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Research Article

PANCREOPROTECTIVE AND ANTI-DIABETIC ACTIVITY OF *PHYLLANTHUS AMARUS* SEEDS METHANOLIC EXTRACTS IN ALLOXON INDUCED DIABETIC RATS

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ABSTRACT

A *Pancreoprotective and Anti-Diabetic activity of Alloxon induced Pancreas were reversed by using methanolic extracts of 150 mg/kg and 250 mg/kg in rats. Seeds of Phyllanthus amarus extracts were found to have Pancreoprotective properties in rats with Alloxon induced Panceatic damage as judged from histopathalogical changes and Anti hyperglycemic activity of methanolic extracts of 150mg/kg and 250mg/kg in Diabetic rats. The methanolic extracts showed improvement in different parameters associated with diabetes like body weight, lipid profile and biochemical parameters. Methanolic extracts also showed improvement in regenation of β-cells of pancreas in Diabetic rats. Histopathological studies strengthen the healing of Pancreas by methanolic extracts of Phyllanthus amarus seeds showing panceoprotective and Anti-Diabetic activity.*

KEYWORDS: *Phyllanthus amarus, Diabetes mellitus, Pancreoprotective, Alloxon induced and Methanolic extracts.*

INTRODUCTION

P*hyllanthus amarus* Schum & Thonn. (Family: Euphorbiaceae) seeds were selected for assessing Panceoprotecrive and Anti-Diabetic potential. However, till date no scientific evaluations are conducted on seeds for confirming its role as organ protective agent. Thus, the present study is designed to investigate the activities of *Phyllanthus amarus* seed extracts on Panceoprotecrive and Anti-Diabetic activity in rat models. Plant based medicaments have been employed since the dawn of civilization for prolonging the life of man for combating various ailments [1].

Phyllanthus amarus Schum & Thonn belong to Euphorbiaceae found in suburban areas of Thirupati town ship. Upon literature survey it was found that plant is mostly untouched. However there is report that the leaves containing lignans and flavonoids known to possess antioxidant activity. However, this plant is relatively virgin and pharmacological and phytochemical profile is incomplete. But traditionally it is being used for treating cardiovascular problems, influenza, dropsy, diabetes, jaundice, asthma and bronchial infections. In India it is used as antifungal, antibacterial and antiviral agent *etc*. keeping the literature review and hypothesis of antioxidant principles and organ protection in view the seeds of *Phyllanthus amarus* was selected for assessing the pancreoprotective and anti-diabetic potential.

Pancreas is an organ of most importance not only as an exocrine organ but also as an endocrine organ of our body. Diabetes mellitus is a common metabolic disorder with micro and macro vascular complication that results in significant morbidity and mortality. It is considered as one of the leading causes of death in the world [2, 3]. Diabetes is well known all over the world even in India it existed since prehistoric age. The practical usage of juices of various plants achieved the lowering of blood glucose levels by 10-20% [4]. Diabetes is a big challenge to the health in most developed countries because of their food habits. Since diabetes is the top five of the most significant diseases and still gaining significance [5]. Hence in such situations exploring new cures from plant sources will always give a positive approach with minimal side effects. Even though an enough work is done with the benefits of medicinal plants but still lot more work is remaining for evaluating their biological importance. Keeping in mind the severity of disease made an effort to treat with this disease which is a global problem.

In this study the prolonged effect (i.e. Up to 14 days) of the methanolic extracts of seeds of *Phyllanthus amarus* in fasting blood glucose (FBG) and biochemical parameters such as serum total cholesterol, LDL, HDL, creatinine, urea and alkaline phosphatase were studied in alloxon induced Diabetic rats.

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MATERIALS AND METHODS

Chemicals:

The toxicant Alloxon monohydrate was obtained from Sigma chemicals (St. Louis, USA). The remaining chemicals used for this study were of analytical grade.

Plant Material:

The seeds of *Phyllanthus amarus* Plant were collected from local gardens of Tirupathi. The Plant was identified and authenticated by Dr. Madava Chetty, Dept. of Botany, Sri Venkateshwara University, Tirupathi, A.P, India

Preparation of Extract:

The crushed and dried seeds of *Phyllanthus amarus* were extracted from methanol by Soxhlet extraction concentrated by rotary vaccum [6] and dried by evaporation until a semi solid mass are produced which is stored in refrigerator below 10^{o}C using air tight container.

