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ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of Antidiabetic Activity of *Vitex Altissima* in Alloxan Induced Diabetes Animal Model

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ABSTRACT
*Different allopathic drugs are available in market to manage diabetes such as Sulfonyl ureas, Biguanides, Gleptines etc. but associated with various side effects on long term usage, but more emphasis **is** being placed on the use of medicinal plants due to easy availability, easy consummation with low cost, and with no well-reported side effects. According to our Ayurdeva system of medicine large number of medicinal plants are available to manage diabetes which are free from side effects with the same potency of allopathic drugs. All these factors provoked us to initiate the present study of antidiabetic activity of Vitex altissima in alloxan induced diabetes animal model. In this study diabetes was successfully induced with the help of alloxan. The diabetic animals were treated with Ethanolic & Petroleum ether extracts of Vitex altissima for 10 days which significantly reduced the elevated blood glucose levels and other altered parameters of Cholesterol, Triglycerides, LDL, Urea, Creatinine and Total Protein. Among the two extracts of Vitex altissima Petroleum ether extract has shown comparatively significant anti-diabetic activity in alloxan induced diabetes in animal model.*
Keywords: *Vitex altissima, Diabetes, Alloxan, Animal model.*

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic syndrome characterized by Hyperglycemia, Hypertriglyceredemia and hypercholesterolemia [1]. It is caused by inherited and/or acquired deficiency in production of Insulin by the pancreas or by the ineffectiveness of the insulin produced [2]. Diabetes is crudely grouped into two types: Insulin dependent diabetes mellitus (IDDM) and non–insulin dependent diabetes mellitus (NIDDM), Both these types are associated with excessive morbidity and mortality [3]. It is estimated that there are 180 million diabetics globally, and by 2030, that number could have doubled. According to reports from the World Health Organisation, diabetes mellitus is one of the leading causes of death in the modern era, with people in Southeast Asia and the Western Pacific region being especially susceptible. [4].

There are numerous allopathic and synthetic drugs available to treat diabetes, but they are all associated with side effects and on the other hand persistence of hyperglycemic conditions for a longer period resulting in serious complications and cause damage to the heart, blood vessels eyes, kidney and nerves, moreover, increases the risk of heart diseases and stroke [5]. The limitations with the existing management/treatment necessitate a search for help among arsenal of herbs available to humans**.** Numerous drugs present in the plants are useful in managing diabetes mellitus. Plants are the major source of drug and are available in the market as extracts directly or indirectly from the plant sources [6]. Plants have been utilized as drugs throughout history for both curative and preventative purposes. Due to their widespread availability and low cost, medicinal herbs have been utilised extensively to treat diabetes all over the world [7].

Vitex altissima is commonly known as Ganduparu, Nemiliadogu (peacock chaste tree) belongs to family Verbenaceae belongs to single genus, *Vitex*. This genus comprises of a number of species which are widely known for the presence of variety of biological principles medical significance. It is a woody plant reaching to a height of 20 meters characterized by greyish bark becomes scaly with maturity. The leaves are trifoliolate or palmate, compound and opposite. They are elliptic or elliptic-lanceolate in shape with acuminate apex and cuneate base. Ethno medicinal information from tribal people of Sirumalai hills of Western Ghats of Tamil Nadu revealed that extract of this plants used by Paliyan tribe as a remedy for

various skin diseases and having rich antioxidant principles. There is, however, no report on the antidiabetic activity of *Vitex altissima* in the literature. Yet, this plant is known for its possession of various medicinal alkaloids saponins & tannins [8] and showing the antibacterial, anti-inflammatory [9] & antioxidant activities [10]. Hence, this study was aimed at investigating the anti-diabetic activity of leaves of *Vitex altissima* by animal models.

