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ANTI DIABETIC EFFECT OF BARK EXTRACT OF *PHYLLANTHUS ACIDUS*

Shilali K[1] and Ramachandra YL[2]*

[1]Research scholar, Department of Biotechnology, Kuvempu University, Shankaraghatta- 577451, Shivamogga, Karnataka, India.
[2]Department of Biotechnology, Kuvempu University, Shankaraghatta- 577451, Shivamogga, Karnataka, India.

ABSTRACT

The present study was carried out to evaluate the antidiabetic activity of bark ethanol extract of *Phyllanthus acidus* in alloxan induced diabetic rats. The ethanol extract at 500 mg/kg exhibited moderate anti-hyperglycemic activity in diabetic rats. The ethanol extract (PAE) also showed improvement in parameters like body weight and lipid profile as well as regeneration of pancreatic beta cells in diabetic rats. The 28 days study shows the markers of diabetic conditions restoring back to near normal levels. Histopathological studies reinforce the healing of pancreas, by PAE, as a possible mechanism of their antidiabetic activity.

Key Words: Hyperglycemia, *Phyllanthus acidus*, Glucosylated hemoglobin, Creatine kinase, Histopathology.

INTRODUCTION

Diabetes mellitus, a common pancreatic islet disorder caused by an inability to produce insulin or a defect in its utilization. It is a chronic metabolic disorder with vascular components that is characterized by disturbances in carbohydrates, lipids and protein metabolism [1]. Long-term complications of diabetes mellitus include retinopathy, nephropathy, neuropathy, microangiopathy and increased risk of cardiovascular disease [2, 3, 4].

To achieve glycemic control, therapeutic agents like insulin, sulfonylureas, biguanides and thiazolidinedione derivatives etc are used. However, on chronic usage most of these agents produced several side effects, including hypoglycemic coma, insulin resistance, hypersensitivity, cholesterol jaundice, abdominal pain, anorexia and metallic taste [5].

In addition, increased cost of treatment and high rates of failure made it difficult to afford and use these agents for a prolonged period. Until the time insulin was invented, this disorder was managed principally by using medicinal plants due to their low cost, easy accessibility and less side effects. There are numerous traditional medicinal plants reported to have hypoglycemic properties such as Allium sativum (Garlic), Azadirachta indica (Neem), Vinca rosea (Nayantara), Trigonalla foenum (Fenugreek), Momordica charantia (Bitter ground) and Ocimum santum (Tulsi) [6].

The plants of genus Phyllanthus (Euphorbiaceae) are widely distributed and long been used in traditional medicines [7]. Presence of potential phytoconstituents in the genus Phyllanthus has led to some promising findings in several disorders [8]. A few species of this genus are also reported to possess antidiabetic activity [9, 10]. Thus the plant is up taken to study its action over insulin dependent diabetes.

Corresponding Author

Y L Ramachandra
Email: shilali.k@gmail.com

MATERIALS AND METHOD

Chemicals and Kits

Alloxan was procured from Sigma Aldrich, St. Louis, USA. Ready to use Citrate buffer and CMC were purchased from Himedia, Mumbai. All biochemical kits were procured from ERBA diagnostics, India.

Plant material and preparation of extracts

Stem bark of *P. acidus* was collected from Balehonnur forest area of Western Ghats, Chikkamagalur (Dist.), Karnataka. Stem bark was cleansed thoroughly,

shade dried, pulverized mechanically and sieved (sieve no. 10/44). The extract was defatted using petroleum ether in Soxhlet apparatus. Further, hot extraction was carried out with defatted material (600 g) successively with chloroform (1.5 L, 45°C, ≈15 cycles) and ethanol (2 L, 50°C, ≈15–17 cycles) to get ethanol extract. The extract was dried in vacuum. Ethanolic extract (PAE) was stored in desiccators to avoid oxidation until further studies.

Grouping and drug treatment

Adult healthy male swiss albino rats with body mass of approximately 200–225 g were used. The animals were conditioned at room temperature and at natural photoperiods for 1 week before study. A commercial balanced diet and tap water *ad libitum* were provided. The animals were initially divided into two groups, the first group (6) received saline solution intraperitonially (i.p.) and it was kept as control (Group I). The second group (18 rats) was injected with a single intra peritoneal dose of Alloxan at 160 mg/kg [11] of body weight, dissolved in 0.01 M citrate buffer, pH 4.5, immediately before use.

Six days later blood glucose levels were determined in this group in whole blood samples collected from the tip of the tail to confirm the diabetic condition. Diabetic animals were further divided into three groups of 6 rats each.