Experimental Animals:

Male albino rats weighing 130-160g were obtained from animal house of Nizam Institute of Pharmacy and Research Centre, Hyderabad, Telangana. The animals were kept under standard environmental conditions in polypropylene cages. Thus animals were fed with standard pellet rat diet and water was supplied to animals ad libitum. Experimental protocols were approved by AIEC of CPCSEA.

Acute toxicity studies:

The acute oral toxicity study is determined according to the guidelines of Organization for Economic Co-operation & Development (OECD) following the up & down method (OECD guideline No. 423).

The acute toxicity for methanolic extract of *Phyllanthus amarus* seeds were determined on albino mice and Wister rats, maintained under standard conditions. Low (5 mg) and high (2000 mg) dose was selected for the treatment [7]. Animals were observed no death or any complications up to 28 days.

Oral Glucose Tolerance Test:

Rats were divided into six groups containing six animals in each group. All animals fasted before treatment. Group I was kept as vehicle control which received 5% Tween 80 p. o. group II received glucose only, group III received methanolic extract 150 mg/kg, group IV received methanolic extract 250 mg/kg and group V and VI received only extracts (150 mg/kg and 250 mg/kg) only in a vehicle, respectively. The rats of group III and IV were loaded with glucose (3 g/kg, p. o.) 30 minutes after drug administration. Blood samples were collected from puncturing the retro orbital sinus just prior to drug administration, and at 0, 30, 90, 150 minutes after loading glucose. Serum glucose level was measured immediately by using glucose estimation kit [8].

Experimental Design:

Five groups of six rats in each were used in this model [9]. The study was carried out for 14 days. Except Group-I all groups received alloxan 150mg/kg p. o., once a day.

Group-I: Animals (-ve control) administered 1ml saline p. o.

Group-II: Animals (+ve control) administered Alloxan 150mg/kg i. p.

Group-III: Animals (Standard) administered Alloxon + Glibenclamide 5mg/kg, p. o.

Group-IV: Animals administered Alloxon + methanolic extract 150 mg/kg p. o.

Group-V: Animals administered Alloxon + methanolic extract 250 mg/kg p. o.

Plant extract, standard drug Glibenclamide (5 mg/kg) and saline were administered with the help of feeding cannula. Group I serve as normal control, which received saline for 14 days. Group II to Group V are diabetic control rats. Group III receive Glibenclamide (5 mg/kg. p. o). Group IV and Group V (which previously received alloxan) are given a fixed dose plants extract (150 mg/kg, p. o), (250 mg/kg, p. o) for 14 consecutive days.

Collection of Blood Sample and Blood Glucose Determination:

Blood samples were drawn from tail tip of rat at weekly intervals till the end of study (i.e., 2 weeks). Fasting blood glucose estimation and body weight measurement were done on day 0, 1, 7, and 14 of the study. Blood glucose estimation can be done by one touch electronic glucometer using glucose test strips.

On day 14, blood was collected from retro-orbital plexus under mild ether anesthesia from overnight fasted rats and fasting blood sugar and other bio-chemical parameters like Cholesterol [10], HDL [11], LDL [13], Creatinine, Serum urea [14] and Alkaline pohosphatase [15] was estimated.

After scarifying, whole pancreas of all animals were removed and collected in a solution of 10% formalin and processed by the paraffin technique for histopathological studies.

Statistical Analysis:

Results were expressed as mean ± SEM, (n=6). Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Turkey-Kramer multiple comparisons test by using Graph Pad Instat software. P value less than 0.05 was considered to be statistically significant. *P<0.05, P**<0.01 and P***<0.001, when compared with control and toxicant group as applicable.

RESULTS AND DISCUSSION

Glucose Tolerance:

The methanolic extracts of *Phyllanthus amarus* seeds (150mg/kg and 250g/kg) on glucose tolerance test are shown in Fig. 1. The supplementation of *Phyllanthus amarus* seeds enhanced the glucose tolerance in the fasted normal rats. The serum glucose level was lowered significantly (P<0.05) at 90 minutes and (P<0.01) at 150 minutes. Extracts also showed good hypoglycemic effect after 90 minutes of treatment.

