

# Antihyperglycemic activity of the ethanolic seed extract of *Vernonia anthelminticum* willd

A. Karthikeyan<sup>1</sup>, G. Siva, S. P. Sujitha, D. A. P. Rex<sup>2</sup>, N. Chidambaranathan<sup>3</sup>

Department of Biotechnology, <sup>1</sup>PG and Research Department of Microbiology, <sup>2</sup>Department of Biochemistry, JJ College of Arts and Science, Pudukkottai - 622 404, <sup>3</sup>Department of Pharmacology, K. M. College of Pharmacy, Madurai - 625 414, India

The investigation was carried out to study the effects of *Vernonia anthelminticum* Willd seed extract on blood glucose level. The antihyperglycemic efficacy of the ethanolic extract of the seed was evaluated in normal, glucose and alloxan induced diabetic rats. The extract exhibited significant hypoglycemic activity in all three animal models when compared with the control group. The activity was also comparable to that of the effect produced by a standard antidiabetic agent gliclazide, 25 mg/kg (p.o.). The results also indicated dose dependent effect. The hypoglycemia and antihyperglycaemia produced by the extract may be due to increased uptake of glucose at tissue level or increase in pancreatic beta-cell function or due to inhibition of intestinal absorption of glucose. The study indicated that the ethanolic extract is a potential antidiabetic agent and lends scientific support for its use in folk medicine.

**Key words:** Alloxan, antidiabetic, blood glucose, gliclazide, *vernonia anthelminticum*

## INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia, hypertriglyceridaemia and hypercholesterolemia, resulting from defects in insulin secretion action or both.<sup>[1,2]</sup> Regions with greatest potential are Asia and Africa, where DM rate could raise 2-3 folds than the present rates. Many herbal medicines have been recommended for the treatment of diabetes. Plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones.<sup>[3]</sup> *Vernonia anthelminticum* willd family Asteraceae is a well-known plant in traditional Indian system of ayurvedic medicine. Found in India in the tropical wild forest, Pakistan, Srilanka, Nepal and Myanmar. Its fruit and seeds are bitter and considered thermogenic, anthelmintic, astringent, anti-inflammatory, anodyne, expectorant, depurative, demulcent, purgative, diuretic, febrifuge, galactagogue antioxidant and toxic. The drug is also useful as a medicinal cure for asthma, skin disease, pruritus, hecorderma, dyspepsia, fever, ophthalmopathy and are very specific for round worm and threadworm.<sup>[4]</sup>

Seeds of *Vernonia anthelminticum* chiefly contain two novel and known steroids from benzene, acetone and ethanolic extracts. It has also been identified as (24 alpha/R) - stigmasta - 7 - en - 3-one (1), (24 alpha/R) - stigmasta 7,9 (11) - dien - 3 - one (2), (24 alpha/R) - stigmasta - 5, 22 - dien - 3 - Beta - 01(3) and (24 alpha/S) - stigmasta - 7,22 - dien - 3 - beta - 01.<sup>[5]</sup> The novel steroids were tested against several human pathogenic bacteria

and fungi. In Unani system, it is used for treating diabetes.<sup>[6]</sup>

Although acclaimed traditionally as anti-diabetic, there are no scientific studies regarding the effect of *Vernonia anthelminticum* on blood glucose level. In the preseny investigation, the anti-diabetic activity of ethanolic extract of *Vernonia anthelminticum* seed is being reported.

## MATERIALS AND METHODS

### Animals

Male albino rats weighing 200-250 gms were fed on standard diet and water *ad libitum*. The animals were housed at room temperature (24±2°C) on a reversed day night cycle (06:00 hrs to 18:00 hrs). The animals described as fasted were deprived of food for 16 hrs. Plant Material Seeds of *Vernonia anthelminticum* willd were collected at Kulasekaram, Tamilnadu, India and taxonomically identified at the Department of Botany, J.J. College of Arts and Science, Pudukkottai. A voucher specimen of the same has been made and deposited (No. JJC - VA - 2007) at departmental herbarium.

