International Journal of Pharmacology and Clinical Research (IJPCR)

IJPCR |Vol.9 | Issue 1 | Jan - Mar -2025

www.ijpcr.com

DOI : https://doi.org/10.61096/ijpcr.v9.iss1.2025.50-56

ISSN: 2349-5448

Research

Antidiabetic Evaluation Of Ethanolic Extract Of *Phyllanthus Retticulatus* In Stz Induced Diabetic In Rats

S. Ganesh*, Dr. Suresh. V

J.K.K.Munirajah Medical Research Foundation, Annai J.K.K. Sampoorani Ammal College Of Pharmacy, Komarapalayam, Namakkal- 638 183 Tamil Nadu. India.

*Author for Correspondence: S. Ganesh. M.Pharm.
Email: ganeshsubramanian96@gmail.com

Published on: 05 Mar 2025

Published by:
DrSriram Publications

Abstract

The possible protective effect of ethanolic extract of *P.retticultus* leaves (EEPR) on diabetes and diabetes-induced oxidative stress was evaluated in Streptozotocin (STZ)-induced diabetic male adult wistar albino rats.. Experimental animals were divided into five groups viz., group-1 control normal saline, group-2 diabetic control, group-3 test dose for 200mg, group-4 test EEPR for 200mg/kg body weight,p.o, group-5 standard dose of glibenclamide0.5mg/kg,b.w.p.o. Diabetes mellitus (DM) was induced in groups II and III mice by a single intraperitoneal injection of Streptozotocin (50 mg/kg body wt). Group I (control mice) received an equal volume of normal saline. Group III mice were further treated with EEPR (200 mg/kg body wt, p.o.) for a period of 21 days. Body weight and fasting blood glucose (FBG) levels were measured at periodic intervals during the test period. At the end of treatment period, blood was collected by cardiac puncture under mild ether theo pental sodium and serum was isolated to analyze its lipid profile i.e. serum total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL). The homogenates of hepatic, pancreatic and renal tissues were also analyzed for both enzymatic and non-enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS) and total protein (TP).

Keywords: Phyllanthus Retticulatus

INTRODUCTION

Herbs are staging come back and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that were regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern Age surpassed the importance, fora while. However, the blind dependence on the Synthetics is over and people started returning to the naturals with hope of safety and security. Over the three-quarters of world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or other was used for medicinal purposes.

It is estimated that world market for plant derived drugs may account for about Rs.2,00,000 crore. It has been estimated that in developed countries like United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China, India and Bangladesh, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India, Bangladesh than to rest of the world. These countries provide two third of the plants used in modern system of medicine and the healthcare system of rural population depend on indigenous systems of medicine. Of the 2, 50,000higher plant species on earth, more than 80,000 are medicinal. The drugs are derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc.

Traditional plant medicines are herbal formulations, which might of fera natural key to unlock diabetic complications. Medicinal plants plays an important role in the management of diabetes mellitus especially in developing countries where there sources are meager. Medicinal plants used to treat anti hyperglycemic conditions are of considerable interest for ethno-botanical community as they are recognized to contain valuable medicinal properties in different parts of the plant and a number of plants have shown varying degree of anti-hyperglycemic activity (Sowmya PRet.al.,2009).

Plant profile

Fig 1: *Phyllanthus retticulatus poir*

In traditional culture medicinal plants are used all over the world and they are becoming increasingly popular in modern society as natural alternatives to synthetic chemicals 1. *Phyllanthus reticulatus* (Family – Euphorbiaceae) commonly known as pancoli or karineli, (Synonym: Kirganelia reticulata Poir.) is a large glabrous or pubescent shrub with smooth or lanticellate branches growing from in height. The plant is widely present in Tropical Africa, Srilanka, South East Asia, China, Malaysia and throughout India, mostly in hedges or waste places. Fruiting and flowering season of plant is from July to March. Leaves of the plant contain tannic acid and gum. Fruit of the plant becomes purplish black when ripe. The leaves are used as diabetic and cooling medicine. It is reported to be used as diuretic, alternative, attenuant, astringent and as antidiarrhoel .

Regional and other names

- Sanskrit : Pulika,Krishna- kamboji
- Bengali : Panjulil
- Gujarati : Datwan
- Hindi : Panjuli
- Kannada : Pulaveri, Anamsule
- Malayalam : Niruri, Nireli
- Marathi : Pavana
- Oriya : Jandaki
- Tamil : Abaranji, Karunelli, Kattukilanelli
- Telugu : Nallapurugudu, pulaguwa, Phulsar

Classification

- Domain : Eukaryota
- Kingdom : Plantae
- Claudus : Angiosperm
- Order : Malpighiales

- Family : Euphorbiaceae
- Tribe : Phyllantheae
- Genus : Phyllanthus
- Species : P. reticulatus

Geographical range

Phyllanthus retticulatus is found in West Indies, Central and South America and Tropical Asia. In India it is seen in Peninsular India, Kerala, Karnataka and Tamil Nadu.