MATERIAL AND METHODS

Plant material

The Fresh and healthy leaves of *Vitex altissima* were obtained from the forests of Tamilnadu in the Kancheepuram region. The sample specimen was authenticated by Dr. Madhav Chetty, Department of Botany & Sri Venkateswara University, Tirupathi, India. The leaves of *Vitex altissima* were collected and washed thoroughly. Then the surface water is removed by air drying. Then these leaves were subjected to shade drying for 3 to 4 days till the colour of the leaf changes to buff/ pale yellow colour and the leaf becomes brittle. Now these leaves were powdered and passed through 100-120 mesh to obtain a coarse fine powder.

Preparation of extracts

Two extracts namely Ethanolic extract & Petroleum ether extract were prepared with powder by using the solvents Ethanol & Petroleum ether.100 gms of powdered material was extracted initially with 500 ml of Ethanol for 48 hrs. at room temperature. The extract was filtered with sterile Whatman No.1 filter paper into a clean conical flask. The pulp in the Whatman filter paper was again extracted with 300 ml of Ethanol and the procedure was repeated. Now both the extracts were pooled and transferred into rotary flash evaporator for the evaporation of solvent, the evaporated extract was preserved at 4^0C in an airtight bottle until further use. The same procedure was followed for the preparation of petroleum ether extract also.

Male Albino rats weighing 180 gm to 210 gms were used for this experimental study. The animals were procured from NIN, Hyderabad and the animals were acclimatized for a period of 07 days before the study. Standard temperature of (26 ± 2^0 C), relative humidity (45-55%) and 12 hrs dark/light cycle were maintained in the quarantine. All the animals were fed with rodent pellet diet and water was allowed *ad-libitum* under strict hygienic conditions. Ethical clearance for performing the experimental animals was obtained from Institutional Animal Ethical Committee (IAEC with experiment number 2013/1587-01862016). Acute toxicity studies were conducted as per the OECD (Organization for the Economic Co-operation and Development) guidelines.

Induction of diabetes:

Alloxan (2,4,5,6 – tetra pyrimidine; 2,4,5,6 – Pyrimidine tetrone) is an oxygenated pyrimidine derivative [11] and was originally isolated in 1818 by Brugnatelli and got its name in 1838 by Frederich Wohler and Justus Von Liebig [12]. The animals were fasted for 16 hrs and accessed drinking water only before the induction of Diabetes. Fasted rats were given a single intra-peritoneal injection of 120 mg/kg of Alloxan monohydrate in sterile saline to induce diabetes. The hyperglycemic rats with glucose levels greater than 250 mg/dl were separated and put into different groups after receiving alloxan injections for five days. Each group contained six animals. The grouping of animals was as follows [13].

Grouping

Group –I: Normal control.
Group –II: Served as Diabetic control and received alloxan monohydrate and treatment with Vehicle.
Group III: Alloxan monohydrate and treatment with Ethanolic extract (250 mg/kg body weight, p.o) Served as test group.
Group IV: Alloxan monohydrate and treatment with Ethanolic extract (500 mg/kg body weight, p.o) Served as test group.
Group IV: Alloxan monohydrate and treatment with Pet. Ether extract (250 mg/kg body weight, p.o) Served as test group.
Group VI: Alloxan monohydrate and treatment with Pet. Ether extract (500 mg/kg body weight, p.o) Served as test group.
Group VII: Alloxan monohydrate and treatment with (Glibenclamide- 10mg/kg body weight, p.o).

All groups of animals received the appropriate drug/doses beginning on the same day (Day 0) and for the following 10 days. Animals in all groups were given free access to the usual meal and water during this treatment period.

On the fourth day, the seventh day, and the tenth day (the last day of treatment), blood sugar levels were estimated. Blood samples were taken on the final day following treatment by puncturing the animal's retroorbital plexus and centrifuging them for 20 minutes at 8,000 rpm. After centrifugation the serum was separated to estimate cholesterol, triglycerides, HDL, LDL, serum urea, serum creatinine, and total protein.

STATISTICAL ANALYSIS

The mean and standard error mean were used to express all the result values. One-way analysis of variance (ANOVA) and Tukey's multiple comparison were used to examine the statistical significance between the groups.

