



Anti-Hyperglycemic and Anti-Hyperlipidaemic Activities of *Bauhinia variegata* L on Streptozotocin Induced Diabetic Rats

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ABSTRACT

To evaluate ethanolic and aqueous extracts of *Bauhinia variegata* L Caesalpiniodeae (caesalpiniaceae) in normal and streptozotocin (STZ) induced diabetic rats. Diabetes was induced by intraperitoneally (i.p) streptozotocin (50 mg/kg) in adult male albino Wistar rats. Blood glucose levels were determined after oral administration of *B. variegata* L (200 mg/kg b. wt) in diabetic groups. Blood glucose levels were determined on 7th, 14th and 21st day after oral administration of ethanolic and aqueous extracts of *Bauhinia variegata* L (200 mg/kg) and standard drug exhibited (500 µg/kg) in diabetic rats. The effect of extracts of *B. variegata* L 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 *B. variegata* possesses a hypoglycemic effect.

Key words: *Bauhinia variegata* L Glibenclamide, Hyperglycemia, Streptozotocin.

INTRODUCTION

The incidence of diabetes is increasing Worldwide; it affects 230 million people of which 30 million are in India. It has been estimated that by the year 2025, the global incidence of diabetes would increase to 350 million. Management of diabetes is a huge burden. While therapeutic insulin production is not adequate to meet demands, the recombinant DNA approach to diabetes

management originally considered as a panacea has faced several problems. It is hypothesized that the ultimate therapy for type I and type II diabetes lies in the herbal approach. However, herbs are not in exhaustible natural resources, and the demand for herbal medicines cannot be met by cultivation only. Plant tissue culture is a boon and can help produce large quantities of the herbal material. However, it is speculated that plant materials produced through tissue culture are deficient in secondary chemicals of therapeutic importance. *B. variegata* L is used in the treatment of diabetics and bark is used as an anticarcinogenic and mutagenic [1], anti-inflammatory [2,3], stem used as cytotoxic [4], hepatoprotective [5], antitumor [6]. The following compounds were isolated from various parts of this plant i.e galactoside binding lectine [7], a new phenanthraquinone from the stem [8] flavanone and a dihydrodibenzoxepin [9], A new flavone glycoside, 5-hydroxy 7,3',4',5'-tetra-methoxyflavone 5-O-beta-D-xylopyranosyl-(1-->2)-alpha-L-rhamnopyranoside [10].

MATERIALS AND METHODS

Plant material

Leaves of *B. variegata* L was collected from foot hill of Kolli hills, Namakkal District, Tamil Nadu, India and authenticated by Dr P. Jayaraman, Plant anatomy Research centre, Chennai, Tamil Nadu, India. Voucher specimens (BV/0221/06) were deposited at our College Museum for future reference.

Preparation of the extract

The powdered material of leaves of *B. variegata* L was extracted separately using ethanol by Soxhlet technique and water by cold maceration [11]. The extracts were dried under reduced pressure. The dried extract 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 [12].

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 five groups separately and were treated orally with ethanolic and aqueous extracts of *B. variegata* L at 50, 100 and 200 mg/kg, body weight doses. 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 writhing reflex, respiratory rate and convulsion.

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 [13]. 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 200 mg/kg of ethanolic and aqueous extracts of *B. variegata* L 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 [14, 15, 16].

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.

RESULTS AND DISCUSSION**Preliminary chemical test**

Our phytochemical studies indicated that ethanolic and aqueous extracts of *B. variegata* L 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 200 mg/kg b. wt. acute toxicity studies revealed the non-toxic nature of the ethanolic and aqueous extracts of *B. variegata* L The result obtained from the LD₅₀ study indicates that ethanolic and aqueous of *B. variegata* L is safer to use in animals even at a dose of 200 mg/kg p.o.

Antihyperglycemic activity

The effects of treatment with ethanolic and aqueous extracts of *B. variegata* L 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 *B. variegata* L 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 and aqueous extracts (200 mg/kg) given upto 21 days after STZ

treatment, showed decreased blood glucose levels significantly from 238.6 ± 12.28 to 128.42 ± 20.18 and 242.2 ± 22.20 to 102.88 ± 12.42 mg/dl, where as glibenclamide (500 μ g/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 *B. variegata* L on STZ induced diabetic rats

Groups Treatment/Dose	0 day (mg/dl)	After 7 days	After 14 days	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	$220.83 \pm 19.0^*$	$214.5 \pm 10.60^*$	$211.33 \pm 20.30^*$	$208.16 \pm 17.38^*$
Glibenclamide (500 μ g/kg)	$232.33 \pm 13.9^{***}$	$184.83 \pm 12.8^{***}$	$129.83 \pm 19.20^{***}$	$94.5 \pm 5.46^{***}$
Ethanollic extract 200 mg/kg	$242.2 \pm 22.20^{***}$	$198.24 \pm 18.12^{***}$	$138.68 \pm 12.02^{***}$	$102.88 \pm 12.42^{***}$
Aqueous extract 200mg/kg	$238.6 \pm 12.28^{**}$	$198.22 \pm 16.32^{**}$	$172.8 \pm 22.42^{**}$	$128.42 \pm 20.18^{**}$

The values are mean \pm 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, 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.

Table 2: Anti-hyperlipidaemic effects of extracts of *B. variegata* L 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	$134.83 \pm 1.96^*$	$140.00 \pm 1.63^*$	$25.67 \pm 1.16^*$	$93.50 \pm 2.38^*$	$30.33 \pm 1.22^*$
Glibenclamide (500 μ g/kg)	$96.67 \pm 3.83^{**}$	$98.50 \pm 2.07^{**}$	$38.33 \pm 6.72^{**}$	$62.33 \pm 1.44^{**}$	$24.67 \pm 0.57^{**}$
Ethanollic extract 200mg/kg	$98.42 \pm 3.28^{**}$	$98.82 \pm 2.62^{**}$	$38.67 \pm 1.26^{**}$	$64.67 \pm 1.52^{**}$	$24.83 \pm 0.47^{**}$
Aqueous extract 200 mg/kg	$100.82 \pm 2.42^{**}$	$110.22 \pm 2.48^*$	$42.46 \pm 2.98^{**}$	$66.28 \pm 2.42^{**}$	$27.22 \pm 0.68^{**}$

The values are mean \pm 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.

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.

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