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Research Article

Evaluation of Antidiabetic Efficacy Herbal Ethanolic Extracts in Alloxan-Induced Diabetes Rats

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ABSTRACT

Diabetes is traditionally treated with the huge climber plant *Bauhinia vahlii* Wight & Arn. The evaluated of antidiabetic effect of *Bauhinia vahlii* ethanolic extracts leaves and stem at doses of 200 mg/kg and 400 mg/kg using an experimental model by alloxan-induced diabetes in albino rats. The acute toxicity tests revealed that the extracts were not harmful, as there were no deaths at the dosages 2000 mg/kg. The groups were divided into herbal extracts doses, standard drug Glibenclamide at 5 mg/kg, diabetic control group, and normal control group. Blood sugar concentrations were regular monitor and measured for 21st days, and the levels of glucose in the blood after fasting were measured on days 0, 7, 14, and 21st in each of the animal groups. The remarkable antidiabetic effects significant (p<0.001) at dose of 400 mg/kg of *Bauhinia vahlii* leaves extract comparable to glibenclamide diabetes control. This beneficial effect is probably due to the existence of flavonoids and phenolic substances, which might enhance the release of insulin.

Introduction

Diabetes mellitus arises either from a genetic or developed deficit in the pancreas's capacity to generate insulin or from the inefficacy of the insulin that is generated. Elevated blood sugar levels can harm numerous bodily functions, especially the blood vessels and nerve system (Jadon *et al.*, 2024). Type 1 diabetes is characterized by elevated blood sugar levels as a result of decreased insulin production, as opposed to type 2 diabetes, which is defined by elevated blood sugar levels due to insufficient insulin utilization by the body (Marshal *et al.*, 2004). Among these two categories, type -2 diabetes poses a significant contemporary challenge, representing approximately 95% of the total diabetic individuals, which is affected by around 246 million people (Mycek *et al.*, 2000). About 45,000 different plant species have been source of medicinal compounds to have therapeutic or medicinal properties (Pradesh A. 2022). Diabetes is typically treated with insulin injections, diet, exercise, and oral antidiabetic drugs (Farzaei *et al.*, 2017).

Diabetes mellitus is a worldwide disease that affects

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in the primary cause of heart attacks, kidney failure, blindness, and lower limb amputations (Balakumar *et al.*, 2016). More than 75% of diabetics will live in developing nations by 2025, up from 62% in 1995 (Eseyin *et al.*, 2010). Diabetes is currently treated using insulin and several oral antidiabetic drugs, such as biguanides and sulfonylureas (Krentz *et al.*, 2005) and many herbs have been shown to produce hypoglycemic effects (Rajan *et al.*, 2012). The World Health Organization states that around 1200 plant species are utilized globally to treat diabetes mellitus, and many of these plants have demonstrated beneficial hypoglycemic action following laboratory testing (Patel *et al.*, 2012). The majority of plants contain alkaloids, carotenoids, flavonoids, glycosides, and terpenoids are commonly used to antidiabetic effects (Aba *et al.*, 2018).

Bauhinia vahlii is climber shrubs belong to family Caesalpiniaceae and used as an antidiabetic drug in traditional Indian medicine (Saravanan *et al.*, 2019). It climbing shrub is found over India in the Eastern and Western Ghats, Assam, Central India, Bihar and highly altitude of the Himalayan region (Nigam. *et al.*, 2021). This plant is known as "Malanjhana" in Sanskrit and "Maljan" in Hindi (Krishnamoorthy *et al.*, 2004 and Chauhan *et al.*, 2013). The bioactive compounds of *Bauhinia vahlii* leaves and stem are stigmasterol, kaempferol, β-sitosterol, campesterol, betulinic acid, quercetin, isoquercetrin, and quercetin 3-glycoside (Shukla *et al.*, 2020 and Elbanna *et al.*, 2016). *Bauhinia vahlii* leaves and stem have been used as an alternative drugs by the people to treat a variety of ailments, such as fever, wounds, cuts, stomachaches, antidiabetic, and skin disorders (Kirtikar *et al.*, 2005). The plant of *Bauhinia vahlii* shown in figure 1.