A total of 24 rats (n = 6) were randomly divided into four experimental groups (Group I to Group IV) as follows:

Group I: normal rats treated with water orally once a day for 4 weeks.

Group II: diabetic rats treated with water orally once a day for 4 weeks.

Group III: diabetic rats treated with PAE (500 mg/kg b.wt.) orally once a day for 4 weeks.

Group IV: diabetic rats treated with Glipizide (25 mg/kg b.wt.) orally once a day for 4 weeks.

Collection of blood and its serum

At the end of the experiment, rats were fasted overnight and anesthetized with sodium pentothal (intraperitoneally) and 4 mL of blood was withdrawn through the retro-orbital plexus using a glass capillary and collected in tubes. Collected blood was centrifuged for 10 minutes at 3000 rpm. Plasma and serum was separated for the analsysis.

Evaluation of hyperglycemic markers in blood

Blood glucose levels were estimated on 1, 7, 14 and 28 day of experiment and recorded. Body weights were monitored and recorded weekly during the entire experiment.

The plasma obtained was used for glucose and glycosylated hemoglobin ($HbA1_C$) using commercial kits (ERBA diagnostics). The serum samples were used for the estimation of creatine kinase (CK), lactate dehydrogenase (LDH) using commercial kits (ERBA diagnostics).

Histopathology

The animals were sacrificed and pancreas was separated and subjected for histopathological studies. The cleaned tissue was fixed in 10% formalin, dehydrated in gradual ethanol grades (50–100%), cleared in xylene and embedded in paraffin. Sections of 3- 5 micron thickness were prepared, processed in alcohol- xylene series and were stained with haematoxylin and eosin (H–E) dye for photomicroscopic observation for the evaluation of histological changes and quantification of Beta-cells and Alpha-cells.

RESULTS

Biochemical findings

The blood glucose increased in ALX-diabetic rats as compared to normal rats. Treatment of ALX-diabetic rats with test extract PAE reduced the hyperglycemia moderately and when compared with ALX alone treated rats (Table 1).

From the results it is observed that diabetes induced a significant decrease in body weight in ALX-induced diabetic rats when compared to controls. Test extract, PAE treated diabetic rats showed augmented body weight when compared with ALX alone treated rats (Table 2). Rats lost their weight after ALX treatment and recovered moderately on treatment with PAE. Whereas, on treatment with Glipizide diabetic animals gained body weight.

$HbAI_C$ levels were higher in the ALX-induced diabetic rats compared to the control rats. The supplementation of PAE decreased the $HbAI_C$ level of the ALX induced diabetic rats. Antidiabetic activity of the test extract PAE at 500 mg/kg body wt dose was moderately affective. The creatine kinase in alloxan treated rats increased, which was redeemed to normal levels by the drug Glipizide. However, the PAE treated rats showed moderate decrease in the level of creatine kinase. The similar pattern was seen in the estimation of lactate dehydrogenase.

Histopathology

Section studied shows pancreatic lobules separated by connective tissue septa. The pancreatic lobules consist largely of the exocrine acini and their intralobular ducts (Figure 1). The section of the normal pancreas (Figure 1-A) the lobules show small, round, light-staining islets of langerhans. The center of islet cells consist of aggregates of small Beta-cells (75%, Short-arrow), while the periphery comprises of large Alpha-cells (20%, Long-arrow). Intervening these cells are seen thin walled capillaries. The pancreatic section of alloxan treated rat showed number of islets appears quantitatively reduced in number [compared to Standard Group]. The center of

islet cells consists of quantitative decrease in Beta-cells (35%, Fig. Short-Arrow), while the periphery comprises of Alpha-cells (60%, Fig. Long-Arrow). Also seen are some degenerated Beta cells within the islets. The animals treated with Glipizide shows quantitative increase in the number of islets. The center of islet cells consists of Beta-cells (70%, Fig. Short-Arrow), while the periphery comprises of Alpha-cells (25%, Fig. Long- Arrow). The action of PAE shows number of islets appears quantitatively increased in number (compared to alloxan treated group Group). The center of islet cells consists of Beta-cells (55%, Fig. Short-Arrow), while the periphery comprises of Alpha-cells (40%, Fig. Long-Arrow).