MATERIALS AND METHOD

Collection of Plant *phyllanthus retticulatus*

The whole plant of *phyllanthus retticulatus* used for the present study was obtained from Nandivaram, Guduvancherry, Chennai, TamilNadu, India.

Authentication of plant

The plant material was identified and authenticated by Dr.V. Aravindhan, Assistant Professor, Department of botony, kongunadu arts and science college, Coimbatore–641029, Tamilnadu.

Sample Extraction

The sun-dried and ground plant materials (2.5kg) were successively extracted by continuous cold percolation over a 48h period with petroleum ether (60–80°), ethyl acetate, and, finally, with methanol at room temperature. The extracts were filtered and concentrated with a rotary evaporator at 40–50°C and reduced pressure and subsequently defatted to obtain the dried petroleum ether (PHRPE), ethyl acetate (PHREA), and methanol (PHRME) extracts. The yields of the extracts were 0.44, 0.68, and 6.84%, respectively.

Percentage yield

The percentage yield of hydro alcoholic extract was 6.84 % w/v and it was preserved in refrigeration for further use.

Chemicals

All the Chemicals used in the study were of analytical grade. The following chemicals were used for the experimental study.

Table 1: Name of the chemicals and their source

S.No	Materials	Sources
1.	Absolute alcohol	S.d. fine chemicals Ltd, Mumbai
2.	Chloroform	S.d. fine chemicals Ltd, Mumbai
3.	Copper sulphate	Qualigensfinechemicals,Mumbai
4.	Creatinine kit	Spandiagnosis Ltd,Bangalore
5.	DNS(3,5-dinitrosalicylicacid)	Sigma chemical Co.,USA
6.	Ethanol	S.d. finechemicals Ltd, Mumbai
7.	Glucose test strips	One touch Horizon test strips
8.	HDL kit	Span diagnosis Ltd, Bangalore
9.	Hydrochloric acid	Qualigens fine chemicals, Mumbai
10.	Hydrogen peroxide solution	Qualigens finechemicals, Mumbai
11.	Sodium hydrogen carbonate	S.d. finechemicals Ltd, Mumbai
12.	LDL kit	Spandiagnosis Ltd,Bangalore
13.	Petroleum ether	Sigma chemical Co.,USA
14.	p-nitro phenyl gluco pyranoside	S.d. fine chemicals Ltd, Mumbai
15.	Pyrogallol	S.d. fine chemicals Ltd, Mumbai
16.	SGOT kit	Span diagnosis Ltd, Bangalore
17.	SGPT kit	Span diagnosis Ltd, Bangalore
18.	Sodium hydroxide	Qualigens fine chemicals,Mumbai
19.	Total protein kit	Span diagnosis Ltd,Bangalore
20.	Total cholesterol kit	Span diagnosis Ltd,Bangalore
21.	Triglycerides kit	Span diagnosis Ltd,Bangalore
22.	Urea kit	Span diagnosis Ltd, Bangalore

23.	VLDL kit	Span diagnosis Ltd, Bangalore

Experimental design

In the present study, Swiss albino rats (male), which weighed between 20-25 g were used. The animals were obtained from the International Centre for Diarrheal Disease Research, Bangladesh (ICDDRB). All animals were kept under ambient temperature with 12 h light followed by a 12 h dark cycle. The animals were acclimatized for one week prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

Histopathology

At the end of the study, all the animals were sacrifices under light ether anesthesia. The rats were sacrificed by decapitation and blood samples were collected by bleeding of retro-orbitol plexus using micro capillary technique from all the groups of overnight fasted rats and serum was separated to study the biochemical parameters. The relevant organs like pancreas, liver were removed and dissected out and washed with ice-cold saline. The organs were preserved in 10% formalin solution for histopathological studies.

Statistical analysis

The data were expressed as mean ± standard error (SEM). The significance of differences among the groups was assessed using one way analysis of variance (ANOVA). The test followed by Dunnet's and foster test p values lees than 0.05 were considered as significance.

