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ORIGINAL ARTICLE

Evaluation of The Antidiabetic Activity of *Vitex altissima* in Streptozotocin and Nicotinamide Induced Diabetes Animal Model

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ABSTRACT
Diabetes mellitus is a metabolic disease marked by low blood sugar levels and malfunctioning metabolism. Glibenclamide, metformin, and glutephrine are just a few of the allopathic pharmaceuticals that can be used to treat diabetes; however, they have negative side effects when taken over an extended period of time, and tolerance develops when using many treatments. Many traditional medicinal plants that have the same therapeutic effect as allopathic medications but are completely devoid of side effects are available to treat diabetes, according to the Ayurvedic medical system.All of these factors led us to start the current investigation into Vitex altisiima's antidiabetic properties in animal models of diabetes produced by streptozotocin and nicotinamide. This investigation included rats with glucose levels > 200 mg/dl after 48 hours. Streptozotocin was used to induce diabetes at a dose of 50 mg/kg body weight, followed by nicotinamide at a dose of 120 mg/kg body weight.For 21 days, the diabetic animals were given extracts from Vitex altissima derived from ethanol and petroleum ether. This led to a substantial decrease in the raised levels of blood glucose as well as other parameters such as cholesterol, triglycerides, LDL, urea, creatinine, insulin, SGOT, and SGPT. Additionally, the reduced levels of HDL and total protein were elevated. In an animal model of diabetes caused by streptozotocin and nicotinamide, Vitex altissima petroleum ether extract showed superior anti-diabetic efficacy among the two extracts.
Keywords: *Vitexaltissima, Diabetes, Streptozotocin, Nicotinamide, Animal model.*

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INTRODUCTION

Hyperglycemia and decreased metabolic activity are hallmarks of the metabolic disease known as diabetes mellitus. 7.8% of adult Indians are affected by the diabetes prevalence. *[1]*. In the last three decades, diabetes incidence has dramatically increased and is still rising in low and middle-income nations. The use of synthetic or allopathic drugs causes adverse effects and the persistence of hyperglycemia for a longer time period, both of which increase the risk of heart disease and stroke as well as major difficulties and harm to the major organs [2]. Many diabetic individuals found it challenging to maintain glycemic control due to the progressive decline in β cell activity [3]. It is usual practice to combine multiple hypoglycemic medications with polyherbal formulations to improve glucose control [4]. Numerous plants have been discovered to be supportive in the management of diabetes. The primary drug source is plants; extracts made directly or indirectly from plant sources are sold on the market [5]. Plants have been utilized as medicine across the world for both curative and preventative purposes. Due to their widespread use and accessibility, medicinal herbs have been utilized extensively to treat diabetes around the world [6].

Vitex altissima is commonly known as Ganduparu, Nemiliadogu (peacock chaste tree) belongs to the family Verbenaceae and fit in to a single genus, *Vitex*. It is a woody plant reaching a height of 20 meters characterized by greyish bark that becomes scaly with maturity. The leaves are opposite, compound, trifoliolate, or palmate. Their shape is elliptic or elliptic-lanceolate, with a cuneate base and acuminate apex. Indigenous medical practices from the Paliyan tribe in the Sirumalai hills of Tamil Nadu's Western

Ghats have shown that the plant's extract has potent antioxidant properties and is used as a treatment for a variety of skin conditions.
However, there is no report of *Vitex altissima* antidiabetic effect in the literature. However, this plant is renowned for having a range of secondary plant metabolites like alkaloids saponins & Tanins [7] and showing Antibacterial, anti-inflammatory [8] & anti-oxidant activities [9]. So, this was intended to study the anti-diabetic effects of *Vitex altissima* leaves on animal models.

MATERIAL AND METHODS

Plant material

The Fresh and healthy leaves of *Vitex altissima* were plucked from the forests of Tamil Nadu in the region of Kancheepuram. The collected leaves were authenticated by Pro. Madhav Chetty, Department of Botany & Sri Venkateswara University, Tirupathi, Andhra Pradesh. After the leaves were cleaned, they were dried in the shade for three to four days, or until they were brittle and changed colour to buff or pale-yellow hue. Now these leaves were powdered to 100-120 mesh into coarse fine powder.

