



Hypoglycemic and anti oxidant activity of *Phyllanthus longiflorus*

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ABSTRACT

The hypoglycemic activity of aerial parts of *Phyllanthus longiflorus* (PHL) was studied against alloxan induced hyperglycemic rats. The activity was assessed by various parameters such as blood glucose level and serum level of triglyceride (TG), total cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL) and high density lipoprotein. A significant decrease in blood glucose, TC, TG, LDL, VLDL and a significant increase in HDL was observed after drug/extract treatment. The extract exhibited free radical scavenging activity on DPPH and superoxide anion free radical and also shown reducing property. From the results of the present study, it was concluded that PHL possessed potent hypoglycemic activity, probably by its antioxidant activity.

Key words: Hypoglycemic, alloxan, anti oxidant, *P.longiflorus*.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder caused by the relative or absolute lack of insulin or its action on cells. Approximately 5 % of world population suffers from diabetes. Since time immemorial, natural products have been used for the treatment of human disease and now, they still remain as important source for the development of anti diabetic drugs. *Phyllanthus longiflorus* (Tam-Nallapullati) belonging to the family Phyllanthaceae is a small bush with obliquely obovate – oblong leaves, pink flowers and small capsular fruits (0.4cm long), distributed in the hills of Kerala and Tirunelveli up to an altitude of 600m [1]. Several species of the genus *Phyllanthus* were reported to possess anti tumour, anti inflammatory, hepatoprotective, diuretic, and anti bacterial activities [2, 3, 4, 5, 6]. *P.amarus* [7], *P.niruri* [8] etc., have been reported for antidiabetic activity. In our previous work, we have reported anti-convulsion [9] and diuretic activity [10] on this plant. The present study was designed to evaluate hypoglycemic effect of aerial parts of *Phyllanthus longiflorus* (PHL) against alloxan induced diabetic rats.

EXPERIMENTAL SECTION

Plant collection and authentication

The plant material was collected from the Western Ghats, Tamilnadu, India during February 2008. It was authenticated by Dr.V.Chelladurai, Govt. Research officer, Botany C.C.R.A.S. Govt. of India, (Retired), Tirunelveli, Tamilnadu, India. A voucher specimen (PHL001) has been deposited for future reference.

Preparation of extract

The powdered plant material was sieved (sieve # 22) to get uniform particle size and then extracted exhaustively with methanol by continuous hot extraction method [11] at 40-50°C for 18-20 hr. The solvent was removed under reduced pressure, dried using rotary evaporator and a green solid mass was obtained. It was then preserved in

desiccators until further use. The extract was then made into suspension using 1 % tween 80 for convenient oral administration.

Estimation of total phenols

Reaction mixture consisting of 0.5 ml of MPHL, 0.5 ml of Folin ciocalteu reagent and 0.5 ml of 10 % sodium carbonate was kept at room temperature for 1 hr. After that period, the absorbance was measured at 700nm using Shimadzu 1700 spectrophotometer. The results were presented as mg/g of gallic acid equivalent [12,13].

Estimation of total flavonoids [14]

MPHL (2.5 ml) was mixed with 1.25 ml of de-ionized water and 0.075 ml of 5% sodium nitrite. 0.15 ml of 10% aluminum chloride, 0.5 ml of 1M sodium hydroxide and 2.5 ml of de-ionized water were added at 6 min interval. The absorbance was measured at 510 nm using Shimadzu 1700 spectrophotometer [15]. The results (amount of total flavonoid) were given in mg equivalent catechin /100 gm of extract. The standard curve was prepared using 5, 10, 20, 40, 60, 80 and 100 mg/L solutions of quercetin in methanol:water (50:50, v/v).

Estimation of superoxide scavenging potential

The superoxide scavenging activity was assessed by the method of McCord and Fridovich [16]. Different concentration of MPHL were added to the reaction mixture containing 0.1 M ethylene diamine tetra acetate, 200 μ l of 0.0015% sodium cyanide, 100 μ l of 1.5 mM nitroblue tetrazolium, 50 μ l of 0.12 mM riboflavin, the final volume was adjusted to 3 ml using 0.2 M phosphate buffer (pH 7.8). The tubes were illuminated under incandescent lamp for 15 min and then the absorbance was measured at 530 nm.