RESULTS AND DISCUSSIONS

Acute Oral Toxicity Study: (OECD^{423} GUIDE LINES)

Acute oral toxicity study was performed as per CPCSEA guidelines (acute toxic class method). The group of six animals were kept fasting for over night and provided only with water, after which the extracts were administered orally at 2mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 200, and 400 mg/kg body weight. (Kumar A et al.,2008)

Effect of EEPR on body weight of STZ induced diabetic rats

It was found that the body weight was decreased significantly when the comparison was made between group I with group II, group III, group IV and group V. -The bodyweight in group II was compared with group III, group IV and group V were increased significantly. The results were shown in (Table-4)

Table 2: Effect of EEPR on normoglycaemic and glucose loaded hyperglycaemic rats (NG –OGTT)

Groups	Test Sample (Mg/Kg)	Blood Glucose Levels (Mg/Kg)					
		0min	30min	60min (Glucose Load)	120min	150min	270min
1	Saline	80.32± 2.34	79.57±4.26	84.73±4.23	127.2±4.23	103.2±2.12	84±3.21
2	EEPR-200	83.73±4.56	85.12±2.34ns	77.33±3.25ns	118.0±3.41	89.32±4.34*	78.82±5.30*
3	EEPR-400	87.61±4.40	79.73± 5.38ns	72.21±5.38**	112±3.44**	79.40±2.31**	73.00±3.54**
4	Standard (Glibencl Amide)	84.16±4.34	67.42±3.67**	58.57±2.28**	98.57±5.30**	72.14±4.15**	60.32±4.67**

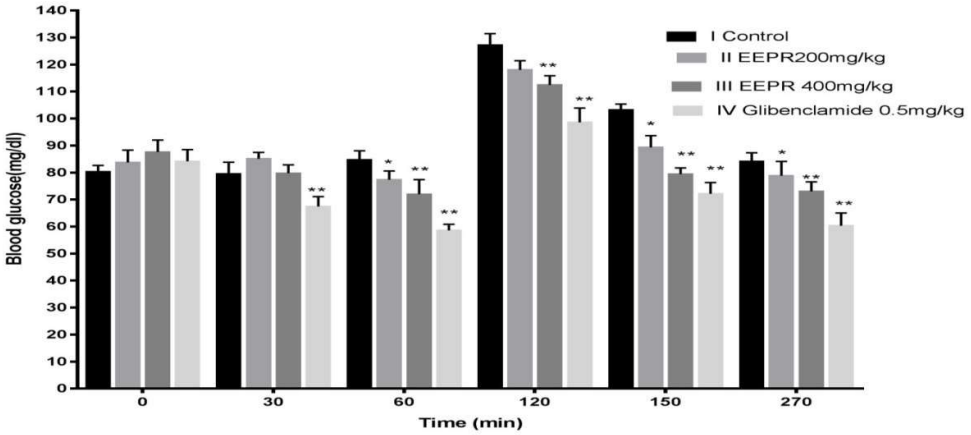

Fig 1:

Table 3: Effect of single dose treatment of EEPR on blood glucose level in STZ induced diabetic

Treatment	Blood glucose level(mg/dl)				
	0min	30min	60min	120min	240min
Saline	83.2±1.32	87.5±2.24	90.2±2.05	92.6±3.27	96.7±2.10
Diabetic Control (STZ)	271.4±2.13	274.8±3.62ns	276.3±3.54ns	282.7±2.61*	292.5±3.56**
STZ+ EEPR (200mg/kg)	275.6±2.62	281.3±2.45ns	268.5±3.51ns	265.1±3.28*	238.2±1.73**
STZ+ EEPR(400mg/kg	270.2±2.67	278.4±3.15ns	264.7±2.45ns	253.2±3.15**	232.5±2.26**
Glibenclamide +STZ	267.3±3.42	259.5±4.26ns	250.7±3.71*	235.2±4.25**	224.1±3.34**

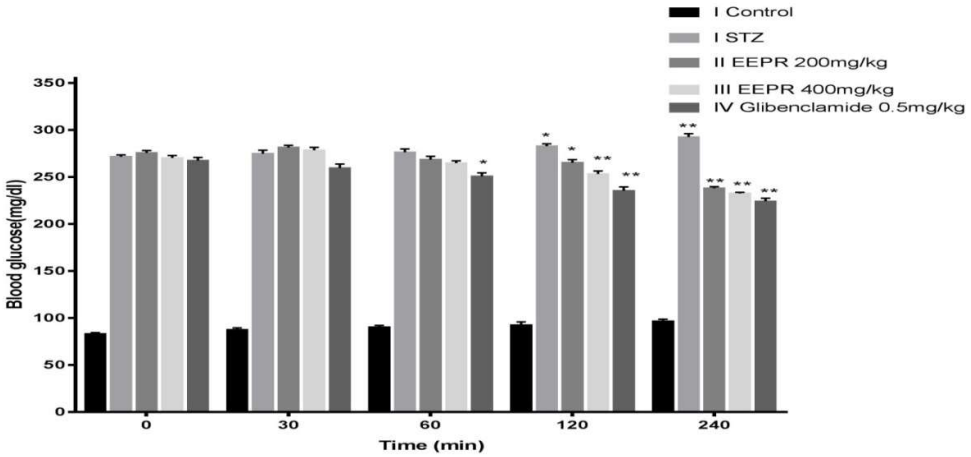

Fig 2:

