

## **Antidiabetic effect of ethanolic leaf extract of *Phyllanthus amarus* in alloxan induced diabetic mice**

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### **ABSTRACT**

*Phyllanthus amarus* Schum. Thonn. is an indigenous medicinal plant, which has a folk reputation in central and southern India as hypoglycemic agent. The present investigation was carried out to evaluate the antidiabetic effect of ethanolic leaf extract of *Phyllanthus amarus* in alloxan induced diabetic mice. Blood glucose levels and body weights of control and diabetic mice were monitored. In the present study activities of liver enzymes such as glucokinase, glucose -6- phosphatase and fructose -1- 6-diphosphatase were also determined. Glibenclamide an antidiabetic oral drug was used as reference in the present investigation. Oral administration of ethanolic leaf extract (400 mg/kg body weight) for 45 days resulted in a significant ( $P < 0.05$ ) decline in blood glucose from 310.20 to 141.0 mg/dl and significant recovery in body weight of diabetic mice. There was also a significant ( $P < 0.05$ ) reduction in the activities of glucose-6-phosphatase and fructose-1-6-disphosphatase in liver, further there was significant ( $P < 0.05$ ) increase in the activity of glucokinase in liver of diabetic mice when compared with that of diabetic control. The study clearly shows that the ethanolic leaf extract of *Phyllanthus amarus* possesses potent antidiabetic activity.

**Keywords:** Diabetes mellitus (DM), alloxan, antidiabetic, glibenclamide, *Phyllanthus amarus*.

### **INTRODUCTION**

Diabetes mellitus (DM) is caused by inherited and/ or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced [1]. This insulin deficiency results in increased concentration of glucose in the blood. Increase in blood glucose damages many of the body's systems, in particular, the blood vessels and nerves. The hyperglycemia caused due to decreased insulin production is called Type-1 diabetes and hyperglycemia due insufficient insulin utilization is called Type-2 diabetes [2]. Out of these two types, Type -2 diabetes is a major problem of today and it account for nearly 95% of total diabetic population of about 246 million [3]. Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. India has about 45000 plant species and among them, several thousands have been claimed to possess medicinal properties [4]. The commonly practiced treatment of diabetes includes oral antidiabetic drugs, insulin injection and management through diet and physical exercise. Apart from currently available therapeutic for the treatment of diabetes, traditional plant medicines are also used throughout the world for treatment of diabetes.

*Phyllanthus amarus* is a small herb common to central and southern India. It can grow to 30-60 cm in height and bloom with yellow flowers. All parts of the plant are used in ayurvedic medicines because of their medicinal properties. Leaves of this plant are reported to contain lignans, alkaloids, flavonoids, galloatnoids and glycosides [5]. Studies on extract of *Phyllanthus amarus* have shown anti hepatitis B activity [6], hepatoprotective[7], anticancerous[8], antimicrobial [9] and kidney stones dissolution properties[10]. Therefore, the present investigation was undertaken to study the antidiabetic effect of *Phyllanthus amarus* ethanolic leaf extract in alloxan induced diabetic mice.

## MATERIALS AND METHODS

### 2.1 Plant material and preparation of extract

The fresh leaves of *Phyllanthus amarus* were collected from Botanical garden of Karnataka University, Dharwad, Karnataka state, India. The plant was identified and authenticated in the Department of studies in Botany Karnataka University, Dharwad, Karnataka state, India. The leaves of the plant were shade dried on a laboratory table for 6 days and reduced to powder by using dry grinder. This powder (100g) was then packed into soxhlet apparatus and extracted using 95% ethanol (40-50°C) [11]. The extraction was carried out for 40h. The extract obtained was dried at 45 °C in hot air oven till green colored semisolid mass was obtained [12]. The yield obtained was 4.5% and the semisolid extract was stored in a refrigerator at 4 °C until further use.

### 2.2 Animals

Laboratory bred normal 3-4 months old adult virgin male Swiss albino mice weighing 25-30 g were used. Animals were maintained under standardized animal housing conditions (temperature 25 ± 2 °C facility with 12 h light/ dark cycle) with unlimited access to pellet diet “Gold Mohar” (Hindusthan Lever Limited, Mumbai) and water *ad libitum* throughout study. Animals described as fasted were deprived of food for 16 h, but had free access to water.

### 2.3 Induction of experimental diabetes

Diabetes was induced by intraperitoneal administration of alloxan monohydrate (150 mg/kg body weight) dissolved in normal saline to the male Swiss albino mice, after an overnight fast (access to only water) of 12 hours to make them more susceptible to developing diabetes [13]. After 72 h mice with diabetes mellitus indicated by glycosuria (indicated by Benedict’s test) and hyperglycemia with blood glucose range of 200 to 350 mg/dl were used for present study [14]. Body weights of all experimental mice were recorded at the start of the experiment and on 46<sup>th</sup> day of the experiment.

### 2.4 Experimental design

Animals were divided into four groups of five mice each. Standard pellet diet and water was provided *ad libitum* to the animals.

