Vol. 9/1 (2009) 27 - 34

JOURNAL OF NATURAL REMEDIES

Effect of *Bauhinia variegata* bark extract on blood glucose level in normal and alloxanised Diabetic rats

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Abstract

The antidiabetic activity of Ethanol (95%) bark extract of *Bauhinia variegata* Linn. was evaluated in alloxan-induced diabetic rats. The various parameters like blood glucose, body weight, total cholesterol, triglycerides, high density lipoprotein, insulin, oral glucose tolerance test and histopathology of pancreas were studied. The results were found to be significant antidiabetic by reducing the blood glucose level, improving body weight, attenuating the altered lipid profile toward the normal and by regenerating the Islets of langerhans. Therefore the present study justifies that ethanol extract of *Bauhinia variegata* exhibits significant antidiabetic activity, so this extract may have Insulin-like properties.

Keywords: Anti-diabetic activity, *Bauhinia variegata* Linn, Islet of Langerhans, plasma biochemical parameters.

1. Introduction

Diabetes is one of the most prevalent chronic disease in the world affecting nearly 25% of the population [1]. Diabetes is the name given to a group of disorders with different etiologies. It is characterized by derangements in carbohydrate, protein and fat metabolism caused by the complete or relative insufficiency of insulin secretion and/or insulin action [2]. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is increasing demand by patients to use the natural products with antidiabetic activity [3].

Bauhinia variegata Linn (Caesalpiniaceae) grows as a medium-sized, deciduous tree found throughout India and it is commonly called Kempumandara in Kannada. *Bauhinia variegata* (BV) is traditionally used in the treatment of constipation, helmintic, inflammatory, diarrhoea, leprosy, tumours, wounds, ulcers, menorrhagia and similarly in diabetes [4].

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No scientific report for its antidiabetic activity has been made so far. Therefore the present paper deals with the study of antidiabetic activity along with antihyperlipidemic effects of Ethanol extract of *Bauhinia variegata* (EBV).

2. Material and Methods

2.1 Plant material

The barks of *Bauhinia variegata* were collected in and around Belgaum district, Karnataka, India and authenticated by Mr. P.S.N.RAO, Joint Director, Botanical Survey of India, Pune (India). The voucher specimen (BSM-1) has been kept in our laboratory for future reference.

2.2 Extract Preparation

The barks were dried in the shade and coarsely pulverized. The powder was then packed into Soxhlet apparatus and subjected to hot continuous percolation using ethanol (95 % v/v) as solvent. The extract was concentrated under vacuum, dried in a vacuum desiccator (yield 11.73% w/w) and then suspended in 5% gum acacia for the pharmacological studies [5]. Extract also subjected for Preliminary Qualitative Phytochemical analysis [6].

2.3 Animals used

Healthy albino Wistar rats weighing 140-180 gms [7], were used and lighting was natural sequence being 12 hours dark, 12 hours light. The conventional laboratory diet was fed with adequate supply of drinking water.

2.4 Experimental procedure

Total duration of study was 15 days [8], and the animals used were rendered diabetic by injecting alloxan monohydrate (dissolved just before use in 0.9% NaCl) through intra-peritoneal (i.p) at the dose of 150mg/kg body weight [9]. Animals having blood glucose level more than 300 mg/dl were separated and divided into different groups of six each [10].

During the period of study and at the end of the treatment period, blood samples were collected by retro-orbital plexus [8], after 16 hours fasting [3], for biological estimations; then rats were sacrificed by excess ether anesthesia, dissected to collect pancreas for histopathological study. All Biochemical estimations were done in a STAR 21 ^{plus} Biochemistry Auto Analyser obtained from the Aspen Diagnostics Pvt. Ltd. Delhi-110009.

2.5 Acute toxicity study

Healthy adult Wistar albino rats of either sex, starved overnight were subjected to acute toxicity studies to determine the nontoxic or safe dose by an up-and-down staircase method. The rats were observed continuously for 2h for behavioral, neurological and autonomic profiles and, after a period of 24 and 72h, for any lethality or death [11].