Preparation of extracts

Two extracts namely Ethanolic extract and petroleum ether extracts were prepared with powder by means of the solvents Ethanol and petroleum ether. Initially, 500 ml of ethanol was used to extract 100 g of powdered material for 48 hours at room temperature. The extract was put into a clean conical flask after being sieved over sterile Whatman No. 1 filter paper. The method was repeated, this time extracting the pulp from the Whatman filter paper with 300 ml of ethanol. Now together extracts were combined and put into a rotary flash evaporator to allow the solvent to evaporate. The evaporated extract was now stored at 4°C in an airtight bottle till usage. Petroleum ether extract was also prepared using the same process.
Male Albino rats weighing 180 ±20 gms were utilized for this experimental study. The animals obtained from NIN in Hyderabad and acclimated for seven days prior to the study. The quarantine was kept at a constant (26 ± 2^0C), relative humidity of 45–55%, and a 12-hour darkness/light cycle. Every animal was housed in meticulously hygienic circumstances, given an infinite supply of water, and fed a diet consisting of rodent pellets. The Institutional Animal Ethical Committee provided its approval for using experimental animals. (IAEC with experiment number 2013/1587-01862016). The OECD's (Organisation for Economic Co-operation and Development) criteria were followed for conducting acute toxicity studies.

Induction of diabetes

The Male Albino rats were split up into the subsequent seven sets of 6 animals respectively:
Group –I: Normal control (No diabetes induction & No treatment)
Group –II: Diabetic control (Only Diabetic induction & No treatment)
Group III: Test group (Induction of diabetes &V.A Ethanolic extract 250 mg/kg body weight p.o)
Group IV: Test group (Induction of diabetes &V.A Ethanolic extract 500 mg/kg body weight p.o)
Group IV: Test group (Induction of diabetes & V.A pet.ether extract 250 mg/kg body weight p.o)
Group VI: Test group (Induction of diabetes & V.A pet. ether extract 500 mg/kg body weight p.o)
Group VII: Standard (Induction of diabetes & Glibenclamide- 10mg/kg body weight)
To induce diabetes in overnight-fasted rats, a single intraperitoneal (i.p.) injection of newly arranged Streptozotocin (STZ) at a dose of 50 mg/kg body weight, followed by a 120 mg/kg dose of Nicotinamide (NIC) in a volume of 0.5 ml/kg body weight of 0.1 M citrate buffer (pH 4.5) [10]. The STZ + NIC treated rats were shown to have diabetes through measurement of their fasting blood glucose levels 48 hours after induction. After receiving an injection of STZ + NIC for 24 hours, the rats were administered 5% w/v glucose solution (2 ml/kg body weight) in order to avoid hypoglycemia mortality. Rats classified as diabetes had abstaining blood glucose levels better than 200 mg/dl, and they remained split into six groups at random (Group –II, III, IV, V, VI & VII). Using oral gavage, the seven groups received their prescribed drugs once a day for 21 days in a row. On the first, seventh, fourteen, and twenty-first days of treatment, blood samples were drawn by piercing the retro-orbital plexus. A glucometer was used to measure the blood glucose levels. By using the remaining blood, the plasma insulin level was assessed on the same day of glucose estimation.
On the last day of the study after three hours of the treatment, the plasma samples were drawn from all the experimental animals by puncturing retro orbital plexus puncture and collected in EDTA tubes for Biochemical analysis [11]. Hemoglobin and glucose were estimated using the full blood sample. The serum was used to estimate biochemical indicators such as urea, creatinine, protein, liver glycogen, total serum cholesterol, serum triglycerides, high-density lipoprotein, and low-density lipoprotein. Serum glutamate oxaloacetate transaminase and glutamate pyruvate transaminase.