### Chemical Preparation

Gliclazide was obtained from Avinash health products (Pvt) Ltd, Chennai. 2 ml of suspension of gliclazide was also made with sterile distilled water. The glucometer kit used was purchased from Life scan. All the animal experimentations were carried out after prior permission from the institutional ethical committee of J.J. College of

**For correspondence:** Dr. A. Karthikeyan, Department of Microbiology, JJ College of Arts and Science (Affiliated to Bharathidasan University, Tiruchirappalli) Pudukkottai, Tamil Nadu, India 622 404. E-mail: mdukarthimicro@yahoo.com

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Arts and science, Pudukkottai, Tamilnadu (India).

### Preparation of Extract

The powdered dried seeds were defatted by extraction with petroleum ether (60-80°C). The defatted material was then extracted with ethanol (95%) for 72 hrs by hot percolation method and subjected to vacuum distillation. The final product (8.5 gms) was then freeze - dried and stored in the refrigerator.

### Effects of Alcoholic Extracts in Normal Glycemics and Glucose Tolerance in Rats

Fasted rats were divided into five groups of six rats each. Groups 1 serve as normal control. Group 2 received 500 mg/kg (p.o.) of ethanolic extract. Group 3 (glucose control) received distilled water (p.o.). Group 4 and Group 5 received 500 µg/kg (p.o.) of ethanolic extract and gliclazide 25 mg/kg (p.o.), respectively. After 30 min, extract and standard drug administration, the rats of all groups were orally treated with 25/kg of glucose.<sup>[3-5]</sup>

Blood samples were collected from retro orbital plexus at 30 and 90 min after glucose loading. Serum was separated by centrifugation of blood at 2000 rpm for 15 min and serum glucose level was determined by glucometer [Table 1].

### Effects of Vernonia Anthelminticum Seed Extracts on Alloxan - Induced Hyperglycemia

Hyperglycemia was induced by a single I.P. injection of 120 mg/kg of alloxan monohydrate in sterile saline. After 5 days of alloxan injection, the diabetic rats (glucose level >250 mg/dl) were separated and divided into five groups

of six animals each. Group 1 serves as diabetic control and was given saline solution (10 ml/day). Group 2 was given alloxan I.P. injection (120 mg/kg). Group 3 received standard drug gliclazide 25 mg/kg (p.o.) and Group 4 and 5 were treated with ethanolic extract at a dose of 500 mg/kg and 1000 mg/kg, respectively. Blood samples were collected from retro orbital plexus at zero time (before receiving the extract, gliclazide) 1, 3, 7 and 10 day following the treatment. Serum was separated by centrifugation of blood at 2000 rpm for 15 min and serum glucose level was determined by glucometer [Table 2].

### Statistical Analysis

Results are expressed as mean ± S.E.M. The significance of the data was evaluated using ANOVA and were considered statistically significant when  $P < 0.01$  and  $P < 0.05$ . The statistical analysis was carried out using InStat 2.1 software.

## RESULTS AND DISCUSSION

In the present study, the hypoglycemic activity of ethanolic seed extract of *Vernonia anthelminticum* was evaluated in normal, normal hyperglycemic and alloxan induced diabetic rats. The effects of *Vernonia anthelminticum* extract on glucose tolerance and normal rat are summarized in Table 1, the treatment of normal rat with ethanolic extract and gliclazide, a known hypoglycemic drug, resulted from a significant decrease ( $P < 0.01$ ). In blood glucose levels after (90 min) oral drug administration when compared with Group 3 glucose (2 g/kg) also shows a significant decrease ( $P > 0.01$ ). The maximum glucose level tolerance was observed at the

**Table 1: Hypoglycemic activity of ethanolic seed extract of *V. Anthelminticum***

Groups	Treatment (P.O.)	Plasma glucose level (mg/dl)		
		0 min	30 min	90 min
I	Control (Distilled water)	75.61 ± 0.47	75.22 ± 0.44	75.13 ± 0.52
II	Extract (500 mg/kg)	72.86 ± 1.21	61.77 ± 1.10*	57.55 ± 0.81*
III	Glucose (2 g/kg)	78.62 ± 1.83	162.5 ± 1.17	133.51 ± 1.66
IV	Glucose + Extract	75.6 ± 0.48	131.54 ± 1.33*	115.8 ± 1.60*
V	Standard (25 mg/kg +Glucose)	73.85 ± 0.90	120 ± 10 ± 2.17*	100.94 ± 1.45*