Table 1. Effect of test extracts on blood glucose levels during treatment

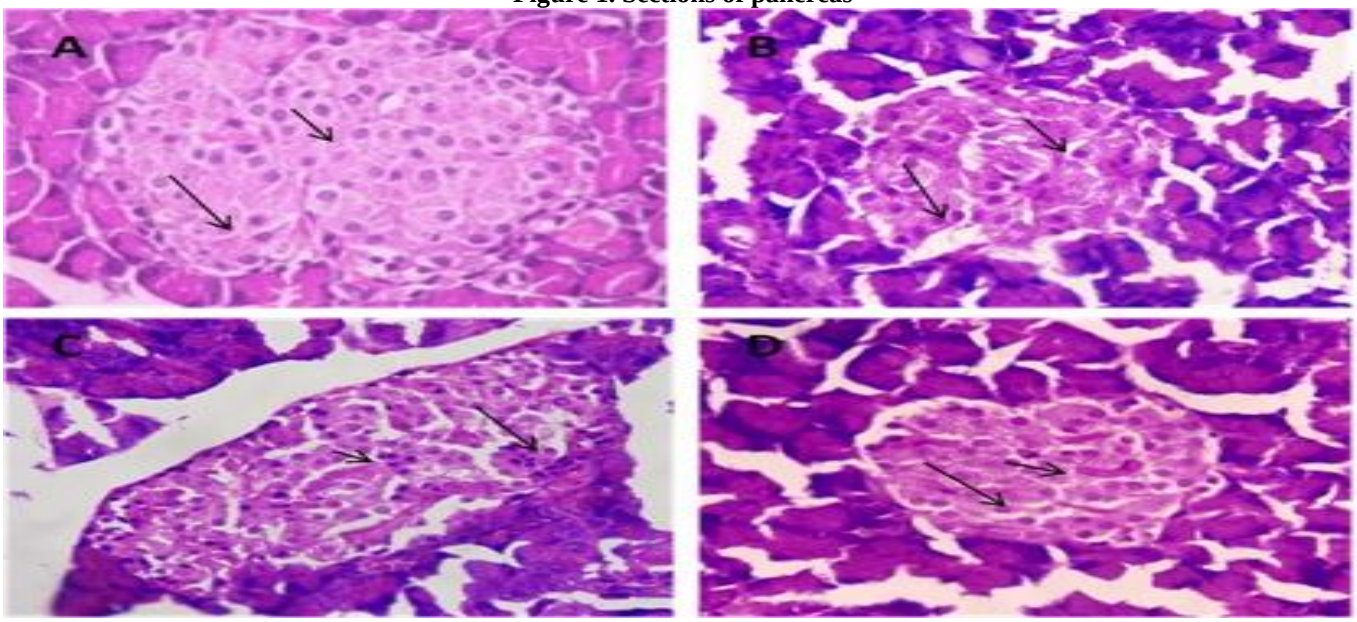
Groups	Treatment	Blood glucose level in mg/dl			
		0 day	7th day	14th day	28th day
I	Control	111.00 ± 8.56	105.16±9.37	103.16 ± 5.61	107.83±6.76
II	ALX treated control	264.66±13.12	271.83±11.68	280.33± 12.80	299.66±20.52
III	ALX + PAE (500 mg/kg)	263.5±10.24	247.00±9.25	230.66± 10.68	214.33±15.95
IV	ALX + GLI (25 mg/kg)	262.16±14.15	197.50±18.24	161.83±11.85	142.83±14.58

Table 2. Effect of test extracts on ALX induced changes on the body weight and blood glucose, $HbA1_C$, CK and LDH

Groups	Treatment	Body weight (g)	$HbA1_C$ (%)	CK (IU/L)	LDH (IU/L)
I	Control	245.5± 15.39	1.31± 0.07	206.3±6.71	90.33±8.66
II	ALX treated control	148.0±12.51	4.033±0.20	290.3±12.86	157.5±17.09
III	ALX + PAE (500 mg/kg)	170 ±18.23	3.197±0.13	264.5±12.61	136.7±6.47
IV	ALX + GLI (25 mg/kg)	215.3±12.4	2.765±0.16	235.5±6.65	117.3±7.03

Values are expressed as mean ± SD for six animals in each group values $P<0.05$ and $P<0.001$ between control versus diabetic control, Diabetic control versus treated groups.

Figure 1. Sections of pancreas



A. Normal pancreas, B. alloxan treated pancreas, C. Glipizide treated pancreas D. PAE treated pancreas.

DISCUSSION

Management of diabetes with the agents devoid of any side effects is still a challenge to the medical system. This concern has led to an increased demand for natural products with antihyperglycaemic activity, having fewer side effects.One of the most potent methods to induce experimental diabetes mellitus is chemical induction by Alloxan [12]. It is a well- known diabetogenic agent that is used to induce Type I diabetes in experimental animals [13]. Alloxan has a structure very similar to glucose and hence GLUT2 glucose transporter in the beta cell plasma membrane accepts the alloxan molecule. The alloxan acts

on the glucukinase which functions as a glucose sensor for glucose induced insulin secretion [14] and also by the formation of reactive oxygen species that cause tissue damage [15].

