



Anti-hyperglycemic and anti-hyperlipidemic activities of *Premna corymbosa* (Burm.F.)Rottl on Streptozotocin induced diabetic rats

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Abstract

Evaluation of ethanolic and aqueous extracts of *Premna corymbosa* (Burm.F.)Rottl (Verbenacea) in normal and streptozotocin (STZ) induced diabetic rats. Diabetes was induced by intraperitoneal (i.p) injection of streptozotocin (50 mg/kg) in adult male albino Wistar rats. Blood glucose levels were determined after oral administration of a dose of *P. corymbosa* (400 mg/kg b. wt) in diabetic groups. Blood glucose levels were determined on 0, 7th, 14th and 21st day after oral administration of ethanolic and aqueous extracts of *P. corymbosa* (400 mg/kg) respectively and standard drug (glibenclamide) exhibited (500 µg/kg) in diabetic rats. The effect of extracts of *P. corymbosa* on blood glucose levels and serum lipid profile like Total cholesterol, triglycerides, phospholipids, low density, very low density and high density lipoprotein were measured in the diabetic and non diabetic rats. There was significant reduction in Total cholesterol, LDL cholesterol, VLDL cholesterol and improvement in HDL cholesterol in diabetic rats. These results indicate that *P. corymbosa* possesses a hypoglycemic effect.

Key words: *Premna corymbosa*, Glibenclamide, Hyperglycemia, Streptozotocin.

Introduction

Diabetes mellitus is a chronic metabolic disorder affecting approximately 10% of the global population. Besides hyperglycemia, several other factors including dislipidemia or hyperlipidemia are involved in the development of micro and macro vascular complications of diabetes which are the major cause of morbidity and death [1]. Plants have played a major role in the introduction of new therapeutic agents. A medicinal plant, *Galega officinalis*, led to the discovery and synthesis of metformin [3]. Despite considerable progress in the treatment of

diabetes by oral hypoglycaemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. In recent times there has been a renewed interest in the plant remedies [4, 5].

Premna corymbosa (Fam: Verbenacea) is a small plant found in and around of Kolli hills, Namakkal District, Tamil Nadu, India. All parts of this plant are medicinally important in the traditional system of medicine in India

Materials and Methods

Plant material

Leaves of *P. corymbosa* was collected from Kolli hills, Namakkal District, Tamil Nadu, India and authenticated by Dr P. Jayaraman, Plant Anatomy Research Centre, Chennai, Tamil Nadu, India. Voucher specimens (PC/0220/06) were deposited at our College Museum for future reference.

Preparation of the extract

The powdered material of leaves of *P. corymbosa* was extracted separately using ethanol by Soxhlet technique and water by cold maceration [9]. The extracts were dried under reduced pressure. The dried extract (22.6 g) was stored in desiccator and was subjected to various chemical tests to detect the presence of different phytoconstituents like alkaloids, tannins, cardiac glycosides and traces of flavonoids etc.

Preliminary phytochemical screening

The ethanolic and aqueous extracts were subjected to preliminary screening for various active phytochemical constituents [6].

Animals

Male albino Wistar rats, 9-12 weeks old with average weight of 150-180 g were purchased from M/S Venkateshwara enterprises (P) Ltd, Bangalore and used for the study. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each experiment, the animals were fasted for at least 18 h. The experimental protocols were approved by Institutional Animal Ethical Committee (JKKMMRF/CP/Ph.D/001/2008)

Toxicity studies

The animals were divided into six groups separately and were treated orally with ethanolic and aqueous extracts of *P. corymbosa* at 100, 200 and 400 mg/kg, body weight doses. The animals were continuously observed for 1 hr., then frequently for 14 days. The animals were observed continuously for the initial 4 h and intermittently for the next six h and then again at 24 h and 48 h following drug administration. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion [7].

Streptozotocin-induced diabetic rats

Streptozotocin (STZ), purchased from Sigma aldrich chemical Co., Bangalore, was dissolved in ice-cold normal saline immediately before use. Diabetes was induced in rats by intraperitoneal

(i.p) injection of streptozotocin at a dose of 50 mg/kg [8]. Forty eight hours after streptozotocin administration, blood samples were drawn from tail and glucose levels determined to confirm diabetes. The rats were divided into 5 groups as follows, first group served as normal control, received food and water. Second group served as diabetic control, received 0.5 ml of 5% Tween 80; third group served as diabetic control, received glibenclamide (500 µg/kg), fourth and fifth groups, (diabetic rats) received 400 mg/kg, b.wt. of ethanolic and aqueous extracts of *P. corymbosa* respectively. The treatment was continued daily for 21 days. Blood drop was collected from the tail for glucose estimation, just before drug administration on 1st day and 1 h after sample administration on days 7, 14 and 21 (Table 1).

Anti-hyperlipidaemic activity

Total cholesterol, HDL- C, LDL-C, VLDL-C, phospholipids and triglycerides were analyzed from serum. Total cholesterol was estimated according to Liebermann Burchard Reaction Method. LDL cholesterol was estimated indirectly by Friedwald's method. Triglycerides (TG) were determined using Hantzsch condensation method [9, 10, 11].