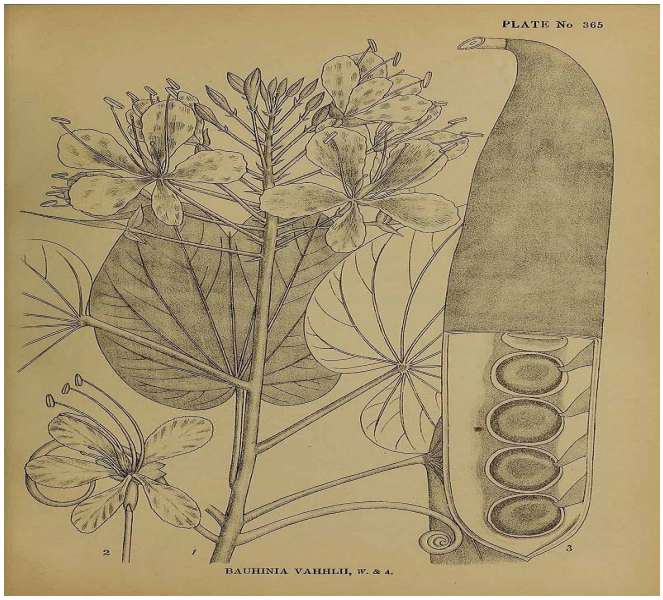

Figure 1: Plate Photo of *Bauhinia vahlii* Leaf, Flower and Pod with Seeds
(Kirtikar, K. R., and Basu, B. D. 2005)

MATERIALS AND METHODS

Herbal Parts

The leaves and stem of *Bauhinia vahlii* were collected from the Chandi Devi Temple in Uttarakhand, India's Hardwar District. The plant sample was recognized and verified to be authentic by Dr. S. K. Sinha, Scientist-E, Botanical Survey of India, Allahabad.

Extracts Preparation

Bauhinia vahlii leaves and stem air dried coarsely powdered 40 mesh size (Handa *et al.*, 2008) have extracted separately from polar solvent 95% ethanol with hot percolation method by soxhlet apparatus at a temperature of 45 to 50ºC (Evans *et al.*, 1996). The ethanolic extracts of *Bauhinia vahlii* leaves and stem*;* (EEBVL) and (EEBVS) were prepared by filtering the extracts, distilling the filtrate at a low temperature (55–60ºC), and then evaporating it under low pressure. The two extracts, EEBVL and EEBVS, were employed for in vivo antidiabetic evaluation after being finely suspended in 1% w/v aqueous normal saline.

Wistar Albino Rats Animal

For the toxicological activity and antidiabetic assessment, 150–200g Wistar albino rats of either sex were chosen. The animals were acclimated to standard laboratory conditions at 25±2˚C and maintained on a 12-hour light-dark cycle (Yousaf *et al.*, 2017) and given regular animal feed with water ad libitum (Sun *et al.* 2010). The Institutional Animal Ethical Committee (IAEC) accepted the experimental procedures and animal care protocols under approval number 837/ac/04/CPCSEA.

Acute Oral Toxicity

The OECD 423 standards, 2000 mg/kg of EEBVL and EEBVS were given as a single dosage. A tenth and a fifth of the fatal dose (LD50) were taken into concern for pharmacological evaluation. Following an oral acute toxicity activity, the doses were selected LD50 determination (OECD, 2001).

Statistical Study

All of the study's graphs were created using Graph Pad Prism. * ($p<0.05$), ** ($p<0.01$), and *** ($p<0.001$) were statistically significant when compared to the control group. The data were presented as Mean ± SD (n = 6) and were examined using the Bonferroni test and two-way ANOVA.

