International Journal of Botany Studies
ISSN: 2455-541X
Impact Factor: RJIF 5.12
www.botanyjournals.com
Volume 2; Issue 6; November 2017; Page No. 93-96

Evaluation of *in-vivo* antidiabetic activity of methanolic extract of *Phyllanthus amarus* schum

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Abstract
Diabetes is metabolic disorders which results from pancreatic beta cells dysfunction & insulin resistance and are characterized by hyperglycemia. The *in-vivo* anti-diabetic potential of *P. amarus* was also determined so as to justify the traditional usage of the plant in treating diabetes. The result of the present study confirmed that the methanolic extract of *P. amarus* possess significant anti-diabetic activity *in-vivo*, this shows that the plants has the potential for the development of drugs in combating diabetes.

Keywords: diabetes, *phyllanthus amarus*, hyperglycemia, *in-vivo*

Introduction
Diabetes mellitus is the third leading cause of death after heart disease and cancer. In many developed countries the complications of diabetes affect the eye, kidney, and nervous system. It is a major cause of blindness, renal failure, amputation, heart attacks, and stroke. It should, however, be noted that diabetes incipidus is another disorder characterized by large volumes of urine excretion due to ADH deficiency (Satyanarayan, 2004) [1].

Type I Diabetes Mellitus (IDDM)
It is a chronic autoimmune disease associated with selective destruction of insulin producing pancreatic β-cells. The onset of clinical disease represents the end stage of β- cell destruction leading to type 1 DM. Type-1 is also known as childhood or juvenile diabetes as most people develop it at childhood. (Homsi and Lukic., 1993; Conget., 2002) [2].

Type 2 Diabetes mellitus (NIDDM)
This form of DM is formerly called noninsulin- dependent or adult (older the 40 years of age) diabetes mellitus. Now DM2 is increasingly diagnosed in young people, adolescents and children. DM2 comprises 80% to 90% of all cases of DM. The relative importance of defects in insulin secretion or in the peripheral action of the hormone in the occurrence of DM2. The intimate relationship between the secretion of insulin and the sensitivity of hormone action in the complicated control of glucose homeostasis, it is practically impossible to separate the contribution of each to the etiopathogenesis of DM2 (Conget., 2002) [2].

Phyllanthus amarus is a branching annual glabrous herb belonging to the family *Euphorbiaceae* which are found in tropical and subtropical countries of the world. It is 30-60 cm in height with distichous leaves which are subsessile elliptic-oblong, obtuse, rounded base. Flowers of the plant are yellowish, whitish or greenish in color.Fruits are globose like smooth capsules present underneath the branches and seeds are trigonous, pale brown with longitudinal parallel ribs on the back. Traditionally the plant is used in several health problems like diarrhoea, dysentery, dropsy, jaundice, intermittent fevers, urinogenital disorders, scabies and wounds.

Materials and Methods
Collection of Plant Material
The Indigenous plant *Phyllanthus amarus* were collected from different locations of Bhopal (M.P.) region. The plants were acknowledged by a senior Botanist Dr. Tayaaf Safi Principal Gandhi P.R. College Bhopal.

Preparation of Extract
Plant material was washed with water and then allowed to dry in shade for about 3 to 4 weeks. Dried plant materials were grinded by using the electronic grinder. The powder of the whole plants of *Euphorbia hirta* L. was extracted according to (Harborne and Baxter., 1995) [4]. The dried plants sample was powered and filed into the soxhlet using petroleum ether and methanol respectively. Almost all the chlorophyll and lipid was deposited on the side of the flask and removed carefully. The extracts were stored in refrigerator till any further use.

Experimental Animals
Male and female Wistar rats of body wt. 200–215 gm were obtained from central animal house, Pinnacle Biomedical research Institute (PBRI). The animals were fed on standard pellet diet (Hindustan Lever, Mumbai, India) and water add libitum. The albino waster rats used in the present study were maintained in accordance with guidelines of the CPCSEA, India and the study approved by the ethical committee.
The diabetic rats (glucose level > 240 mg/100ml) were divided into

5 Groups of 6 Rats Each
Groups divide in a such way that –
Group I : Normal Vehicle
Group II : Streptozotocin control
Group III : Standard (Glibenclamide 600 µg/kg)
Group IV : Dose 200mg/kg
Group V : Dose 400 mg/kg

Induction of Diabetes

Diabetes was induced by Streptozotocin (S.D. Fine Chemicals Ltd., Boisa). Streptozotocin is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (that is beta cells) when administered to rodents and many other animal species (Sikarwar *et.al*, 2009) [5]. Animals were fasted for 12n hours before the experiment.

The animals were injected intraperitoneally (i.p.) at a dose of 60 mg/kg body weight streptozotocin freshly dissolved in sterile normal saline.