Values (mg/100 ml) are mean ± S.E.M. (n=6), determine at different time (min) after treatment. A statistically significant difference of the values when compared with the zero time (\* $P < 0.01$ )

**Table 2: Effect of ethanolic seed extract of *V. anthelminticum* on alloxan-induced diabetic rats**

Group	Treatment	Dose	Plasma glucose level (mg/dL)				
			Basal value	Day 1	Day 3	Day 7	Day 10
I	Normal control	Saline 10 ml/kg	71.60 ± 2.24	75.50 ± 1.68	78.50 ± 2.66	80.00 ± 2.78	82.50 ± 1.62
II	Diabetic control	Alloxan 120 mg/kg	348.00 ± 6.81	352.50 ± 6.01	356.00 ± 6.32	358.00 ± 7.15	353.50 ± 7.97
III	Standard drug	Gliclazide 2 mg/kg	324.00 ± 9.28	312.50 ± 7.14	262.00 ± 5.96	216.00 ± 9.80*	188.50 ± 9.03*
IV	Ethanol extract of <i>V. anthelminticum</i>	500 mg/kg	323.50 ± 10.8	303.00 ± 7.24	290.00 ± 7.69	262.50 ± 9.89*	248.50 ± 9.69*
V	Ethanol extract of <i>V. anthelminticum</i>	1000 mg/kg	328.00 ± 2.82	301.50 ± 9.43	272.50 ± 4.17	218.00 ± 6.32**	202.50 ± 7.29**

Values (mg/100 ml) are mean ± S.E.M (n=6), determined at different time (Day) after treatment. A statistically significant difference of the value when compared with the diabetic control (\* $P < 0.01$ ; \*\* $P < 0.05$ )

30<sup>th</sup> min for ethanolic extract.

The blood glucose levels of the diabetic rats treated with ethanolic seed extract at doses of 500 and 1000 mg/kg showed significant differences at 7<sup>th</sup> and 10<sup>th</sup> day from initials levels ( $P<0.05$ ;  $P<0.01$ ) [Table 2]. The doses of the crude extract produced a maximum glucose lowering in diabetic rat's serum and with the higher dose, a significant time dependent hypoglycemic effect was shown throughout the period of study.

Based on the results of this study it was concluded that the ethanolic extract of *Vernonia anthelminticum* seeds, given orally at a dose of 500-mg/kg b.w. possesses significant hypoglycemic activity in normal, glucose loaded and diabetes induced rats. It is generally considered that alloxan treatment causes permanent massive destruction of beta cells of the islets of langerhans resulting in reduced synthesis and the release of insulin.<sup>[7-9]</sup> It was well established that leaves and seeds of certain plant constituents produce hypoglycemia by increasing the secretion of insulin from pancreas and that these compounds are active in mild alloxan induced diabetes whereas they are inactive in intense alloxan diabetes.<sup>[5,10]</sup> In another report, it was proved that some flavanoids and saponins isolated from *Curculigo orchioides* significantly reduce the blood glucose levels.<sup>[11-16]</sup> Further, flavonoids and glycosides may stimulate the secretion of insulin in beta-cells of pancreas.<sup>[6]</sup> In glucose-loaded animals, it is possible that the extract may enhance the secretion of insulin increasing glucose uptake.<sup>[17,18]</sup> In conclusion, it could be proved that the ethanolic extract of seeds of *Vernonia anthelminticum* possesses a hypoglycemic agent (24 alpha/R) - stigmasta -7-en-3-one (1), (24 alpha/R) - stigmasta 7,9 (11)-dien-3-one (2), (24 alpha/R) - stigmasta -5, 22-dien-3-Beta-01(3) and (24 alpha/S) - stigmasta -7, 22-dien-3-beta-01(5). However, chemical and pharmacological investigations are necessary to identify the latter and confirm its mechanism of action and its antidiabetic potential.

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