Evaluation of Antidiabetic Activity Alloxan Induced Diabetes Model

Intraperitoneal injections of 120 mg/kg of alloxan monohydrate in 0.9% w/v NaCl were used to induce diabetes in rats that had fasted overnight (Jain *et al.*, 2010). In order to prevent hypoglycemia, the rats were then given a 10% glucose solution for the following 24 hours (Jarald *et al.*, 2008). Rats with significant hyperglycemia

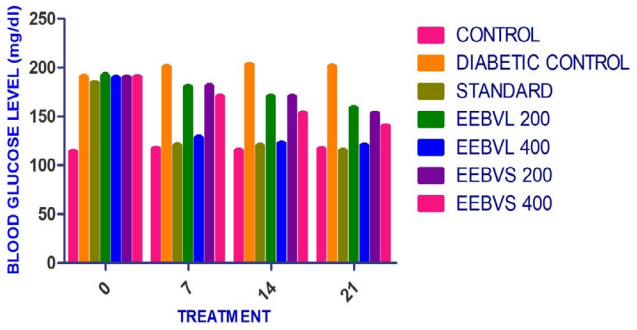

Figure 2: Blood Glucose Level Effects of EEBVL and EEBVS in Alloxan-Induced Diabetic Rats

(fasting blood glucose > 250 mg/dl) were chosen for the investigation and used 72 hours following injection (Nwoye *et al.*, 2010). For each of these animal groups, fasting blood glucose levels were assessed on days 0, 7, 14, and 21st.The course of treatment was maintained for twenty-one days in a row (Ewenighi *et al.*, 2015). Seven groups (n = 6) were formed from the selected diabetic animals as follows:

- Normal control (Saline vehicle 10 ml/kg p.o) as a Group I
- Diabetic control (Alloxan + vehicle 120 mg/kg bw i.p.) as a Group II
- Diabetic rats were treated with standard drug glibenclamide (5 mg/kg p.o.) as a Group III
- Diabetic rats treated with EEBVL (200 mg/kg p.o.) as a Group IV
- Diabetic rats treated with EEBVL (400 mg/kg p.o.) as a Group V
- Diabetic rats treated with EEBVS (200 mg/kg p.o.) as a Group VI
- Diabetic rats treated with EEBVS (400 mg/kg p.o.) as a Group VII

Blood Collection, Estimation of Biochemical Parameter and Body Weight

An Accu-Chek active test meter and glucose oxidase peroxidase reactive strips were used to take blood from a rat tail vein in order to measure the blood sugar level (Ekun *et al.*, 2018). In order to get additional plasma profiles, capillary tubes were used to draw blood from the rats' retro orbital plexus while they were under mild ether anesthesia. Heparin was then added to Eppendorf tubes containing the blood. After centrifuging the plasma for five minutes at 5000 rpm, the lipid profiles of triglycerides and cholesterol were examined. The Erba diagnostic kit was utilized to estimate total cholesterol. Enzokit-Ranbaxy was utilized to quantify serum triglycerides (Kusmeirczyk, *et al.*, 2020). Standard enzymatic techniques were used with an automated analyzer to measure the plasma profiles (Maroo *et al.*, 2003). The rats' body weight was measured twice using a digital scale from KERN (EMB), Germany, Macline: once for the initial body weight (g) and once for the final body weight (g) on the twenty-first day of the treatment period (Nagappa *et al.*, 2003).

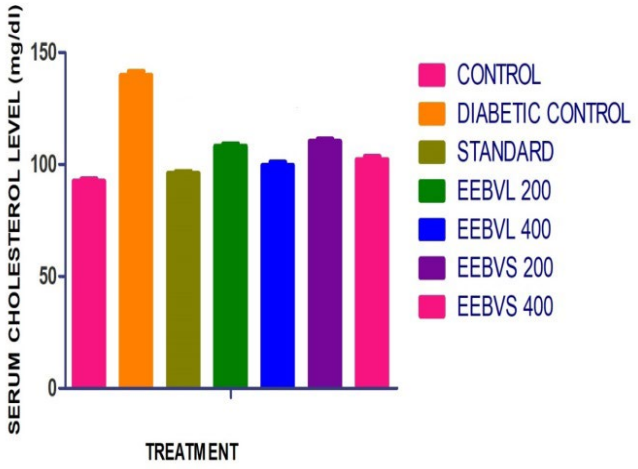

Figure 3: Effect of EEBVL and EEBVS Serum Cholesterol Level Profile in Alloxan Induced Diabetic Rats