Results and Discussion

Evaluation of antidiabetic acitivity

The result obtained with diabetic rats treated with methanolic extract of *Phyllanthus amarus* prompted us to perform a study with crude methanolice extract in view to determine the nature of active principle. The results of methanolic extract which are expressed as change in blood glucose levels are as shown in (Table 1 & Fig 1, 2, 3, 4). The methanolic extract of whole plant of *Phyllanthus amarus* showed significant ($p<0.05$) reduction in blood glucose levels. The blood glucose levels were reduced considerably within seven days of drug administration. Methanol extract doses of 200 mg/kg and 400mg/kg reduced blood glucose levels when compare to diabetic control group. Maximum effect was shown by standarad drug Glibenclamide by lowering blood glucose efficacy all the time. The pretreatment with streptozotocin induces diabetes in rats and blood glucose levels were 225-294 mg/dl. After administration of the crude methanol extracts, the glucose levels were found to decrease significantly from 289.66mg/dl to 202.5mg/dl (200mg/kg), 291.5mg/dl to 173.33mg/dl (400mg/kg) on 0 day after drug administration i.e., on 7th day from 202.5mg/dl to 14th day 176.66mg/dl (200mg/kg), 173.33mg/dl to 155.16mg/dl (400mg/kg) on 21th day 143.33mg/dl (200mg/kg), 120.66mg/dl (400mg/kg).

Table 1: Showing effect of methanolic extract on blood glucose level (mg/dl) in normal and Streptozotocin-induced diabetic rats (*P. amarus* Schum) Data represented in Mean±SD, n=6, *p<0.05 compared to diabetic control and **p<0.05 compared to vehicle normal.

S. No	Treatment	Dose	0 Day	7 Day	14 Day	21 Day
1.	Vehicle (Normal saline)	10ml/kg	85.73±8.51	87.43±7.82	88.48±7.96	89.25±7.45
2.	STZ Control	60mg/kg	262.166±13.67**	267.33±14.47**	271.83±14.41**	275.5±13.30**
3.	Glibenclemide	600µg/kg	265.3±22.19*	174.33±13.54*	122.5±7.09*	98.5±8.93*
4.	Extract D1	200mg/kg	289.66±14.51*	202.5±28.75*	176.66±25.42*	143.33±17.31*
5.	Extract D2	400mg/kg	291.5±18.68*	173.33±16.46*	155.16±14.63*	120.66±12.19*

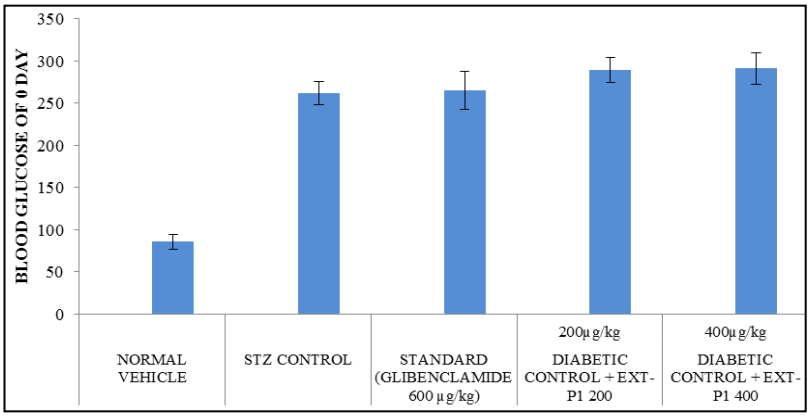

Fig 1: Showing blood glucose levels on day-0 Blood glucose levels on 0 day (*P. amarus* Schum)

