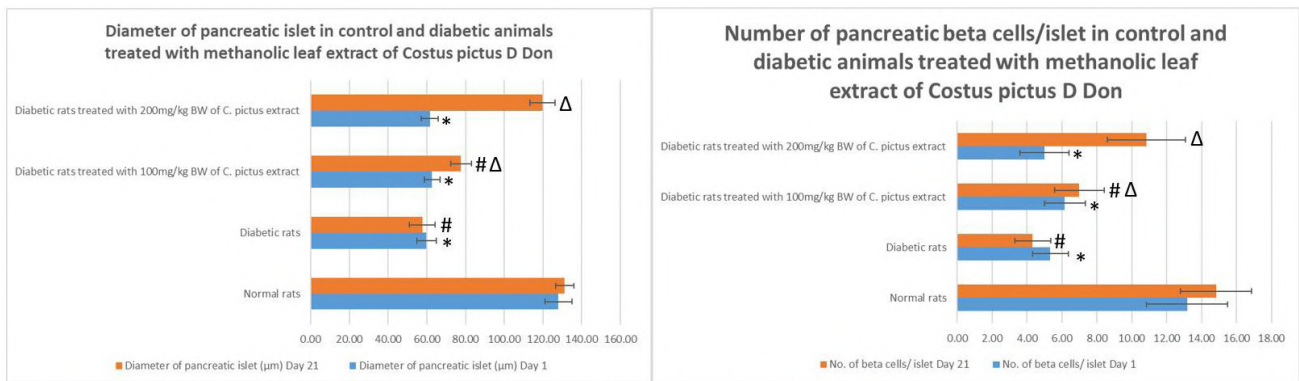
Histology and histo-morphometric analysis

After 21 days of treatment with methanolic leaf extract, all the animals were sacrificed using sodium pentobarbital (120 mg/kg BW, i.p). The pancreas was excised carefully, washed with saline, and used for studying histological changes using hematoxylin and eosin stain (H&E). Histo-morphometric analysis was performed by measuring the diameter of pancreatic islets of Langerhans and counting the number of beta cells per section. The diameter of pancreatic islets was assessed

using a liner scale & an ocular micrometer (Olympus research microscope) and the number of islets per square centimeter using an ocular grid.

RESULTS

The fasting blood glucose and insulin levels were significantly higher in the animals induced with diabetes with 35 mg/kg of Streptozotocin. The raised levels of insulin and fasting glucose were reduced to near normal in animals treated with the plant extract for 21 days (Fig. 1).



Data expressed in Mean ± SD and analyzed for statistical significance ($P < 0.001$) by Tukey multiple comparison test. * - day 0 data compared with control. # - Day 21 data compared with control, Δ - data compared with diabetic rats.

Fig. 3.- Histomorphometric changes in islet cells of animals in control and treatment groups.

The histological study in pancreatic sections stained with H&E showed that in normal control (Group 1) the normal architecture of islets with the normal number of β -cells was seen. In Diabetic control (Group 2), streptozotocin caused severe necrotic changes in pancreatic islets, especially in the center of islets with a higher concentration of β -cells. Nuclear changes, karyolysis, and distortion of normal architecture were visible. Relative

reduction of the size of the islets and severe reduction of β -cells was clearly seen. A study of the pancreas treated with 100 mg/kg of *Costus pictus* D Don methanolic leaf extract the section showed an increased size of islets and hyperchromic nuclei in the section stained with H&E, and also in Group 4, which was treated with 200 mg/kg of *Costus pictus* D Don methanolic leaf extract, there was a relative increase of granulated and normal