RESULTS AND DISCUSSION

Alloxan-Induced Diabetic Rats' Blood Glucose Level

EEBVL reduced blood glucose levels in fasting normal rats

Table 1: Effects of Ethanolic Extract from *Bauhinia vahlii* Leaves and Stem on Blood Sugar Levels in Alloxan-Induced Diabetic Rats

Group	*Treatment*	*Dose*	*Blood Glucose Level (mg/dl) (Mean ± SD)*			
			0 day	*7th day*	*14th day*	*21st day*
I	Normal Control	10 ml/kg p.o.	114.00±1.10***	114.83±0.98***	115.33±0.82***	116.83±1.17***
II	Diabetic Control (Alloxan)	120 mg/kg i.p.	190.50±1.52	200.67±1.03	203.17±0.98	204.33±1.03
III	Standard (Glibenclamide)	5 mg/kg p. o.	184.50±0.84**	120.50±1.05***	120.17±1.17***	115.17±0.98***
IV	EEBVL	200 mg/kg p.o.	191.83±1.60*	180.33±1.23**	170.21±1.05**	158.83±0.98**
V	EEBVL	400 mg/kg p. o.	189.33±1.63*	128.54±1.17***	122.57±0.98***	120.57±0.84***
VI	EEBVS	200 mg/kg p.o.	190.17±0.98*	181.24±1.56**	170.21±0.89**	153.33±0.82**
VII	EEBVS	400 mg/kg p. o.	190.67±0.82*	170.22±1.37**	153.33±1.03**	140.21±0.98**

All value was shown as Mean ± SD (n=6). The data were statistically *(p<0.05), **(p<0.01) and *** (p<0.001) evaluated in relation to the diabetes control group using a two-way ANOVA and the Bonferroni test.

Table 2: EEBVL and EEBVS' Effect on Alloxan-Induced Diabetic Rats' Serum Lipid Profile

Group	*Treatment*	*Dose*	*Cholesterol (mg/dL) (Mean ± SD)*	*Triglycerides (mg/dL) (Mean ± SD)*
I	Normal Control	10 ml/kg p.o.	92.55±0.95***	82.2±0.91***
II	Diabetic Control (Alloxan)	120 mg/kg i.p.	137.83±1.17	115.32±1.00
III	Standard (Glibenclamide)	5 mg/kg p.o.	96.04±0.87***	87.21±0.97***
IV	EEBVL	200 mg/kg p.o	108.21±0.98**	99.31±0.84**
V	EEBVL	400 mg/kg p.o.	99.54±1.58***	91.10±1.17***
VI	EEBVS	200 mg/kg p.o.	110.27±1.07**	104.41±0.89**
VII	EEBVS	400 mg/kg p.o.	102.20±1.35**	94.21±0.82**

All values were displayed using mean ± SD (n=6). The results were statistically evaluated using one-way ANOVA, Dunnett's test * ($p<0.05$), ** ($p<0.01$), and *** ($p<0.001$), and they were compared with the diabetic control group.

Table 3: Body Weight Effects of EEBVL and EEBVS in Alloxan-Induced Diabetic Rats

Group	*Treatment*	*Dose*	*Mean initial body weight(g) (Mean ± SD)*	*Mean final body weight(g) (Mean ± SD)*	*Percentage (%) change in body weight*
I	Normal Control	10 ml/kg p.o.	178.67±1.03***	184.17±1.33***	2.99
II	Diabetic Control (Alloxan)	120 mg/kg i.p.	183.83±1.17	198.00±1.26	7.16
III	Standard (Glibenclamide)	5 mg/kg p.o.	177.50±1.05***	185.67±1.03***	4.40
IV	EEBVL	200 mg/kg p.o	184.33±1.21**	196.17±1.17**	6.04
V	EEBVL	400 mg/kg p.o.	178.00±1.26***	188.33±1.51***	5.49
VI	EEBVS	200 mg/kg p.o.	179.83±1.47**	191.17±1.37**	5.93
VII	EEBVS	400 mg/kg p.o.	176.67±1.21**	187.17±1.47**	5.61

All value was shown as Mean ± SD (n=6). The data were statistically analyzed using two-way ANOVA and the Bonferroni test * ($p<0.05$), ** ($p<0.01$), and *** ($p<0.001$) in comparison to the diabetes control group.