2.6 Effect of EBV on Fasting blood glucose levels (FBGL) in Normal rats

Animals fasted overnight (16 h) were randomly divided into four groups of six rats: Group I, received the vehicle (5% Gum acacia, p.o.), and served as Normal control; Group II, received the test extract 250 mg/kg, p.o.; Group III, received the test extract 500 mg/kg, p.o.; Group IV received the std. drug Glibenclamide 600 µg/kg b.w. p.o. Plasma glucose was measured on 0 day, 1st, 4th, 7th, 10th and 15th day of the experiment, using Glucose Oxidase/Peroxidase (GOD/POD) method using a standard kit obtained from Span Diagnostics Ltd. Surat, India.

2.7 Assessment of EBV on alloxan-induced diabetic animals

Diabetic rats were divided into five groups of six rats: Group I, Normal control (received vehicle only); Group II, Diabetic control (vehicle+alloxan); Group III EBV 250 mg/kg; Group IV 500 mg/kg; and Group V Std. Drug Glibenclamide 600 µg/kg, p.o.

Plasma glucose was measured on 0 day, 1st, 4th, 7th, 10th and 15th day of the experiment, and weekly Body weight measurement were done on before alloxan-induction (Initial value), 1st day, 7th, 15th day of the study. On day 15th, plasma Total cholesterol (cholesterol esterase, cholesterol oxidase and Peroxidase method), Plasma Triglycerides (GPO- Trinder method), Plasma High density lipoprotein (HDL) cholesterol (Precipitation method), plasma total protein (Biuret, End point method), plasma insulin (Radio Immuno Assay) were estimated.

Pancrease were removed after sacrificing the animal and was collected in 10% formaline solution, and immediately processed by the paraffin technique. Section of 5 μ thickness were cut and stained by haematoxylin and eosin (H and E) for histological examination. The photomicrographs of histological studies are presented in fig.1.

2.8 Effect of EBV on Glucose tolerance in Normal rats

In this Oral Glucose Tolerance Test [3], fasted rats were divided into four groups of six rats each. Group I served as normal control and received vehicle only. Groups II and III received ethanol extracts at a dose of 250 mg/kg bodyweight 500 mg/kg and as a fine Gum acacia suspension respectively and group IV received the standard drug Glibenclamide as an aqueous suspension at a dose of 600 μg/kg body weight. After 30 min of extract administration, the rats of all the groups were orally treated with 2 g/kg of glucose. Blood samples were collected from the retro-orbital plexus just prior to glucose administration and at 30 and 90min after glucose loading. Plasma was separated by centrifugation and blood glucose levels were measured immediately by using Glucose Oxidase/Peroxidase (GOD/POD) method.

2.9 Statistical analysis

Results are expressed as the mean ± SEM. Statistical analysis was carried out using one-way analysis of variance followed by Neuman-keul's test for comparison between groups. $P<0.05$ was considered as a significant [12].

3. Results

In Normal rats (Table-1) daily treatment with EBV of 250 mg/kg b.w. and 500 mg/kg b.w. led to a significant fall in the BGL. The effect seems to reach maximum on 4th day of the treatment period with EBV 500 mg/kg. Glibenclamide also significantly reduced the fasting blood glucose level in normal rats. In alloxan-induced hyperglycemic rats (Table-2) a significant reduction in the blood glucose level was observed as compared to diabetic control with both doses of EBV (250 mg/kg b.w. and EBV 500 mg/kg b. w.) and Glibenclamide treated groups.

In the body weight measurement (Table-3), normal vehicle control animals were found to be gained in their body weight. In alloxan-induced diabetic rats showed a significant reduction in the body weight, which is reversed by extract treated groups (EBV 250 mg/kg b.w. and EBV 500 mg/kg b.w.) and Glibenclamide (std. drug) treated group during 15 days treatment.

Plasma Total cholesterol and Triglycerides levels were decreased significantly by EBV 250 mg/kg and EBV 500 mg/kg b.w. and Glibenclamide after 15 days treatment. Where as plasma HDL, total protein, and insulin levels were increased by extract treated groups (EBV 250 mg/kg b.w. and EBV 500 mg/kg b.w.) and Glibenclamide during 15 days study (Table-4).

The effect of EBV on glucose tolerance is reported in Table-5. Both doses of EBV and Glibenclamide have prevented the increase in blood glucose levels significantly after glucose administration; the maximum glucose tolerance was observed at the 30th min with all treated groups.