Histopathologicl
Pancreas

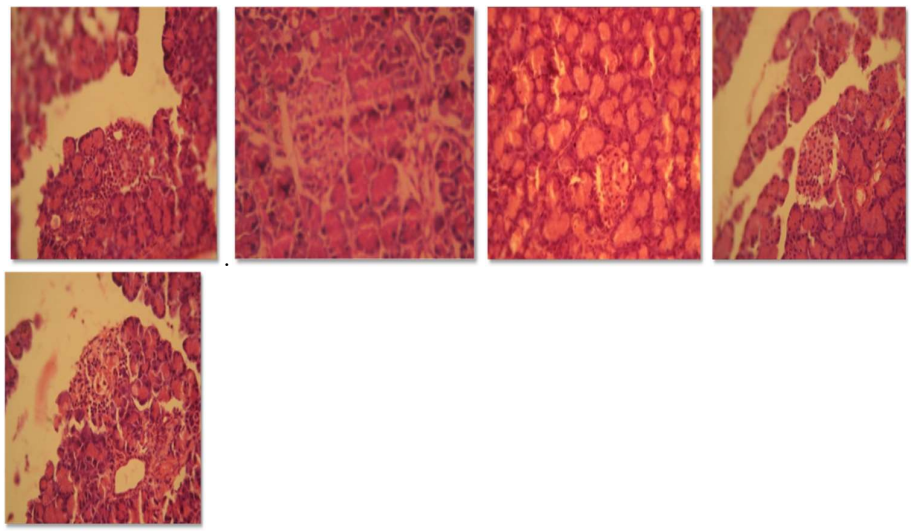

Fig 3:

1. Normal control - Haematoxylin & Eosin stained section shows pancreas with normal islets and acini.
2. STZ GROUP- H & E stained section shows damaged and atrophic islet with acini.
3. EEPR 200 mg/kg-H & E stained section shows small pancreatic islet cells.
4. EEPR 400 mg/kg- H & E stained section shows hyperplastic islet with acini.
5. Glibenclamide treated - H & E stained section shows preserved cells

It is well known that the toxic glucose analogue STZ selectively destroys insulin hormone producing pancreatic beta-cells in rodents, there by causing insulin-dependent diabetes in these animals, which has similar characteristics as observed in humans In the present study, a single intra peritoneal injection of STZ monohydrate (50 mg/kg) led to significant pancreatic damage as was judged by pertinent biochemical and histopathological changes. Fourteen days continuous treatment with methanol extract (200 and 400 mg/kg), ethyl acetate fraction (100 and 200 mg/kg), and mother liquor (200 and 400 mg/kg) moderately alleviated the elevated levels of blood glucose, triglycerides, total cholesterol as well as other biochemical parameters, but the highest reduction in blood glucose concentration, triglycerides, total cholesterol, creatinine, and urea occurred with the largest dose of ethyl acetate fraction (400 mg/kg) of *P. retticulatus*. Histopathological examination of pancreas also revealed highest protection with the *P. retticulatus* ethyl acetate fraction (400 mg/kg). To the best of our knowledge, this is the first study to report the antidiabetic effects of *P. retticulatus* extracts in alloxan-induced diabetes in the rat model. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) through redox reaction.

It appears that alloxan-induced toxic injury in the pancreatic β-cells is initiated by the free radicals formed via redox reaction Oxidative stress resulting from metabolic syndrome can damage the cell membranes and components, including lipids, proteins, glutathione, metabolic enzymes and DNA. In our study, alloxan-induced diabetes resulted in significant oxidative stress related changes in the pancreas and kidneys. There was a marked increase in lipid peroxides (TBARS, SAG) and reduction in GSH concentration in both pancreas and kidney tissues.