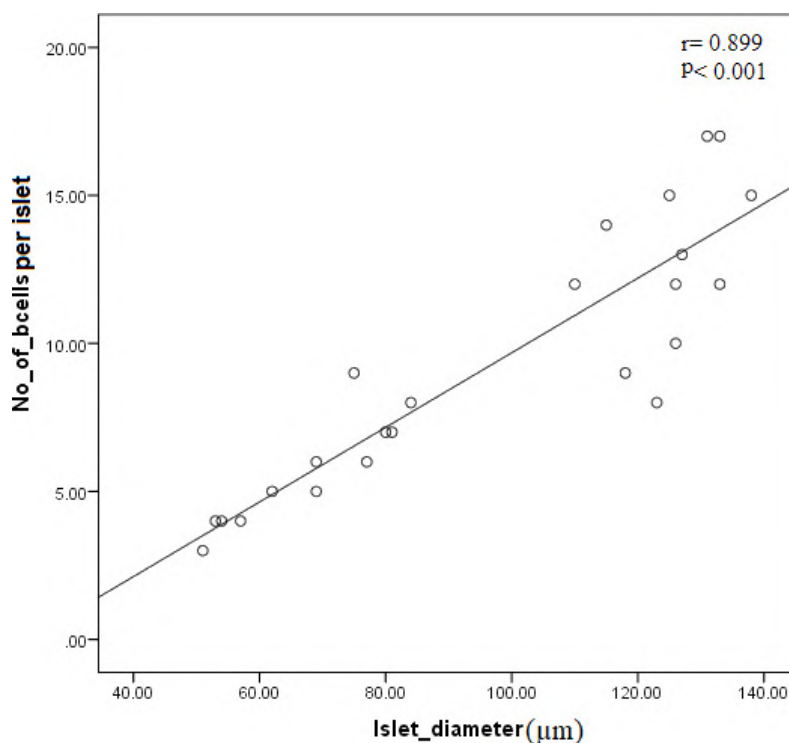


Fig. 4.- Linear relationship between the increase in islet diameter and number of beta cells per islet in the control and treatment group as analyzed by Pearson correlation coefficient.

β -cells, (Fig. 2). The islets size and the number of beta cells increased significantly in the islets of Group 4 in comparison with a diabetic group, which signifies the regeneration of islets or beta cells in the group that received plant extract (Fig. 3). Pearson correlation coefficient was used to analyze the linear relationship between the increase in the diameter of islet to the number of beta cells (Fig. 4), which was statistically evident with p value < 0.001 .

The data about fasting glucose levels, fasting insulin levels, the diameter of pancreatic islets cells, and the number of beta cells per islets were expressed in Mean \pm SD and comparison between groups was analyzed for statistical significance ($P < 0.001$) by Tukey multiple comparison test.

DISCUSSION

Streptozotocin (STZ) damages pancreatic β -cells by the alkylating property induced by the cytotoxic nitrosourea compound, and result in decreased insulin production and poor glycemic control (Graham et al., 2011). The same was evident in our study with the increased blood glucose level and decreased insulin levels in animals induced with diabetes. Histological observation on pancreatic beta cells of these animals also revealed necrotic changes in islets of Langerhans and decreased number of beta cells. Similar changes were also observed in the study by Arora et al. (2021) in STZ-induced diabetic Sprague-Dawley rats and also by Jin et al. (2008) in STZ-induced diabetic rats treated with aucubin. In diabetic control, the pancreas section showed moderate hyperplasia of islet cells, severe congestion in pancreatic parenchyma, and mild infiltration of inflammatory cells. In diabetic animals treated with MECP, the pancreas section showed mild hyperplasia of islet cells and congestion of pancreatic parenchyma (Nandumane et al., 2011).

In our study, the histo-morphometric analysis revealed that the necrotic changes induced by STZ in diabetic animals were restored back to near normal when the diabetic animals were treated with both 100 mg/kg and 200 mg/kg of the plant extract for 21 days. This was evident with increased granulation of the cells in the islets,

increased islet cell diameter, and increased cell count per section. Increased granulation of cells signifies the restoration of cell architecture after tissue damage.

The diameter of the islets in the diabetic animals treated with 100 mg/kg and 200 mg/kg were almost three-fold larger when compared to diabetic animals. Similarly, there was an two-fold increase of cells in the animals treated with both doses of the plant extract. These changes point to the fact that the methanolic leaf extract of *Costus pictus* D Don exerts a regenerative effect on the pancreatic beta cells in the animal model. The blood glucose level which was elevated in diabetic animals was also kept well under control in diabetic animals treated with both doses of the plant extract. Similarly, the decreased insulin level was normal in diabetic animals treated with the plant extracts, and rationalized the increased islet cell and diameter, proving the efficacy of the plant extract in maintaining glucose homeostasis. Our result was in accordance with the study by Gireesh et al. (2009), where the authors found that the antihyperglycemic effect of *Costus pictus* extracts correlates with the circulating insulin level by the stimulation of the surviving pancreatic beta cells (Jin et al., 2008).