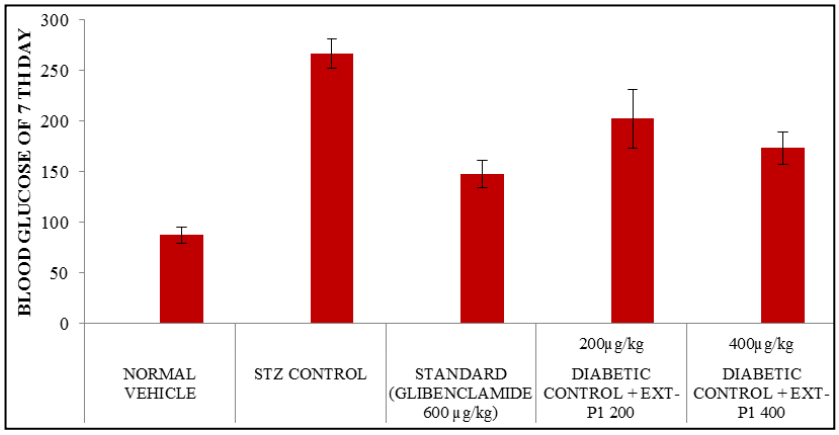

Fig 2: Showing blood glucose levels on day-7th Blood glucose levels on 7th day

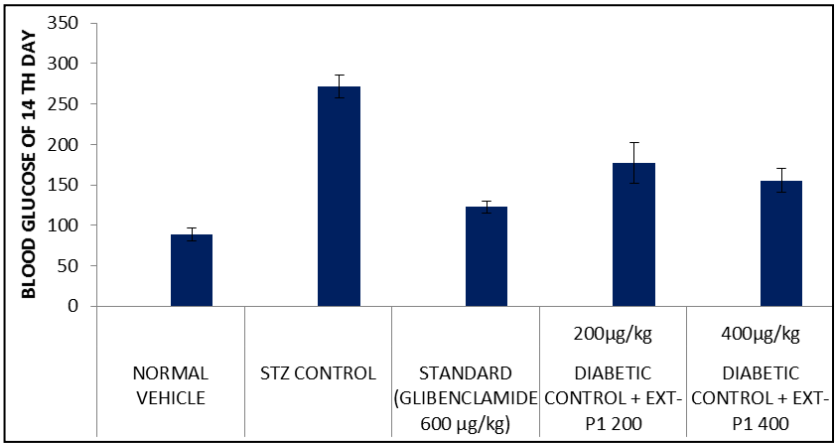

Fig 3: Showing blood glucose levels on day-14th Blood glucose levels on 14th day

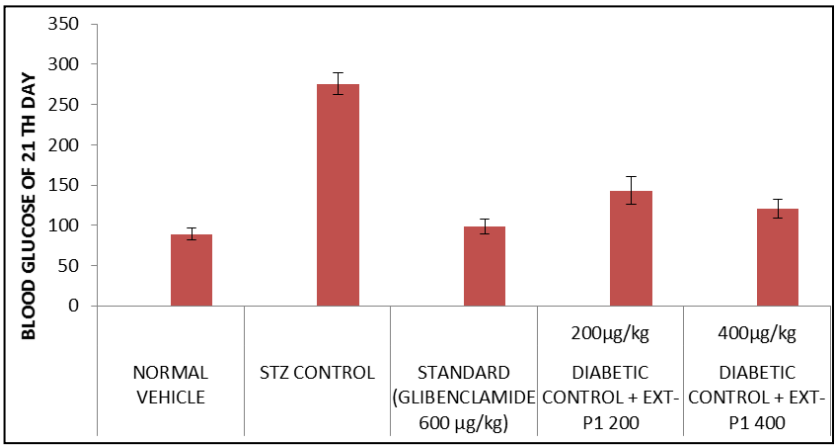

Fig 4: Showing blood glucose levels on day-21th Blood glucose levels on 21th day

Discussion

Medicinal plants are used for treatment of various infectious disease [6]. Streptozotocin (STZ) is used in experimental type-I diabetes mellitus, which causes pancreatic islet β-cell cytotoxicity. It results in imminent β-cell necrosis. The development of STZ induced SOD anions on mitochondria accelerate diabetic complications [7]. In the present study it was observed that on administration of Streptozotocin diabetes was induced which cause significant increase in blood glucose levels (Table 1 & Fig 1,2,3&4) (Arunachalam and Parimelazhagan, 2012) [8].

Blood glucose levels were higher than the normal levels which were 267.33±14.47, 174.33±13.54, 202.5±28.75, 173.33±16.46 in *P. amarus*. After the administration of methanol crude extract of *P. amarus* Linn with doses of 200mg/kg b.w. and 400mg/kg b.w there was significant ($p<0.05$) decrease in blood glucose levels which were 143.33±17.31, 120.66±12.19 as compared to the diabetic control group blood glucose level i.e. 275.5±13.30 within 7 days of administration. Blood glucose level reduces and body weight also reduces. The results are in agreement with the view that blood glucose levels were reduced on administration of crude extract of *Phyllanthus amarus* (Matthew *et al.*, 2012; Ashish and Swapnil., 2011) [9]. The antidiabetic effect can be attributed to the presence of polysaccharides, terpenes and tannins, steroids and alkaloids [10].

Conclusion

Diabetes is a metabolic disorder which results from pancreatic beta cells dysfunction & insulin resistance. It is characterized by hyperglycemia. The plant *P. amarus* was selected to evaluate its efficacy against streptozotocin induced diabetic rats. By analyzing the result we have concluded that secondary metabolites isolated from the plant *P. amarus* possess and showed significant antidiabetic activity *in vivo*. Further investigation is required to purify the exact bioactive compound which is responsible for its antidiabetic property. It shows that the *P. amarus* has the potential for the development of drugs in combating diabetes.

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