Table 1. Effect of *Bauhinia variegata* bark extract on blood glucose level of non- diabetic (normal rats) albino rats after prolonged treatment

Groups	Treatment (n=6)	Dose	Blood Glucose Level mg/100ml (Mean±SEM)					
			Basal value (0 day)	1st day	4th day	7th day	10th day	15th day
I	Normal Control	Vehicle	75.54±1.04	79.83±0.93	80.50±0.76	79.83±0.47	80.0±0.57	79.11±0.47
II	Ethanol extract	250 mg/kg b.w	78±0.73	57.83±0.47***	44.33±0.66***	44.33±0.49***	44.83±0.60***	56.5±0.61***
III	Ethanol extract	500 mg/kg b.w	76.5±0.76	54.07±0.60***	41.17±0.47***	43.33±0.66***	41.5±0.42***	50±0.51***
IV	Glibenclamide	600 µg/kg b.w	75. 83±0.65	73.83±0.60***	68.5±0.42***	64.17±0.60***	58.5±0.42***	56.5±0.76***

One-way ANOVA followed by Newmann-Keuls test. Values are expressed as mean±SEM; n= number of animals. P>0.05 is considered as non-significant. ***P<0.001 as compared to normal control group.

Table 2. Effect of *Bauhinia variegata* bark extract on blood glucose level of alloxan induced diabetic albino rats after prolonged treatment

Groups	Treatment (n=6)	Dose	Blood Glucose Level mg/100ml (Mean±SEM)					
			Basal value (0 day)	1st day	4th day	7th day	10th day	15th day
I	Normal Control	Vehicle	75.54±1.04	79.83±0.93	80.50±0.76	79.83±0.47	80.0±0.57	79.11±0.47
II	Diabetic Control	Vehicle	334.7±3.13+++	326.0±3.09+++	321.3±1.45+++	321.7±0.88+++	332.3±0.98+++	339.7±0.66+++
III	Ethanol extract	250 mg/kg b.w	319.3±1.25	132.7±2.24***	128.5±0.56***	119.5±1.11***	117.5±0.42***	118.8±0.47***
IV	Ethanol extract	500 mg/kg b.w	323.3±5.48	122.7±1.43***	120.3±0.49***	118.0±0.57***	115.5±0.56***	104.8±0.94***
V	Glibenclamide	600 µg/kg b.w	327.0±6.82	276.3±2.43***	228.3±4.4***	192.7±2.17***	161.2±1.74***	138.7±1.43***

One-way ANOVA followed by Newmann-Keuls test. Values are expressed as mean±SEM; n= number of animals. P>0.05 is considered as non-significant. P<0.05 is considered as significant. +++P<0.001 as compared to normal control group. ***P<0.001 as compared to diabetic control group.

Table 3. The effect of 15 days treatment with bark extract of *Bauhinia variegata* on body weight in alloxan induced diabetic rats.

Groups	Treatment (n=6) P.O	Average body weight (g) ±SEM			
		Initial value	Day 1	Day 7	Day15
I	Normal Control, vehicle only (NC)	142±0.57	156.0±0.57	181.7±0.61	204.5±0.76
II	Diabetic control (DC)	143.5±0.57	135.5±0.42+++	129.5±0.76+++	110.5±0.76+++
III	Ethanol extract (250 mg/kg) (EBV2)	141.5±0.42	129.8±1.4***	150.8±0.93***	197.0±0.81***
IV	Ethanol extract (500 mg/kg)(EBV1)	143.5±0.76	133.5±0.42	154.8±0.60***	203.0±1.23***
V	Glibenclamide (600 µg/kg) (GLB)	140.5±0.76	132.70.61*	131.3±0.49	142.3±1.81***

One-way ANOVA followed by Newmann-Keuls test. Values are expressed as mean±SEM; n= number of animals. P>0.05 is considered as non-significant. *P<0.05 as compare to diabetic control group. +++P<0.001 as compared to normal control group. ***P<0.001 as compared to diabetic control group.

Table 4. Effect of Ethanol bark extract of *Bauhinia variegata* on plasma Profile in alloxan-induced diabetic albino rats after 15 days of treatment.