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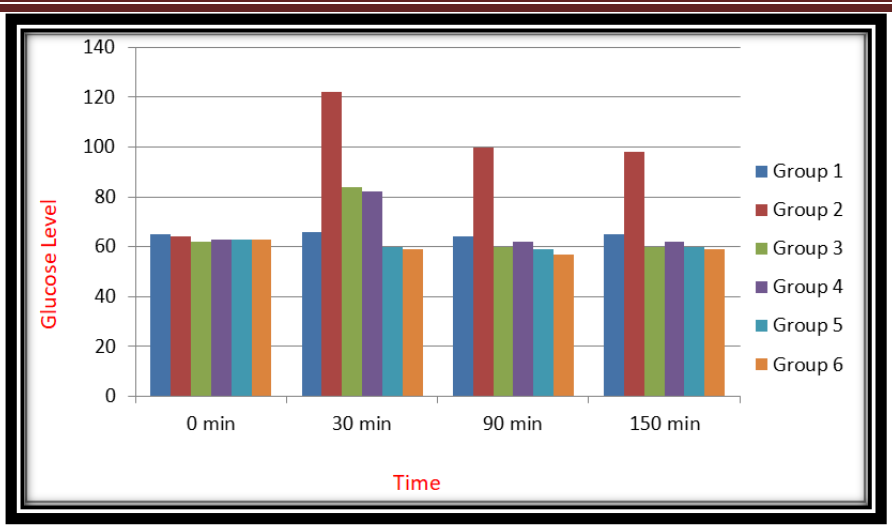


Fig. 1: Effect of methanolic extract of *Phyllanthus amarus* seeds on Glucose tolerance test

Acute Toxicity Study:

This study showed no mortality up to the dose of 2000mg/kg body weight. Hence the extracts can be used for long term.

Blood Glucose Levels of Alloxan Induced Pancreotoxicity:

The methanolic extract exhibited significant ($p<0.01$) anti-diabetic activity in alloxan-induced diabetic rats and improved the condition of Diabetes mellitus as indicated by parameters like body weight & lipid profile along with serum creatinine, serum urea and serum alkaline phosphatase.

In diabetic control there was a significant increase in blood glucose levels when compared to normal control ($p<0.01$). Diabetic rats treated with *Phyllanthus amarus* seeds extract 150mg/kg body weight showed significant decrease in blood glucose levels when compared to diabetic control ($p<0.01$). Diabetic rats treated with Glibenclamide showed significant decrease ($p<0.05$) in blood glucose levels.

Body Weight of Rats on Alloxan Induced Pancreotoxicity:

Body weight decreased on administration of alloxan in group II when compared to normal control ($p<0.01$) and administration of plant extracts showed an increase in body weights when compared to diabetic control rats ($p<0.01$) (Table 2).

Biochemical estimations on Alloxan induced Pancreotoxicity:

It is observed that all biochemical parameters except HDL increase in group II, but *Phyllanthus amarus* seeds extracts and glibenclamide (5 mg/kg) reversed the above alloxan induced changes in group III, IV and V (Table 3).

Histology of Pancreas on Alloxan induced Pancreotoxicity:

Histological examination revealed extensive damage to the islets of Langerhans and reduced dimensions of islets in animals treated with alloxan only whereas partial restoration of normal cellular population and enlarged size of β-cells with hyperplasia was observed by methanolic extracts. Restoration of normal cellular population size of islets with hyperplasia by glibenclamide was also observed.

Histopathological studies reinforce the healing of pancreas, by *Phyllanthus amarus* seeds extract, this shows that it has anti-diabetic activity (Fig. 5).

Table No. 1: Blood glucose levels of different groups of animals on different days treated with *Phyllanthus amarus* seeds extract on Alloxan induced Pancreotoxicity

S. No	0th day	1st day	7th day	14th day
GROUP I	71.33±1.20	74±5.00	75.35±3.74	79.00±5.19
GROUP II	83.35±7.42	200.15±18.5	185.00±6.05	115.00±5.05
GROUP III	77.33±1.20	183.35±7.56	130.00±1.65**	80.19±3.33**
GROUP IV	82.33±12.75	194.33±20.48	145.00±15.68*	85.67±1.56*
GROUP V	88.53±12.75	198.50±19.59	155.75±19.68*	98.33±1.86

Values are expressed as mean ± SEM; n = 6 animals in each group; **= P≤ 0.05 (significant) Experimental groups were compared with standard; *= P ≤ 0.01 (significant) Experimental groups were compared with control