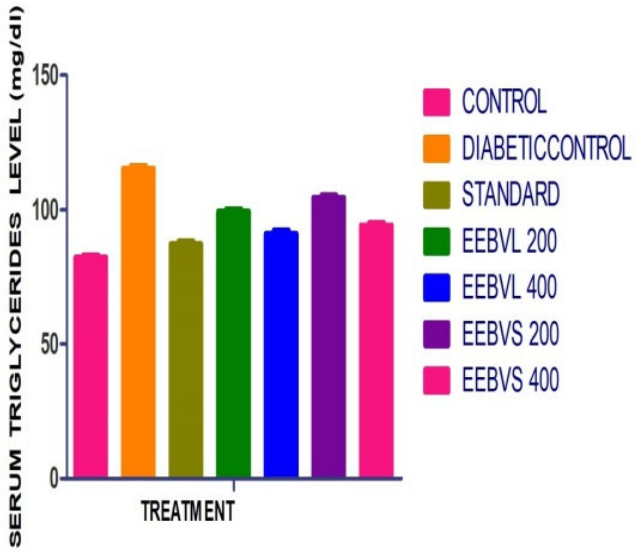

Figure 4: Effect of EEBVL and EEBVS on Serum Triglycerides Level Profilebin Alloxan Induced Diabetic Rats

in the Alloxan-induced diabetes model from 189.33±1.63 mg/dL to 120.57±0.84 mg/dL at 400 mg/kg, while blood glucose levels in the diabetic control group dropped from 190.50±1.52 to 204.33±1.03 mg/dL. On the 21st day, the glibenclamide 5 mg/kg group's peak blood sugar levels significantly ($p<0.001$) decreased from 184.50±0.84 mg/dL to 115.17±0.98 mg/dL, showing serious hypoglycemia. The most remarkable result was a significantly ($P<0.001$) reduction in EEBVL blood glucose levels at 400 mg/kg. The antidiabetic effects showed in the Tables 1 and Figure 2.

Alloxan-Induced Diabetic Rats' Serum Lipid Profile

EEBVL was found to have good activity in decreasing the blood cholesterol level (99.54±1.58 mg/dL) and triglyceride level (91.10±1.17 mg/dL) at 400 mg/kg when compared to the diabetes control group (137.83±1.17 mg/dL and 115.32±1.00 mg/dL). Consequently, compared to diabetic control rats, the administration of EEBVL at 400 mg/kg, p.o. bw for 21 days significantly ($p<0.001$) decreased the serum levels of triglycerides and cholesterol. The outcome was showed in Figures 3 and 4 as well as Table 2.

Body Weight Change in Alloxan-Induced Diabetic Rats

The final body weight in the diabetic control group increased from 183.83±1.17 g to 198.00±1.26 g, while the end body weight in the EEBVL at 400 mg/kg was only slightly different from the initial body weight (178.00±1.26 g). The result was shown in Table 3.

CONCLUSION

The study's findings provide that ethanolic extracts of *Bauhinia vahlii* leaves and stems. The antidiabetic effectiveness of 200 mg/kg and 400 mg/kg ethanolic extracts of *Bauhinia vahlii* leaves and stem (EEBVL and EEBVS) was evaluated in an albino rat model of alloxan-induced diabetes. Ethanolic leaf extracts showed a quick, long-lasting, and statistically significant antidiabetic activity impact at dosages of 400 mg/kg. EEBVL revealed a significant (p<0.001) antidiabetic effect at 400 mg/kg when compared to the diabetes control in this test model. The presence of flavonoids and phenolic compounds, which may increase the release of insulin, is most likely the cause of this positive effect.

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DECLARATION OF INTERESTS

The authors declare that there is no conflict of interest.

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This research received no external funding.

DATA AVAILABILITY

The data supporting the funding of this study are available.

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