Statistical Analysis

Every value was given as Mean + Standard Error. The statistical implication amongst the groups was examined using Tukey's multiple comparisons and one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Result of extracts on blood glucose levels

The results of Table – 1 reveal that, administration of STZ & NA has successfully induced the Diabetes in Rats which we confirmed by comparing with normal control group where the STZ & NA were not administered to this group. But, After the treatment with the *Vitex altissima* the elevated blood glucose levels have been decreased dose-dependently. The blood glucose levels have been significantly reduced when related to the Diabetic control group where we did not treat the animals with any drug. The Glucose levels comparatively decreased from Day 1 to Day 7, Day 7 to Day 14 and Day 14 to Day 21 as the treatment continued. Finally, the high doses of Ethanolic & Pet. ether extracts of *Vitex altissima* has decreased the Blood glucose levels very significantly on Day 21 which is closer to the standard group animals where we treated with standard Glibenclamide at a dose of 10mg/kg body weight.

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

S.No	Treatment group	Fasting Blood Glucose (mg/dl)			
		Day-0	Day-7	Day-14	Day-21
1.	Normal control	89.33 ± 2.060	87.33 ± 1.498	87.66 ± 1.838	87 ± 1.366
2.	Diabetic control	256.83 ± 3.250	256 ± 2.769^{+++}	249 ± 1.366^{+++}	246 ± 1.862^{+++}
3.	V.A Eth. extract (250mg/kg)	246 ±1.390	232.5 ± 2.291*	208.33 ± 4.578***	196.66 ± 1.256***
4.	V.A Eth. extract (500 mg/kg)	253.33 ± 1.961	225.33 ± 3.33**	203.16 ± 1.682***	192. ± 3.088***
5.	V.A Pet. Eth. extract (250 mg/kg)	249.5 ± 2.012	236.5 ± 1.586*	220.66 ± 2.431***	206.16 ± 2.212***
6.	V.A Pet. Eth. extract (500 mg/kg)	251.5 ± 1.996	221.66 ± 2.716***	198.16 ± 3.081***	181.16 ± 1.869***
7.	Glibenclamide- 5 mg/Kg	250.83 ± 1.759	218 ± 4.531***	195 ± 3.183***	178.5 ± 1.522***

Values are articulated as Mean ± SEM

$^{+++}$ P < 0.001, $^{++}$P < 0.01, $^{+}$P < 0.05 when related to normal control.

*** P < 0.001, **P < 0.01, *P < 0.05 when linked to diabetic control.

In this study, the Diabetes was successfully induced by the combination of STZ and nicotinamide combination which increased the blood glucose levels by more than 200 mg/dl values. The mechanism behind this is the STZ is a strong free radical generator that particularly damages and degenerates the Pancreatic cells at this dose of 50 mg/kg body weight and resembles the diabetes of IDDM. But, in this study, we used the combination of Nicotinamide along with STZ and Nicotinamide has the advantage of the partial defense of Pancreatic cells against the STZ-induced effect of degeneration of pancreatic cells so that the Pancreatic cells are damaged to a small extent and insulin levels were decreased as a result the blood glucose levels will be raised. It is clearly observed that Nicotinamide protects the STZ effect to some extent and diabetes will be induced due to partial damage of pancreatic cells which resembles the NIDDM [12].

Effect of extracts on plasma insulin levels

The results of Table 2 reveal that the Plasma Insulin levels were decreased in the Diabetes induced animals when related to Normal control. The decreased levels have shown elevated Blood glucose levels in all the Diabetes induced animals.

But after the treatment with the *Vitex altissima* Ethanolic & Pet. Ether extracts, the decreased insulin levels gradually increased as the treatment progressed. Finally, on the 21st day, the higher doses of Ethanolic &Pet. Ether extracts have significantly increased the Plasma insulin levels which was reflected in decreased blood glucose levels which we observed from Table 1.