Estimation of reducing power

MPHL (10 mg in 1ml distilled water) was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferri cyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50°C for 15 min. After the incubation period 2.5 ml of 10% trichloroacetic acid was added. The mixture was centrifuged at 3000 rpm for 15 min, 2.5 ml of supernatant liquid was collected and mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$. The absorbance was measured at 700 nm. Sodium meta bisulphite was used as the reference standard. [17] The reductive ability of the tested samples increased with the absorbance and the concentration at which absorbance was 0.5 was taken as EC_{50} .

Estimation of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) scavenging potential

About 0.1ml of the extract (0.01-5.00 mg/ml) was mixed with 3.9 ml of DPPH in methanol solution (0.1mM) and incubated at 37°C for 30 min. The absorbance of the each reaction mixtures were measured at 517 nm using spectrophotometer [18]. BHA was used as standard and all the experiments were done in triplicates. DPPH scavenging ability was calculated as follows.

$$\text{DPPH radical scavenging activity (\%)} = (1 - \text{Absorbance of the sample} / \text{Absorbance of control}) \times 100$$

Animals

Male albino rats (150-200 g) of Wister strain were procured, housed in standard polypropylene cages and kept under controlled temperature ($24 \pm 20^\circ\text{C}$; relative humidity 60-70%) in a 12 h light-dark cycle. The rats were given a standard rat pellet diet and water *ad libitum*. The protocol for the present study was approved by institutional animal ethics committee (Approval no. 509/02/C/CPCSEA). The dose for the preset study was calculated based on the acute toxicity study [10].

Estimation of blood glucose level [19]

Healthy rats were selected and acute diabetes was induced by the intraperitoneal administration of single dose of 1 % w/v of alloxan monohydrate (120 mg/kg, *i.p.*) in normal saline [20]. 72 hours after alloxan administration, blood samples were collected, the serum was separated and blood glucose was measured using digital glucometer (Apex Biotechnology Corp, Taiwan). Rats having blood glucose level > 150 mg/dl were selected, divided into 4 groups (n=6) and treated as follows. Group I 10% Aqueous tween 80, *p.o.*, Group II: Glibenclamide 5 mg/kg, *p.o.*, Group III and IV: Methanol extract of *Phyllanthus longiflorus* 100 and 200 mg/kg, *p.o.*, respectively. This treatment was continued for fifteen days. The blood glucose levels were measured on days 5, 10 and 15. The rats were weighed daily throughout the experiment and the changes in the body weight was also calculated.

Estimation of biochemical parameters

Blood was collected through retro-orbital plexus of all the rats, under light ether anesthesia, the serum was separated at 3000 rpm for 5 min using micro centrifuge and various biochemical parameters such as blood glucose level and

serum level of triglyceride (TG), total cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL) and high density lipoprotein studied using standard procedures to assess the activity [21].

Statistical analysis

The results were presented as Mean \pm SEM (n = 6). The statistical package Graph-Pad Prism, version 4 for Windows (San Diego, CA, USA) was used in the analysis. p<0.01 was considered significant when compared to control.

Table 1 Effect of *Phyllanthus longiflorus* on blood glucose level

Groups and Dose (mg/kg)	Blood glucose level (mg/dl)				
	Before alloxan	72 hrs after alloxan	After drug administration		
			5 th day	10 th day	15 th day
Tween 80	149 \pm 0.21	172 \pm 0.16	170 \pm 0.47	167 \pm 0.96	166 \pm 0.29
Glibenclamide	155 \pm 0.51	221 \pm 0.46	169 \pm 0.39	157 \pm 0.52	151 \pm 0.97
PHL 100	142 \pm 0.05	167 \pm 0.32	148 \pm 0.19	139 \pm 0.11	139 \pm 0.56
PHL 200	125 \pm 0.12	191 \pm 0.19	137 \pm 0.20	121 \pm 0.09	122 \pm 0.19

Mean \pm SEM (n = 6), p<0.01, measured using GraphPad InStat v. 3.0.10.0 soft ware.