There was significant reduction in lipid peroxides accompanied by marked increase in GSH level by treatment with methanol extract (200, 400 mg/kg), ethyl acetate fraction (200, 400 mg/kg) and mother liquor (200, 400 mg/kg). The greatest attenuation of these biochemical parameters was observed with ethyl acetate fraction of *P. retticulatus* (400 mg/kg) .Histopathological studies of pancreas and renal tissues support noticeable destruction of islets of Langerhans which were then replaced by fibrotic cells in pancreatic tissue and thickening of glomerular membrane, mesangial expansion and inflammation of interstitial cells in renal tissues. These histopathological observations lend additional support and are consistent with the previous alloxan-induced diabetic studies Fourteen days continuous treatment with *P.* retticulatus extracts not only decreased inflammation and lipoid nephrosis in renal tissues in comparison to alloxan diabetic control group, but also significantly reduced morphological abnormalities in kidneys and pancreas, accompanied by regeneration and expanded islet cells in pancreas.

CONCLUSION

Oral administration of *P.* retticulatus extracts to rats significantly improved alloxan-induced biochemical alterations. There was also a significant reduction in lipid peroxidation and also significantly reduced the morphological changes in pancreas and kidneys supporting the biochemical improvements. The findings of this investigation suggest that polyphenol catechin present in *P.* retticulatus may be primarily responsible for the anti-diabetic and renoprotective activity in rats which could be mediated through its flavonol-induced anti-oxidant and anti-inflammatory activities in the pancreas. Further studies are warranted to ascertain the underlying antidiabetic mechanism of *P. retticulatus* extracts containing catechin and other ingredients found in this medicinal plant.

REFERENCES

1. Ivorra MD, Paya M, Villar A. A review of natural products and plants as potential anti diabetic drugs. J Ethnopharmacol 1989;27: 243.
2. Shukia R, Sharma SB, Puri D. Medicinal plants for treatment of diabetes. Indian J Clin Biochem 2000; 15:169.
3. Sowmya PR, Rout KA, Chowdary DM. Plants as source of Novel Anti- Diabetic Drug: Present Scenario & Future Perspectives. Current Trends in Biotech & Pharmacy 2009;3: 37- 55.
4. Swarnalatha Saraf, Mahendra Singh Ashawat. Flavonoids: A Nutrional Protective against oxidative and UV induced cellular damages. Pharmacognosy Reviews 2007; 1:30-38.
5. Fabricant DS, Farn Swarth NR. The value of plants used in traditional medicine for Drug Discovery. Environ Health Perspectives 2007; 109:69-75..
6. Newmann DJ, Gragg GM. Natural Products as sources of New drugs over the last 25 years. J Nat Products 2007; 70:461-77.
7. King H, Aubert RE, Herman WH. Global burden of diabetes,1995 -2025 -Prevalence, numerical estimates and projections. Diabetes Care 1998; 21:1414-1431.
8. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27: 1047-1053.
9. Viswanathan M, McCarthy MI, Snehalatha C, Hitman GA, Ramachandran A. Familial aggregation of type 2 diabetes mellitus in South India. Diab Med 1996; 31: 232-237.
10. Lt Gen SR Mehta, Col AS Kashyap, Lt Col S Das et al.Diabetes Mellitus in India: The Modern Scourge 2009; 65:50-54.
11. The effect of intensive treatment of diabetes on the development and progression of longterm complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. N Engl J Med 1993; 329:977–986.
12. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998; 352:837–853.
13. Goldstein DE, Little RR, Lorenz RA, Malone JI, Nathan DM, Peterson CM. Tests of glycemia in diabetes. Diabetes Care 2004;27(Suppl 1): S91–S93.
14. A Ramachandran, A K Das, SR Joshi et al.Current Status of Diabetes in India and Need for Novel Therapeutic Agents june 2010; 58:7-9.
15. Definition, Diagnosis and classification of diabetes mellitus and its complications, Report of a WHO consultation 10. IDF Diabetes Atlas, 4th edition. International Diabetes Federation, 2009.
16. Siddharth N.S. Containing the global epidemic of diabetes. Journal of Diabetology. 2001 3:11.
17. Davis S.N., Granner D.K. Insulin, oral hypoglycemic agents and the pharmacology of endocrine pancreas. In: Goodman I S; Gilman AG, eds, The Pharmacological Basis of Therapeutics, 9th Ed. New York: Mc-Graw Hill; pp.1996; 1487-1517.
18. Sharma A.K. Diabetes mellitus and its complications: An update, 1st Ed. New Delhi: mhb Macmillan India Ltd. pp.1993; 92-205.
19. Sharma A.K 4th edition. International Diabetes Federation, 2009.
20. IDF Diabetes Atlas, 6th edition. International Diabetes Federation, 20014. Available from http://www.idf.org/diabetesatlas (Acessed january 31,2014).