Table 2: Effect of *Vitex altissima* on plasma insulin levels

S. No	Treatment group	Plasma Insulin levels (µIU/ml)			
		Day-0	Day-7	Day-14	Day-21
1.	Normal control	21.283 ± 0.347	20.816 ± 0.518	20.38 ± 0.248	20.66 ± 0.418
2.	Diabetic control	7.3 ± 0.191	6.81 ± 0.130^{+++}	7.35 ± 0.128^{+++}	7.61 ± 0.149^{+++}
3.	V.A Eth. extract (250mg/kg)	7.13 ± 0.247	8.58 ± 0.144**	8.91 ± 0.124**	10.21 ± 0.289***
4.	V.A Eth. extract (500 mg/kg)	7.16 ± 0.239	9.516 ± 0.391***	10.3 ± 0.258***	12.33 ± 0.218***
5.	V.A Pet. Eth. extract (250 mg/kg)	7.4 ±0.206	8.71 ± 0.149**	8.8 ± 0.106**	10.4 ± 0.281***
6.	V.A Pet. Eth. extract (500 mg/kg)	7.56 ± 0.187	10.98 ± 0.419***	11.3 ± 0.348***	13.16 ± 0.176***
7.	Glibenclamide- 5 mg/Kg	7.83 ± 0.188	11.51 ± 0.353***	11.93 ± 0.374***	13.633 ± 0.164***

Values are articulated as Mean ± SEM

$^{+++}$ P < 0.001, $^{++}$P < 0.01, $^{+}$P < 0.05 when related to normal control.

*** P < 0.001, **P < 0.01, *P < 0.05 when associated to diabetic control.

Effect of extracts on other biochemical parameters

The Results of Table -3 show that the renal functional parameters of Serum Urea and serum Creatinine levels were raised and Total protein values were decreased gradually in the Diabetes induced animals. The augmentation of elevated serum Urea and Creatinine might be due to the osmotic diuretic effect of elevated bold glucose levels in diabetic rats which depletes the extracellular fluid and retains the metabolites such as Urea and Creatinine in the body [13].

After the treatment with the Ethanolic and petroleum ether extracts of *Vitex altissima* has decreased the raised Serum Urea and creatinine levels and increased the total Protein levels in the test group animals. The liver functional parameters of SGOT & SGPT were elevated due to elevated stress on the liver due to free radicals produced by STZ and the treatment with *Vitex altissima* has reduced the elevated SGOT & SGPT levels might be owing to scavenging of free radicals by the test drugs. Reversing all these biochemical parameters is a dose-dependent treatment only because the higher doses of extracts reversed the changed parameters toward the normal values.

Table 3: Effect of *Vitex altissima* on other biochemical parameters

Treatment group	Serum Creatinine (mg/dl)	Serum Urea (mg/dl)	TP (mg/dl)	SGOT (IU/L)	SGPT (IU/L)
Normal control	0.675 ± 0.031	35.83 ± 1.167	7.15 ± 0.226	40.83 ± 1.249	49.66 ± 1.453
Diabetic control	1.73 ± 0.028^{+++}	67.83 ± 1.352^{+++}	3.53 ± 0.248^{+++}	101.16 ± 2.442^{+++}	125.33 ± 2.011^{+++}
V.A Eth. extract (250mg/kg)	1.45 ± 0.038***	54.33 ± 1.054**	4.08 ± 0.224**	90 ± 2.324**	99.33 ± 3.116***
V.A Eth. extract (500 mg/kg)	1.33 ± 0.015***	46.33 ± 0.666***	5.5 ± 0.184***	84.66 ± 2.201***	97.16 ± 1.470***
V.A Pet. Eth. extract (250 mg/kg)	1.40 ± 0.039***	52.33 ± 1.054***	4.16 ± 0.218**	88.83 ± 2.272***	100± 2.436***
V.A Pet. Eth. extract (500 mg/kg)	1.21 ± 0.032***	40.83 ± 0.600***	6.26 ± 0.217***	77.66 ± 1.978***	88.66 ± 1.647***
Glibenclamide- 5 mg/Kg	1.10 ± 0.027***	39.66 ± 0.333***	6.95 ± 0.099***	74.5 ± 1.893***	84.66 ± 1.202***

Values are stated as Mean ± SEM

$^{+++}$ P < 0.001, $^{++}$P < 0.01, $^{+}$P < 0.05 when related to normal control.