RESULTS

No mortality and significant behavioral changes were observed at the dose 2500 mg/kg body weight *Vitex altissima* of both Ethanolic & Pet. Ether extracts and the doses were fixed as 250 mg/kg body weight as low dose and double of this dose 500 mg/kg body weight as high dose for both the extracts in this study.

The Results of Table- I reveal that Diabetes was induced successfully in all the groups of animals except Normal control with the administration of Alloxan. As the course of treatment continued, the increased glucose levels on Days -4, -7, and 10 were brought back to normal. At a greater dose of 500 mg/kg body weight, the ethanolic extract of *Vitex altissima* significantly reduced elevated blood glucose levels by the tenth day. Similar effects of a significant decrease in the blood glucose level were seen in rats administered with pet ether extract of *Vitex altissima* at a dose of 500 mg/kg body weight. The plant extricates exhibited significant antihyperglycemic activities among which pet ether extract of *Vitex altissima* at a dose of 500 mg/kg body weight exhibited a greater significant activity and was compared favorably well with glibenclamide (10 mg/kg body weight) treated rats.

Table 1: Effect of *Vitex altissima* on glucose levels

S. No.	Treatment groups	Glucose (mg/dl)			
		Day-1	Day-4	Day-7	Day-10
1.	Normal control	90.33 ± 1.563	90.5 ± 1.155	91.5 ± 1.432	89.33 ± 1.783
2.	Diabetic control	284.83 ± 3.060	286.33 ±2.963^{+++}	279.83 ± 1.701^{+++}	273.16 ± 1.990^{+++}
3.	V.A Eth. extract (250mg/kg)	285.33 ± 1.926	266.16 ± 2.040**	247.66 ± 2.654***	238 ± 2.543***
4.	V.A Eth. extract (500 mg/kg)	283 ± 1.826	258.83 ± 2.798**	220.83 ± 3.807***	178.33 ± 1.606***
5.	V.A Pet. Eth. extract (250 mg/kg)	276.66 ± 2.418	267.16 ± 1.851**	243.33 ± 1.706***	213.33 ± 3.827***
6.	V.A Pet. Eth. extract (500 mg/kg)	283.16 ± 2.120	260.33 ± 1.145**	206.5 ± 2.262***	160.16 ± 2.822***
7.	Glibenclamide- 10 mg/Kg	279 ± 3.873	213.33 ± 2.108***	184.33 ± 1.801	141.16 ± 2.167***

Values are expressed as Mean ± SEM; $^{+++}$ P < 0.001, $^{++}$P < 0.01, $^{+}$P < 0.05 when compared to normal Control
*** P < 0.001, **P < 0.01, *P < 0.05 when compared to Diabetic Control

Table 2: Effect of *Vitex altissima* on biochemical parameters

S.No.	Treatment group	Cholesterol (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	T.P (g/dl)
1.	Normal control	114.16 ± 2.455	69.66 ± 2.108	37.5 ± 0.957	40.166 ± 1.195	31.5 ± 0.763	0.541 ± 0.017	7.48 ± 0.174
2.	Diabetic control	196.16 ± 2.535^{+++}	166.16 ±1.939^{+++}	12.16 ± 0.703^{+++}	153.83 ±2.151^{+++}	63.16 ± 1.352^{+++}	1.51 ± 0.048^{+++}	4.31 ±0.238^{+++}
3.	V.A Eth. extract (250mg/kg)	168 ± 1.238***	149 ± 1.366**	16.5 ± 0.763**	116 ± 1.653***	47 ± 1.366**	1.24 ± 0.0235**	4.6 ± 0.148^{ns}
4.	V.A Eth. extract (500 mg/kg)	153.16 ± 1.470***	106.16 ± .040***	29.5 ± 1.088***	89.16 ± 2.798***	37.16 ± 0.703***	0.77 ± 0.014***	5.66 ± 0.140*
5.	V.A Pet. Eth. extract (250 mg/kg)	166 ± 2.113***	144.33 ± .216**	17.83 ± 0.872**	119.66 ± .275***	41.83 ± 0.945***	1.08 ± 0.025***	4.9 ± 0.167^{ns}
6.	V.A Pet.Eth. extract (500 mg/kg)	134.33 ± 2.076***	81.83 ± 2.023***	34.16 ± 0.945***	60.33 ± 1.856***	33.5 ± 0.428***	0.650 ± 0.018***	7.28 ± 0.172***
7.	Glibenclamide-10 mg/Kg	123.66 ± 2.261***	73.33 ± 2.060***	34.66 ± 0.614***	42.33 ± 1.563***	32.5 ± 0.763***	0.596 ± 0.017***	7.55 ± 0.183***