- Group I. Normal healthy mice given only vehicle (1% gum acacia)
- Group II. Diabetic control mice.
- Group III. Diabetic mice of this group were given a single dose of glibenclamide (600 µg/kg body weight) 1 ml with vehicle by oral administration daily, for 45 days.
- Group IV. Diabetic mice of this group were given single dose of ethanolic leaf extract of *Phyllanthus amarus* (400 mg/kg body weight) 1ml with vehicle by oral administration daily, for 45 days.

### 2.5 Evaluation of antidiabetic activity

The blood glucose level (BGL) was monitored after alloxanisation in blood samples collected by amputation of the tail tip under mild anesthesia. Using a blood glucose test strip (Glucocard™ 01 sensor) and a glucometer ARKRAY Glucocard 01-mini Blood glucose testing system. After 72 h Swiss albino mice having blood glucose level above 200 mg/dl of blood were selected for the study. Blood glucose levels were tested before the treatments and on 46<sup>th</sup> day of treatment on fasting mice. The mice of all four groups were fasted and sacrificed by cervical decapitation. The liver was dissected out and washed with ice-cold saline immediately. A portion of the tissue was homogenized using a potter- Elvehjem homogenizer, and the homogenate was used for estimation of protein, glucokinase[15], glucose 6-phosphatase, [16]and fructose 1, 6- diphosphatase[17].

### 2.6 Statistical analysis

Values were recorded as Mean ± standard error of the mean. Statistical difference between the means was determined by ANOVA followed by Duncan post Hoc test. P< 0.05 was accepted as significance level.

## RESULTS AND DISCUSSION

Changes in blood glucose level and body weight in normal, diabetic and on treatment of diabetic mice with *Phyllanthus amarus* extract, glibenclamide are presented in Table1. Oral administration of *Phyllanthus amarus* ethanolic leaf extract (400 mg/kg body weight) for 45 days showed significant (P<0.05) reduction in blood glucose (310.20 to 141.0 mg/dl) and an improvement in body weight in diabetic mice compared with untreated diabetic mice. This was almost similar to results obtained with reference drug glibenclamide (312- 133 mg/dl). Effect on the hepatic glucokinase glucose 6-phosphatase and fructose 1-6, diphosphatase, due to administration of *Phyllanthus amarus* ethanolic leaf extract and glibenclamide on diabetic mice is given in Table 2. The activity of hepatic

glucokinase is significantly ( $P < 0.05$ ) decreased while activities of glucose-6-phosphatase and fructose 1-6-diphosphatase were significantly ( $P < 0.05$ ) elevated in alloxan diabetic control mice.

The administration of *Phyllanthus amarus* ethanolic leaf extract for 45 days showed significantly ( $P < 0.05$ ) increased activity of hexokinase in diabetic mice when compared to that of diabetic control group. There was also significant ( $P < 0.05$ ) decrease in activities of glucose -6-phosphatase and fructose 1, 6- diphosphatase in diabetic mice when compared to those of diabetic control group. The administration of glibenclamide to diabetic mice also showed similar results. The results showed that *Phyllanthus amarus* extract caused a significant ( $P < 0.05$ ) reduction in the blood glucose levels in diabetic mice by stimulating the activity of hepatic enzymes involved in glucose metabolism.

**Table1. Changes in blood glucose levels and body weight in control, diabetic and diabetic mice treated with *Phyllanthus amarus* ethanolic leaf extract and glibenclamide.**

Values are mean  $\pm$  S.E.M (n=5).  
Significance vs. control group. \* $P < 0.05$

Groups	Blood glucose Level (mg/dl)		Body weight (gram)		Change in body weight (gram)
	Initial	Final	Initial	Final	
I	105.20 $\pm$ 3.9	110 $\pm$ 3.6	30.40 $\pm$ .10	32.60 $\pm$ 0.18	+2.2
II	310.20 $\pm$ 8.24	352 $\pm$ 5.26	30.28 $\pm$ .22	28.22 $\pm$ 0.14 *	- 4.32
III	312.60 $\pm$ 7.67	133 $\pm$ 7.67 *	30.38 $\pm$ .28	27.28 $\pm$ 0.28 *	- 3.0*
IV	310.20 $\pm$ 7.88	141 $\pm$ 2.3 *	30.40 $\pm$ .16	28.22 $\pm$ 0.14 *	- 2.82*

**Table 2. Effect of *Phyllanthus amarus* ethanolic leaf extract on the activities of hepatic enzymes in control and experimental animals after 45 days of treatment**

Groups	Glucokinase <sup>a</sup>	Glucose-6-phosphatase <sup>b</sup>	Fructose 1-6-diphosphatase <sup>c</sup>
I	135.67 $\pm$ 4.28	0.172 $\pm$ 0.06	0.252 $\pm$ 0.024
II	98.92 $\pm$ 1.89	0.238 $\pm$ 0.038	0.419 $\pm$ 0.07
III	143.78 $\pm$ 3.21 *	0.168 $\pm$ 0.02 *	0.278 $\pm$ 0.023*
IV	125.48 $\pm$ 2.78 *	0.192 $\pm$ 0.028 *	0.282 $\pm$ 0.024*

a moles of glucose phosphorylated/g/h.

b, moles of pi liberated/min/mg.

c, moles of pi liberated/min/mg.