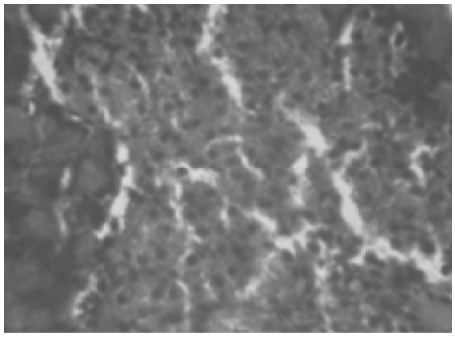
Groups	Treatment (n=6)	Dose	Plasma profiles				
			Plama Total cholesterol (mg/dl)	Plasma Triglycerides (mg/dl)	Plasma HDL (mg/dl)	Plasma Total Protein (g/dl)	Plama Insulin (μ IU/ml)
I	Normal Control	Vehicle only	61±0.57	122.7±0.71	33.5±0.42	5.6±0.05	30.50±0.42
II	Diabetic Control	Vehicle only	178.7±0.88^{+++}	399.0±3093^{+++}	24.67±0.66^{+++}	4.19±0.05^{+++}	14.0±0.36^{+++}
III	Ethanol extract	250 mg/kg b.w	68.33±0.88***	175±0.57***	33.5±0.42***	4.63±0.06***	29.95±0.34***
IV	Ethanol extract	500 mg/kg b.w	62.0±0.57***	181.8±0.70***	33.0±0.57***	5.14±0.04***	26.67±0.33***
V	Glibenclamide	600 μg/kg b.w	73.0±0.57***	137.2±0.60***	26.33±0.66*	5.36±0.09***	22.5±0.42***

One-way anova followed by newmann-keuls test. Values are expressed as mean±sem; n= number of animals. P>0.05 is considered as non-significant. *p<0.05 as compare to diabetic control group. +++p<0.001 as compared to normal control group. ***p<0.001 as compared to diabetic control group.

Table 5. Effect of *Bauhinia variegata* extracts on oral glucose tolerace test (OGTT) in normal albino rats

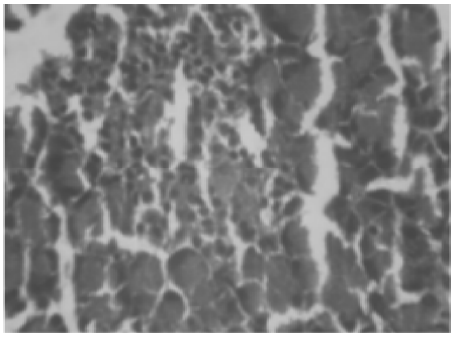
Groups	Treatment (n=6) P.O	Blood Glucose Level mg/100ml (Mean±SEM)		
		Fasting value (FBGL)	30 min	90min
I	Normal Control, glucose (2g /kg)	81.67±0.61	184.7±0.66	104.8±0.94
II	Ethanol extract (250 mg/kg), glucose (2 g /kg)	83.67±0.88	154.2±0.65***	101.5±0.76**
III	Ethanol extract (500 mg/kg), glucose (2 g/kg))	80.83±0.70	154.8±1.01***	94.67±0.88***
IV	Glibenclamide (600 μg/kg), glucose (2 g/kg)	82.67±0.49	170.0±0.57***	105.0±0.57

One-way ANOVA followed by Newmann-Keuls test. Values are expressed as mean±SEM; n= number of animals. P>0.05 is considered as non-significant. **P<0.01as compared to normal control group. ***P<0.001 as compared to normal control group.



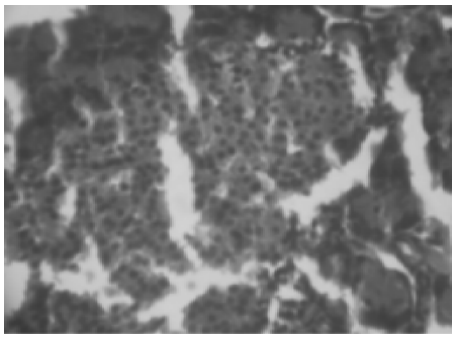
A) Normal Control

(A) Normal Control: Normal number of islets /sqcm cells, absence of extensive damage to the islets, absence of size reduction to islets and absence of hyperplasia to islets of langerhans in pancreas of vehicle-treated normal control rats.