Number of plants has been used traditionally in treatment of diabetes and some have been proven scientifically to have hypoglycemic activity. The PAE has a number of secondary metabolites like tannins, flavonoids, sterols, alkaloids and phenolic compounds which can contribute to the reversion of oxidative stress and hence aid to revive the beta cells in the pancreas. The renewal of β cells in diabetes have been studied in several animal models. The total β cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet beta cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs from the effects of the drug [16]. The present study shows the regeneration of beta cells in PAE treated animals. Though the regeneration is not as significant as that of the drug Glipizide, PAE at 500mg/kg concentration shows moderate anti-diabetic effect. Rather than possessing a direct tissue repair effect, it is likely that the extract, through antioxidant and hypoglycaemic effects, protected the already compromised pancreas from further assault or tissue damage which then allowed the natural repair processes to proceed and restore the tissues [17].

CONCLUSION

The study shows that there is a moderate and gradual decrease in the blood glucose level of the rats treated with PAE. The other markers like body weight, creatine kinase, glycosylated haemoglobin and latate dehydrogenase showed levels more towards normalisation. The histopathological studies show the increased number of beta cell which clearly speaks the ability of the extract to moderately control the diabetic condition.

REFERENCES

1. Pickup JC, Williams G. Textbook of diabetes, 3rd ed., Blackwell Science Ltd, USA, 2003, 103-114.
2. Laar FA, Lucassen PLBJ, Akkermans RP, Lisdonk EH, Rutten GEHM, Weel C. Alpha-glucosidase inhibitors for type 2 diabetes mellitus (Cochrane Review, The Cochrane Library, 2008.
3. Cheng AYY, Fantus IG. Oral antihyperglycemic therapy for type 2 diabetes. *Mellitus Can Med Assoc J*, 172, 2005, 213-226.
4. Aguiar LGK, Villela NR, Bouskela EA. Microcirculação no Diabetes: Implicações nas Complicações Crônicas e Tratamento da Doença, *Arq Bras Endocrinol Metab***,** 51, 2007, 204-211.
5. Chaudhary SK. Quintessence of Medical Pharmacology, 2nd ed., New Central Book Agency (P) Ltd, Kolkata, 2001, 707.
6. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential, *Journal of Ethanopharmacology*, 81, 2002, 81-100.
7. Kirtikar KP, Basu BD. Indian medicinal Plants, 2nd ed., Vol III, International book distributors, India, 1987, 65-69.
8. Calixto JB, Santos ARS, Filho VC, Yunes RAA. Review of plants of the genus Phyllanthus: their chemistry, pharmacology and therapeutic potential, *Med Res Rev*, 18, 1998, 225-234.
9. Higashino H, Suzuki A, Tanaka Y, Pootakham K. Hypoglycemic effects of Momordica charantia and Phyllanthus urinaria extracts in streptozotocin-induced diabetic rats. Nippon Yakurigaku Zashi, 100(5), 1992, 415-421.
10. Garg M, Dhar VJ, Kalia AN. Antidiabetic potential of Phyllanthus urinaria in albino rats. *Int J Green Pharm*, 1, 2006,168-170.
11. Aruna RV, Ramesh B, Kartha VN. Effect of beta carotene on protein glycosylation in alloxan induced diabetic rats. *Indian J Exp Biol*, 32, 1999, 399-401.
12. Etuk EU. Animals models for studying diabetes mellitus. *Agric Biol J N Am*, 1, 2010, 130-4.
13. Viana GS, Medeiros AC, Lacerda AM, Leal LK, Vale TG, Matos FJ. Hypoglycemic and anti-lipemic effects of the aqueous extract from *Cissus sicyoides*. *BMC Pharmacol*, 8, 2004, 4-9.
14. Tiedge M, Richter T, Lenzen S. Importance of cysteine residues for the stability and catalytic activity of human pancreatic beta cell glukokinase. *Arch Biochem Biophys*, 375, 2000, 251-260.
15. Lee JH, Park JW, Kim JS, Park BH, Rho HW. Protective Effect of Amomi Semen Extract on Alloxan-induced Pancreatic β-Cell Damage. *Phytother Res*, 22, 2008, 86-90.
16. Gorray KC, Baskin D, Brodsky J, Fujimoto WY. Responses of pancreatic β cells to alloxan and streptozotocin in the guinea pig. *Pancreas*, 1(2), 1986, 130–138.
17. Okoli CO, Ibiam AF, Ezike AC, Akah PA, Okoye TC. Evaluation of antidiabetic potentials of Phyllanthus niruri in alloxan diabetic rats. *African Journal of Biotechnology*, 9(2), 2010, 248-259.