CONCLUSION

The prevalence of diabetes increases year after year in India, making it the capital of diabetes in the world. The economic burden of the disease treatment on individuals, as well as on the country, is excruciating. This forces people and researchers to look for a safe alternative. Natural products derived from plants can be a better alternative. *Costus pictus* D Don, with its diverse medicinal properties, has proven to be protective to beta cells and also induces regenerative changes in animal models, especially in the dose of 200 mg/kg. However, its efficacy in humans may differ and needs to be evaluated. Basic staining using H&E has been a limitation of this study. Special staining techniques like aldehyde fuchsin method and immunostaining for insulin releasing content and lineage factors of the cells would have added more details of the extract on pancreatic beta cells.

REFERENCES

- ARORA SK, VERMA PR, ITANKAR PR, PRASAD SK, NAKHATE KT (2021) Evaluation of pancreatic regeneration activity of *Tephrosia purpurea* leaves in rats with streptozotocin-induced diabetes. *J Tradit Complement Med*, 11(5): 435-445.
- CHOWDHURY MSH, KOIKE M, MUHAMMED N, MD. HALIM A, SAHA N, KOBAYASHI H (2009) Use of plants in healthcare: a traditional ethno-medicinal practice in rural areas of southeastern Bangladesh. *Int J Biodiver Sci Manag*, 5(1): 41-51.
- GIREESH G, THOMAS SK, JOSEPH B, PAULOSE CS (2009) Antihyperglycemic and insulin secretory activity of *Costus pictus* leaf extract in streptozotocin induced diabetic rats and in in vitro pancreatic islet culture. *J Ethnopharmacol*, 123: 470-474.
- GRAHAMML, JANECEKJL, KITTREDGEJA, HERINGBJ, SCHURMAN H-J (2011) The streptozotocin-induced diabetic nude mouse model: differences between animals from different sources. *Comp Med*, 61(4): 356-360.
- JIN L, XUE HY, JIN LJ, LI SY, XU YP (2008) Antioxidant and pancreas-protective effect of aucubin on rats with streptozotocin-induced diabetes. *Eur J Pharmacol*, 582(1-3): 162-167.
- JOTHIVEL N, PONNUSAMY SP, APPACHI M, SINGARAVEL S, et al. (2007) Anti-diabetic activity of methanol leaf extract of *Costus pictus* D. Don in alloxan-induced diabetic rats. *J Health Sci*, 53(6): 655-663.
- NANCY A, RAJ JB, MANIMEKALAI K (2019) Comparative evaluation of the hepatoprotective effect of *Costus pictus* D Don methanolic leaf extract and silymarin on paracetamol induced liver damage in albino Wistar rats. *Int J Anat Res*, 7(3.1): 6722-6726.
- NANDUMANE VK, RAJASHEKAR S, NARAYANA P, ADINARAYANA S, VIJAYAN S, PRAKASH S, SHARMA A (2011) Evaluation of the anti-cancer potential of *costus pictus* on fibrosarcoma (HT-1080) cell line. *J Natural Pharmaceutical*, 2(2): 72-76.
- RAJ JB, KALAIVANI R (2016) Comparative in-vitro evaluation of anthelmintic property of leaves and rhizome of *Costus pictus* D. Don against albendazole. *Natl J Physiol Pharm Pharmacol*, 6(5): 438-441.
- RAJ JB, KALAIVANI R (2018) In-vitro evaluation of antimicrobial activity of *Costus pictus* D. Don aqueous leaf extract. *Natl J Physiol Pharm Pharmacol*, 8(8): 1107-1109.
- RAJ JB, PARTHASARATHY S, KUMARAPPAN M, SRINIVASAN AR (2022) Effect of *costus pictus* D don methanolic leaf extract on induced prediabetic behavioral change in albino Wistar rats. *Asian J Pharm Res Health Care*, 14: 224-230.
- RAMACHANDRAN A, SNEHALATHA C, VISWANATHAN V (2002) Burden of type 2 diabetes and its complications - the Indian scenario. *Curr Sci*, 83: 1471-1476.
- SETH SD, SHARMA B (2004) Medicinal plants in India. *Indian J Med Res*, 120(1): 9-11.
- WHO.int. (2023) Diabetes. Last assessed on 31Jan 2023. Available from: <https://www.who.int/news-room/fact-sheets/detail/diabetes>