Statistical evaluation

All the data are presented as mean ±SEM. The differences between group were evaluated by one-way analysis of variance (ANOVA) followed by the Dunnett multiple comparisons test. $P < 0.01$ was considered to be significant.

Result and Discussion

Preliminary chemical test

Our phytochemical studies indicated that ethanolic and aqueous extracts of *P. corymbosa* contains alkaloids, flavanoids, glycosides, saponins, terpenes and steroids.

Toxicity studies

In performing preliminary test for pharmacological activity in rats, ethanolic and aqueous extracts did not produce any significant changes in the behavioral or neurological responses upto 400 mg/kg b. wt. acute toxicity studies revealed that the non-toxic nature of the ethanolic and aqueous extracts of *P. corymbosa*. The result obtained from the LD₅₀ study indicates that ethanolic and aqueous extracts of *P. corymbosa* is safer to use in animals even at a dose of 400 mg/kg p.o.

Antihyperglycemic activity

The effects of treatment with ethanolic and aqueous extracts of *P. corymbosa* on blood glucose levels in normal and diabetic rats are reported in Table 1. Blood glucose level of the rats were significantly higher than those in normal rats. The present experiment was conducted to study the anti-diabetic effect of *P. corymbosa* in normal as well as streptozotocin induced diabetic rats.

In STZ (50 mg/kg) induced diabetic rats, the BGL significantly increased from 75.0 ± 4.61 to 208.16 ± 17.38 . Ethanolic extracts and aqueous extracts (400 mg/kg) given upto 21 days after STZ treatment, showed decreased blood glucose levels significantly from 219.5 ± 13.25 to 116.83 ± 19.16 and 237.0 ± 15.0 to 98.83 ± 10.55 mg/dl, where as glibenclamide (500 µg/kg) treated diabetic rats, the BGL significantly decreased from 232.33 ± 13.9 to 94.5 ± 5.46 respectively.

Table 1: Anti-hyperglycemic activity of extracts of *P. corymbosa* on STZ induced diabetic rats

Groups Treatment/Dose	0 day (mg/dl)	After 7 days (mg/dl)	After 14 days (mg/dl)	After 21 days (mg/dl)
Normal control	76.16 ± 5.36	75.66 ± 3.94	75.0 ± 4.96	75.0 ± 4.61
Diabetic control	220.83 ± 19.0*	214.5 ± 10.60*	211.33 ± 20.30*	208.16 ± 17.38*
Ethanollic extract 400mg/kg	237.0 ± 15.0***	190.16 ± 16.14***	132.66 ± 11.01***	98.83 ± 10.55***
Aqueous extract 400 mg/kg	219.5 ± 13.25**	191.0 ± 15.35**	164.5 ± 19.45**	116.83 ± 19.16**
Glibenclamide (500µg/kg)	232.33 ± 13.9***	184.83 ± 12.8***	129.83± 19.20***	94.5 ± 5.46***

The values are mean ± SEM, n=6, When compared with diabetic control *p<0.05, **p<0.001, ***p<0.001 (One way ANOVA followed by Dunnett's, multiple comparison test).

Anti-hyperlipidaemic activity

The lipid profiles in control and experimental rats are depicted in Table 2 in STZ induced diabetic rats, there was a significant (P<0.001) increase of total cholesterol, triglycerides, phospholipids, and low density lipoproteins (LDL) and very low density lipoprotein (VLDL) cholesterol and significant (p<0.001) decreases in high density lipoprotein (HDL) cholesterol in serum compared with normal control. The extracts treated rats were significantly (p<0.001) decreased the total cholesterol, triglycerides, phospholipids and LDL and VLDL cholesterol and significantly (p<0.001) increased HDL cholesterol.

The present experimental result indicated that ethanolic and aqueous extracts exhibited a potent blood glucose lowering properties in STZ diabetic rats. A further exploration of the bioactive molecule responsible for the activity is under investigation in our laboratory.

Table 2: Anti-hyperlipidaemic effects of extracts of *P. corymbosa* on STZ induced diabetic rats

Groups Dose/	Changes in Mg/dL				
	TC	TG	HDL-C	LDL-C	VLDL-C
Normal control	80.50±1.35	70.33±0.76	37.83±0.72	41.00±2.80	18.83±0.79
Diabetic control	134.83±1.96*	140.00±1.63*	25.67±1.16*	93.50±2.38*	30.33±1.22*
Ethanollic extract 400mg/kg	98.17±3.27**	96.00±2.59**	36.67±1.26**	58.67±1.52**	27.83±0.47**
Aqueous extract 400 mg/kg	91.67±2.38**	85.83±1.70**	34.83±0.94**	52.67±1.82**	21.83±0.60**
Glibenclamide (500µg/kg)	100.67±3.83***	98.50±2.07**	38.33±6.72**	62.33±1.44**	24.67±0.57**

The values are mean ± SEM n= 6, when compared with diabetic control, * = p<0.05, ** = p<0.01, (One way ANOVA followed by Dunnett's, multiple comparison tests)

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