Table 2 Effect of *Phyllanthus longiflorus* on various serum biochemical parameters

Groups	Dose (mg/kg)	Biochemical parameters (mg/dl)				
		TC	TG	HDL	LDL	VLDL
Tween 80	10	205 \pm 0.25	175 \pm 0.26	35 \pm 0.16	161 \pm 0.45	49 \pm 0.06
Glibenclamide	5	126 \pm 0.59	65 \pm 0.48	53 \pm 0.27	85 \pm 0.57	27 \pm 0.17
PHL	100	162 \pm 0.65	106 \pm 0.37	42 \pm 0.46	119 \pm 0.11	32 \pm 0.13
PHL	200	153 \pm 0.62	90 \pm 0.19	49 \pm 0.21	110 \pm 0.20	29 \pm 0.09

Values are expressed as Mean \pm SEM (n = 6). p<0.001, measured using GraphPad InStat v. 3.0.10.0 soft ware.

Table 3 Effect of *Phyllanthus longiflorus* on body weight of diabetic rats

Groups and Dose (mg/kg)	Body weight (g)		
	5 th day	10 th day	15 th day
Tween 80	156.27 \pm 1.56	142.15 \pm 0.73	135.70 \pm 2.17
Glibenclamide	162.51 \pm 1.25	157.26 \pm 2.05	160.42 \pm 0.47
PHL 100	161.29 \pm 0.69	145.39 \pm 2.46	153.06 \pm 0.19
PHL 200	158.10 \pm 0.52	147.32 \pm 0.32	152.27 \pm 1.15

Values are expressed as Mean \pm SEM (n = 6). p<0.01, measured using GraphPad InStat v. 3.0.10.0 soft ware.

RESULTS

Antioxidant potential

The Radical scavenging activity (RSA) was determined by the DPPH and superoxide scavenging assay. MPHL inhibited superoxide radical formed by the photoreduction of riboflavin with the IC₅₀ value of 60.5 μ g/ml. For, ascorbic acid it was 9.5 μ g/ml. MPHL inhibited DPPH radical by 93.49 % with EC₅₀ 173.95. BHT exhibited 96.6 % RSA with EC₅₀ 73.84 μ g/ml. Total flavanoid content of MPHL was found to be 0.0127 \pm 0.006 gm QE per 100gm of MPHL and the total phenol content was 0.6592 \pm 0.16 gm GAE per 100gm of MPHL.

Hypoglycemic activity

The results demonstrates that aerial parts of *Phyllanthus longiflorus* produced significant (p<0.01) decrease in serum glucose (table 2), TC, TG, LDL, and VLDL levels (table 2) and a significant (p<0.01) increase in serum HDL level (table 3) at the doses 100 and 200 mg/kg. Significant normalization of body weight of the animal also observed in drug treated animal. The activity was found to be dose dependent and comparable to the standard anti diabetic drug, glibenclamide (5mg/kg).

DISCUSSION

Hypoglycemic activity of aerial parts of PHL was assessed in the present study, which involved measurement of serum glucose, TC, TG, LDL, VLDL and HDL levels on alloxan induced hyperglycemic rats. Alloxan induced hyperglycemia is a well established method for studying hypoglycemic activity of plant drugs. Alloxan induces diabetes by reducing / disturbing insulin secretion in pancreas. This action is thought to mediated through the oxygen, hydrogen peroxide and hydroxyl free radical which causes destruction of β cells of Pancreas [22]. Alloxan induced diabetes was characterized in the present study by an increased serum glucose, TG, LDL, and VLDL [23] levels. The deficient / impairment of insulin secretion were evident by these parameters. Administration of PHL at the tested doses produced significant normalization / reverse of elevated serum glucose, TC, TG, LDL, VLDL and HDL level. The PHL extract exhibited free radical scavenging activity on DPPH and superoxide anion free radical and also shown reducing property. From the findings of the present study, it can be concluded that the existence of

hypoglycemic activity of *Phyllanthus longiflorus* might involve its free radical scavenging ability. The presence of phenolic compounds, flavanoids or other phytoconstituents are responsible for the activity.

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