Values are expressed as Mean ± SEM; $^{+++}$ P < 0.001, $^{++}$P < 0.01, $^{+}$P < 0.05 when compared to normal Control; *** P < 0.001, **P < 0.01, *P < 0.05 when compared to Diabetic Control

Table – 2 showing that Cholesterol, Triglycerides, LDL, Urea & Creatinine levels were increased in Diabetic control group which is marked in diabetic condition. But treatment with *Vitex altissima* and standard drug have reduced the elevated parameters. On the other hand, the HDL & Total Protein levels were significantly reduced in Diabetic control group and these levels were reversed after the treatment with test drug and standard drug, which was observed in Groups III, IV, V, VI & VII.

DISCUSSION

Ethanolic & Petroleum ether extracts of *Vitex altissima* has decreased the blood glucose levels in Alloxan induced Diabetic rats. *Momardica Charantia, Gymnema sylvestre, Ocimum sanctum* etc. plants have shown marked Anti hyperglycemic activity in similar models.

The mechanism to reduce the Serum glucose lies in both the traditional and allopathic drugs might be [14]:

- To stimulate beta cells of pancreatic islets to release insulin.
- To resist the hormones which increase the blood glucose levels.
- To increase the sensitivity of Insulin towards its receptors which is the key mechanism for entry of glucose into cells.
- To decrease the leading out of glycogen.
- To enhance the use of glucose in all the tissues and organs of the body.

An imbalance in lipid parameters might be due to inability of a cell to access enough glucose, they must instead turn to lipids as a source of energy, with insulin also playing a crucial role in regulating the body's lipid levels. Due to a disruption in lipid metabolism, diabetic rats had higher levels of all detrimental lipid parameters like cholesterol, triglycerides, and LDL while the levels of useful lipid parameters like HDL were decreased. Administration of ethanol extract and pet ether extract of *Vitex altissima* containing 500 mg/kg body weight showed a better decrease in the levels of cholesterol, triglycerides, and LDL and an increase in levels of HDL but a greater significance was observed in pet ether extract of *Vitex altissima* containing 500 mg/kg body weight in comparison with the standard drug Glibenclamide.

Elevation in the level of urea and creatine levels in diabetic rats might be due to decline in the levels of carriers necessary to transport metabolites from the body to urine which may be due to excessive amount of glucose thus leading to their retention. Rats treated with ethanol and pet ether extract of *Vitex altissima* containing 500 mg/kg body weight in comparison with the standard drug Glibenclamide but a greater significance was observed in pet ether extract of *Vitex altissima* containing 500 mg/kg body weight

CONCLUSION

From the above results, it can be concluded that both Ethanolic & Pet.Ether extracts of *Vitex altissima* have significantly decreased elevated glucose, Triglycerides, Cholesterol and LDL levels and increased the HDL levels in the alloxan induced diabetic rats but, pet ether extract of *Vitex altissima* containing 500 mg/kg body weight showed a greater significance. Thus, *Vitex altissima* is effective against Diabetes mellitus, and one potential method would be by improving Insulin's sensitivity to its receptors, which was disrupted as a result of the free radical damage caused by alloxan.

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