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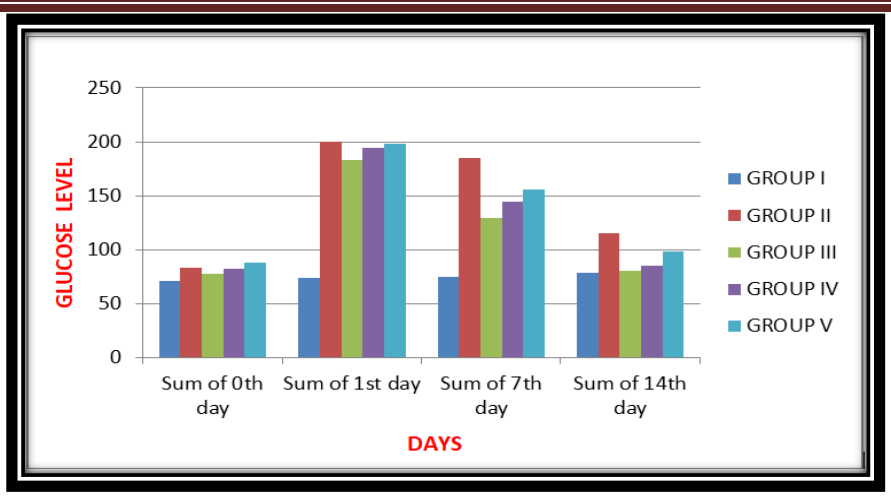


Fig. 2: Blood glucose levels of different groups of animals on different days treated with *Phyllanthus amarus* seeds extract on Alloxan induced Pancreotoxicity

Table No. 2: Body weight measurements for different groups of animals on different days treated with *Phyllanthus amarus* seeds extract on Alloxan induced Pancreotoxicity

S. No	0th day	1st day	7th day	14th day
GROUP I	180.33±7.08	190.33±4.49	189.30±5.23	193.00±7.64
GROUP II	204.57±1.57	182.00±2.31	174.00±3.03	149.00±15.44
GROUP III	180.00±5.77	179.00±2.33	189.59±6.23	195.00±6.79
GROUP IV	192.57±7.37	181.35±11.30	186.00±12.53*	200.67±6.36*
GROUP V	201.00±5.67	173.67±6.33	178.30±6.51**	195.00±1.20**

*Values are expressed as mean ± SEM; n = 6 animals in each group; **= P≤ 0.05 (significant) Experimental groups were compared with standard; *= P ≤ 0.01 (significant) Experimental groups were compared with control*

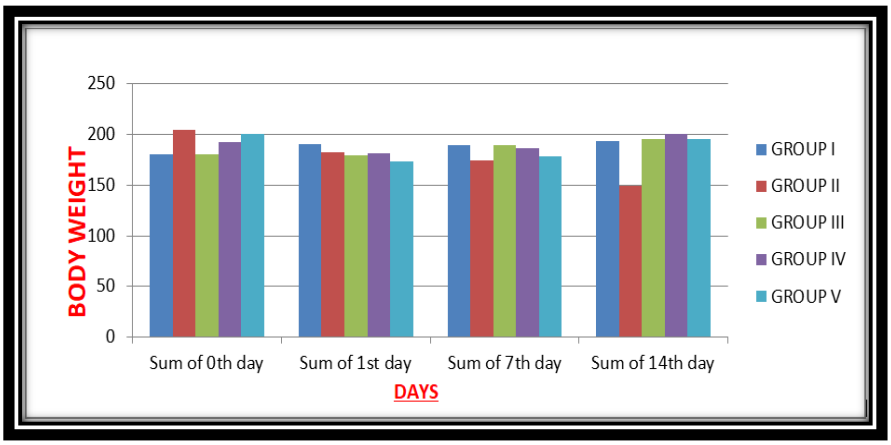


Fig. 3: Body weight measurements of different groups of animals on different days treated with *Phyllanthus amarus* seeds extract on Alloxan induced Pancreotoxicity

Table No. 3: Biochemical parameters measured for all groups from the blood serum treated with*Phyllanthus amarus* seeds extract on Alloxan induced Pancreotoxicity

S. NO.	Cholestrol (mg/dl)	H.D.L (mg/dl)	L.D.L (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	ALP (mg/dl)
Group I	140.36±2.3	35.73±2.5	90.32±1.2	0.53±0.3	30.50±2.2	119±3.5
Group II	270.16±9.5	30.00±0.9	185±11.4	2.4±0.3	61.6±1.6	270.00±2.6
Group III	142.53±5.3	36.73±1.5	92.35±3.1	0.58±0.1	32.44±1.5	130.75±2.5
Group IV	155.46±5.6	35.53±2.1	95.65±2.6	0.65±0.2	33.35±2.0	135.49±5.5
Group V	186.32±2.5	36.22±3.3	120.25±1.5	0.88±0.4	43.3±4.8	146.35±4.9