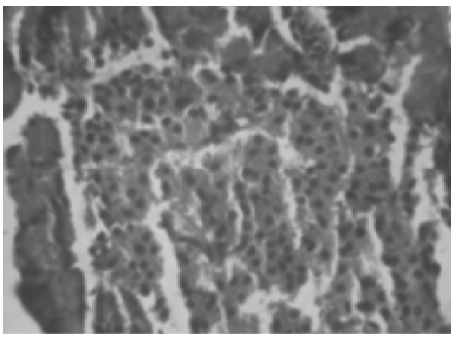
B) Diabetic Control

(B) Diabetic Control: Decrease in normal cellular population of islets of langerhans, extensive damage to islets, reduction in size of islets and absence of hyperplasia was seen in diabetic control rats.



C) GLB Standard Control

(C) GLB Standard Control: Restored the number of islets towards normal population with hyperplasia, restored the cellular size and absence of damage to islets was seen in Glibenclamide treated group.



D) EBL - Treated Ground

(D) EBV (500 mg/kg) treated Group: Restored the number of islets towards normal population with hyperplasia, restored the cellular size and absence of damage to islets was also seen in *Bauhinia variegata* (EBV 500mg/kg body weight) treated group (D).

Fig. 1. Photomicrographs of Rat Pancreas

After keen observation of all the results, EBV 500 mg/kg found to have maximum effect in most of parameters in experimental animals, so histopathology of the same is studied.

Photomicrographs (fig. 1), showed that normal number of islets /sqcm cells, absence of extensive damage to the islets, absence of size reduction to islets and absence of hyperplasia to islets of langerhans in pancreas of a vehicle-treated normal control rats (A). Decrease in normal cellular population of islets of langerhans, extensive damage to islets, reduction in size of islets and absence of hyperplasia was seen in diabetic control rats (B). Restored the number of islets towards normal population with hyperplasia, restored the cellular size and absence of damage to islets was seen in Glibenclamide treated group (C). Restored the

number of islets towards normal population with hyperplasia, restored the cellular size and absence of damage to islets was also seen in Bauhinia variegata (EBV 500 mg/kg body weight) treated group (D).

4. Discussion

The ethanolic extract of the bark of *Bauhinia variegata* (250 mg/kg and 500 mg/kg body weight) have shown significant reduction in blood glucose levels in normal, alloxan-induced diabetic and glucose loaded hyperglycemic normal rats respectively.

The hypoglycemic effects of *Bauhinia variegata* in normal rats and anti-hyperglycemic effect in glucose loaded hyperglycemic normal rats may be due to the potentiation of insulin release from β- cells, this effect is supported by a earlier reports such as extracts of Bauhinia variegata shows insulin secretagogue activity from INS-1 cells [13] and presence of insulin-like protein (which is involved in the carbohydrate metabolism) present in the leaves of *Bauhinia variegate* [14].

It is generally accepted that the alloxan treatment causes permanent destruction of β-cells [3]. In such alloxan-induced diabetic rats, the EBV shows a significant anti-hyperglycemic effect, this could be due to regeneration of β-cells of Islets of langerhans of pancreas. This regeneration effect is supported by the results of histopathological evaluation of pancreas and plasma insulin level. The extracts have also improved the condition of diabetic mellitus by increasing the body weight and attenuating the lipid profiles towards the normal.

5. Conclusion

Earlier reports suggest that the glycosides, alkaloids, flavonoids, steroidal compounds and tannins are responsible for anti-diabetic activity [15, 16]. However, preliminary phyto-chemical study reveals the presence of, steroids, flavonoids and tannins in the alcoholic extract of *Bauhinia variegata*. Thus the anti-diabetic effect produced by the extract of *Bauhinia variegata* may be due to presence of any of these active ingredients. Further studies are in progress to isolate the active principles and to study their mechanism of actions.

6. Acknowledgements

Authors wish to thank the Principal and management of K.L.E. Society's college of Pharmacy, Belgaum for providing the necessary facilities to carryout this research work.

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