*** P < 0.001, **P < 0.01, *P < 0.05 when linked to diabetic control.

Effect of extracts on lipid profile parameters

The results of Table 4 reveal that the Lipid profile parameters such as Triglycerides, Total Cholesterol & LDL levels were raised & HDL levels were decreased significantly in the diabetes-induced animals which we can observe in the Diabetic control group of animals. However, the treatment with Ethanolic and petroleum ether extracts of *Vitex altissima* reversed the altered Lipid profile parameters towards the normal values dose-dependently which we can observe in the test group animals. Among the four test group animals the higher doses of Ethanolic and petroleum ether extracts have shown significant effects when compared with Lower dose group animals [14].

The Elevated levels of TG, Cholesterol, LDL & decreased HDL levels were observed in diabetic control group animals and were reversed after the treatment with *Vitex altissima* extracts may be the result of enhanced insulin effect on intestinal cholesterol absorption, fatty acid and cholesterol production.

Table 4: Impact of *Vitex altissima* on lipid profile parameters

Treatment group	TG (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Normal control	69.5 ± 1.408	116.16 ± 2.007	38.5 ± 0.846	41.33 ± 0.881
Diabetic control	164.5 ± 1.478^{+++}	199.66 ± 3.313^{+++}	12.16 ± 0.600^{+++}	154.33 ± 2.261^{+++}
V.A Eth. extract (250mg/kg)	151.33 ± 1.308**	182.83 ± 3.781**	17.83 ± 0.600***	116 ± 1.033***
V.A Eth. extract (500 mg/kg)	105.5 ± 2.012***	170.33 ± 4.072***	25 ± 0.683***	89.83 ± 2.023***
V.A Pet. Eth. extract (250 mg/kg)	145.16 ± 1.515**	179.16 ± 3.361***	19.33 ± 0.421***	118.33 ± 2.076***
V.A Pet. Eth. extract (500 mg/kg)	80.5 ± 1.607***	160.33 ± 2.459***	29 ± 0.966***	61.5 ± 1.500***
Glibenclamide- 5 mg/Kg	75.16 ± 1.740***	155.66 ± 2.654***	34.16 ± 0.872***	46.83 ± 1.424***

Values are articulated as Mean ± SEM

$^{+++}$ P < 0.001, $^{++}$P < 0.01, $^{+}$P < 0.05 when related to normal control.

*** P < 0.001, **P < 0.01, *P < 0.05 when linked to diabetic control.

In this present study the treatment with Standard drug of Glibenclamide has decreased the elevated blood glucose levels. The underlying mechanism for this might be increasing the production of insulin which we observed in the study and increasing the sensitivity of Insulin towards it's receptors which enhances the entry of glucose into the cells results in decrease of blood glucose levels. The same effects also produced by the both extracts of *Vitex altissima*. The mechanism of lowering blood glucose levels might be the same mechanism as that of Glibenclamide either by increasing the insulin secretions or increasing the sensitivity of insulin towards its receptors [15].

CONCLUSION

Based on the aforementioned findings, it can be inferred that the Vitex altissima ethanolic and ether extracts pointedly reduced the elevated levels of LDL, cholesterol, triglycerides, serum creatinine, SGOT, and SGPT, and augmented the levels of total protein, HDL, and insulin in the rats that were induced to develop diabetes by streptozotocin and nicotinamide. As a result, Vitex altissima is beneficial for treating diabetes mellitus. One potential reason for this action could be an increase in insulin levels or sensitivity to insulin receptors. Among the two extracts the Pet. ether extract at higher doses has shown very good significant anti-diabetic activity.

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