*Values are given as mean ± SEM for groups of six animals each *P < 0.01 (Dunnet -test). Diabetic control was compared with the vehicle control and extract treated groups were compared with the diabetic control.*

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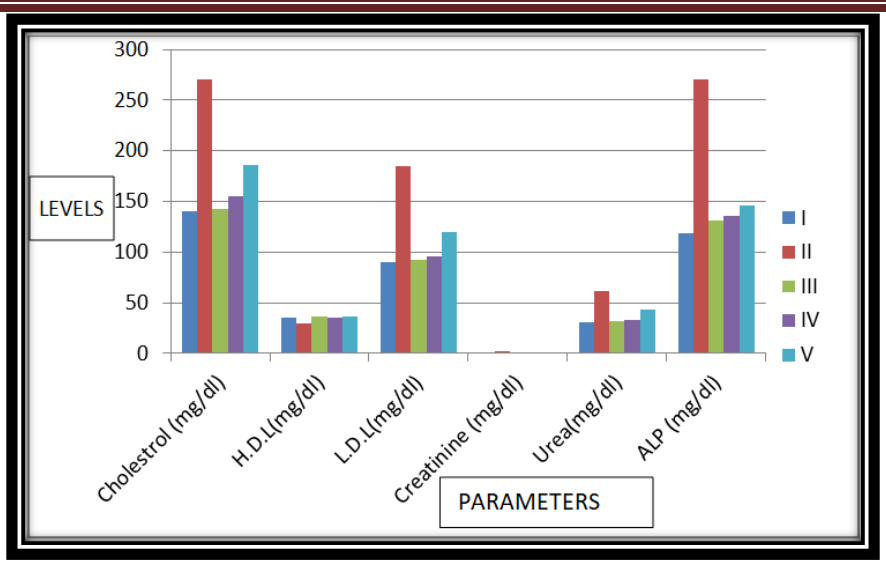


Fig. 4: Biochemical parameters measured for all groups from the blood serum treated with*Phyllanthus amarus* seeds extract on Alloxan induced Pancreotoxicity

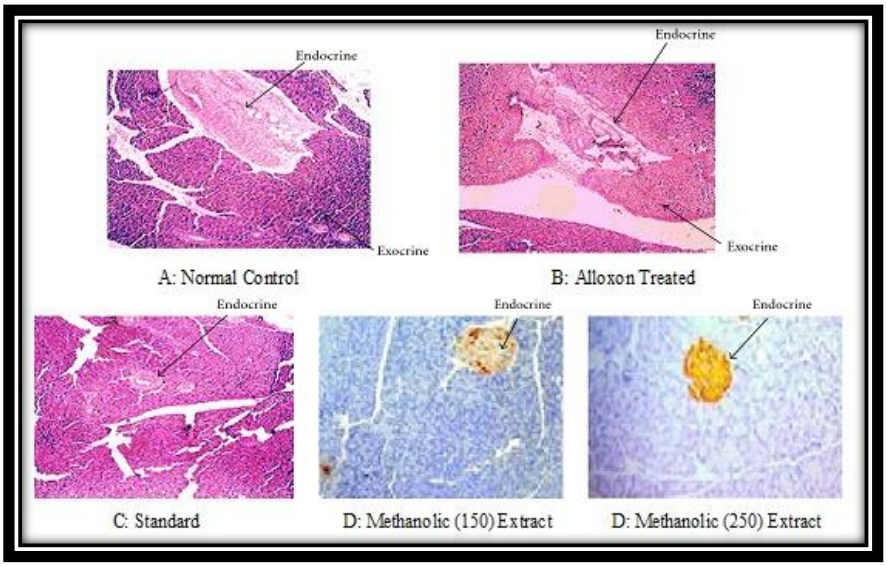


Fig. 5: Histopathological studies of in alloxon induced pancreotoxicity

CONCLUSION

The analysis of results of this study reveals that extracts possess good pancreoprotective and anti-diabetic activity in alloxon-induced diabetic rats. The *Phyllanthus amarus* seeds methanolic extracts of 150mg/kg and 250mg/kg also showed improvement in different parameters associated with diabetes like body weight, lipid profile, histopathological studies and other biochemical parameters. Further studies are required to explain in detail the mechanism of action of *Phyllanthus amarus* seeds at cellular and molecular levels.

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