Values are mean  $\pm$  S.E.M (n=5).

Significance vs. control group. \* $P < 0.05$

Number of plants has been used traditionally in treatment of diabetes and some have been proven scientifically to have hypoglycemic activity. These plant extract contain compound like polysaccharides[18], flavonoids [19], terpenoids and tannins[20], steroid[21], polypeptides [22] and alkaloids [23], and these compounds are responsible for the antidiabetic activity. *Phyllanthus amarus* leaf extract is reported to contain compounds like lighans, alkaloids, flavonoids, galloatnoids and glycosides [5]. The higher blood glucose levels are expected in alloxan induced diabetic mice, since alloxan causes a massive reduction in insulin release, by the destruction of the  $\beta$  cells of the islets of Langerhans and inducing hyperglycemia [24]. In the present study oral administration of *Phyllanthus amarus* extract (400 mg/kg body weight) caused a significant ( $P < 0.05$ ) reduction in the blood glucose and improvement in body weight compared to untreated diabetic mice. The antihyperglycemia action of *Phyllanthus amarus* ethanolic leaf extract may be due to potentiation of pancreatic secretion of insulin. Plants like *T. arjuna*, *Tapinanthus butungii*, *Lagerstroemia speciosa*, *Ficus bengalensis* etc. have also showed anti hyperglycemic and insulin release stimulatory effect [24]. *Phyllanthus amarus* leaf extract is also known for its liver protective action [22]. Liver is an insulin dependent tissue, which plays a vital role in glucose and lipid homeostasis and is severely affected during diabetes. Decreased glycolysis impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver [22].

Insulin influences the intracellular utilization of glucose in a number of ways. It increases hepatic glycolysis by increasing the activity and amount of several key enzymes including glucokinase, phosphofructokinase, and pyruvate kinase [25]. Hepatocytes also contain a form of hexokinase called hexokinase D or glucokinase that is more specific for glucose and differ from other forms of hexokinase in kinetic and regulatory properties [24]. Glucokinase catalyzes the conversion of glucose to glucose -6- phosphate and play a central role in the maintenance

of glucose homeostasis. In the liver this enzyme is an important regulator of glucose storage and disposes [26]. In the present study, the glucokinase activity was decreased in alloxan induced diabetic mice which may be due to insulin deficiency. Insulin stimulates and activates glucokinase in the liver. *Phyllanthus amarus* ethanolic leaf extract or glibenclamide elevates the activity of glucokinase in liver. *Phyllanthus amarus* extract like glibenclamide, may stimulate insulin secretion which may activate glucokinase thereby increasing utilization of glucose and this increased utilization leads to decreased blood sugar level [27]. Insulin decreases gluconeogenesis by decreasing the activity of key enzymes such as glucose-6-phosphatase, fructose 1, 6- diphosphatase, phosphoenolpyruvate, carboxykinase, and pyruvate carboxylase [28]. In the present study, increased activities of glucose-6-phosphatase and fructose-1, 6- diphosphatase were observed in the liver of alloxan-diabetic mice. Glucose 6-phosphatase is one of the key enzymes in the homeostatic regulation of blood glucose levels, it catalyzes the terminal step in both gluconeogenesis and glycogenolysis [28] and fructose 1, 6-diphosphatase, catalyzes one of the irreversible step in gluconeogenesis, and serves as a site for the regulation of these process [29]. Increased activities of these two gluconeogenic enzymes in alloxan induced diabetic mice may be due to insulin insufficiency. In alloxan induced diabetic mice treated with *Phyllanthus amarus* ethanolic leaf extract, activities of these two enzymes were reduced. This may be due to increased insulin secretion which is responsible for the repression of the gluconeogenic key enzymes. The present observation provide evidence that ethanolic extract of *Phyllanthus amarus* leaves exhibited antidiabetic or hypoglycemic activity on alloxan induced diabetic mice.

### CONCLUSION

The present observation provide evidence that ethanolic extract of *Phyllanthus amarus* leaves exhibited antidiabetic or hypoglycemic activity on alloxan induced diabetic mice may be due to enhancing the peripheral utilization of glucose by correcting the impaired liver or kidney glycolysis and by suppression of its gluconeogenic activity similar to insulin. This effect may be due to the presence of phyllanthin ,hypophyllarntin, nirulin, flavonoids ,terpens, tripenes, alkaloids, and other constituents present in the leaves which could act synergically or independently in enhancing the activity of glycolytic and gluconeogenic enzymes. However, Further, comprehensive chemical and pharmacological investigation should be carried out to isolate the active compound and appropriate elucidation of its mechanism of action. The result suggests that it is worth undertaking further studies on possible usefulness of the *Phyllanthus amarus* leaves in diabetes mellitus.

### Acknowledgement

The authors are grateful to the Post Graduate Department of studies in Microbiology and Biotechnology, Karnataka University for providing the necessary facilities.

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