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In vitro and in vivo evaluation of anti-diabetic potential of strobilanthes cuspidata

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Abstract

The interest in medicinal plants stems from their vital role in sustaining life, as diseases have coexisted with humanity, and plant-derived medicines have served as natural remedies. Strobilanthes cuspidata, known for its rich chemical composition, was selected for our study. We conducted extractions from its leaves using various solvents through Soxhlet apparatus and evaluated their pharmacological effects through in vitro and in vivo studies. Our research findings demonstrated promising anti-diabetic activity from multiple extracts. Specifically, 50% inhibition of alpha-amylase activity was observed at 91 µg/ml for the ethyl acetate extract, 80 µg/ml for the aqueous extract, 75 µg/ml for the petroleum ether extract, and 61 µg/ml for acarbose, which served as the positive control. Among all extracts, the ethanolic extract exhibited the most potent anti-diabetic activity, attributed to its higher concentration of bioactive compounds with anti-diabetic properties. This research highlights the therapeutic potential of Strobilanthes cuspidata and suggests that its ethanolic extract could be a promising candidate for developing novel treatments for diabetes mellitus. Overall, our study provides valuable insights into the plant's medicinal properties and supports further exploration of its bioactive compounds for managing diabetes.

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INTRODUCTION

As the world's fifth most significant cause of mortality, diabetes mellitus is an epidemic that is spreading alarmingly. One of the oldest known diseases in the world, diabetes has been dubbed the "Diabetic Capital of the World" and is prevalent in India. The World Health Organisation (WHO) designated diabetes prevalence as a "basic health indicator" for member states in 1997 [1]. The WHO projected that 300 million people worldwide would have diabetes by 2025. Although they can be used to treat and manage

diabetes, chemical compounds are harmful. The current trend uses nutraceutical methods to treat this fatal disease because they are safe and don't have any significant adverse effects. One of the earliest known disorders is diabetes mellitus or just diabetes. The WHO added diabetes prevalence as a baseline health indicator for member states 1997, estimating that 300 million people worldwide would have diabetes by 2025 [2].

As anticipated by the WHO, the number of diabetes patients has recently increased along with microvascular consequences such as diabetic neuropathy, nephropathy, and retinopathy. Every type of diabetes has detrimental impacts on one's health. The disorder is linked to other chronic illnesses and the effects of aberrant glucose metabolism (such as hyperlipidemia, protein glycosylation, etc.) [3].

These include cardiovascular, peripheral vascular, ophthalmic, neurological, and renal problems, as well as depression, and they are the causes of morbidity, disability, and early mortality. Despite being thought of as a lifestyle disease, genetic disorders can potentially cause diabetes.

Along with insulin supplements, several oral hypoglycemic medications, such as sulfonylureas, biguanides, thiazolidinedione, diphenylalanine derivatives, meglitinides, and α-glucosidase inhibitors, are currently available for the treatment of diabetes [4].

Developing novel chemicals, particularly those derived from herbs, to treat diabetes is ongoing due to undesirable side effects. We attempted to investigate the impact of *S. cuspidata* ethanolic extract on antidiabetic activity for the first time using a rat model of diabetes produced by alloxan. The α-amylase enzyme was inhibited in vitro to assess the antidiabetic potential of the isolated compounds.

The Nilgiri Hills' indigenous people use this herb to treat inflammation and joint discomfort. Strobilanthes cuspidata's anti-inflammatory, anti-osteoarthritis, and analgesic properties in vitro. The current study aims to assess the effectiveness of S. cuspidata in the treatment of diabetes to create a herbal medication that is safe for humans to use in treating this condition and its associated consequences [5].

MATERIALS AND METHODS:

Assay for Alpha-Amylase Inhibition. Alpha-amylase analysis was carried out using the described method, which involved adding plant extract at various fixations (50 g/ml to 200 g/ml) diluted in a phosphate buffer to a 96-well plate containing protein from a pig's pancreas. [6] After 10 minutes of hatching at 37°C, 20 millilitres of starch arrangement were added to initiate the response, which was further brooded for 30 minutes at 37°C. After that, the reaction was stopped by applying 75 iodine reagents and 10 1M HCl to each area. A positive control (acarbose, 64 g/ml) and a clear containing phosphate buffer (pH 6.9) instead of the concentrate were prepared. Neither a starch nor a compound control was used for every test. The absorbance was estimated at 580 nm, and the rate of inhibitory action was determined by utilizing the accompanying condition:

% Inhibition = (1 − Absorbance of the untreated (Control) Absorbance of the test well) × 100

***In vivo* anti-diabetic activity of Strobilanthes Cuspidata:**

Both male and female Wistar albino rats weighing 150–200 grams were purchased from the College of Veterinary and Animal Science. For seven days before the experiment, the rats were kept in sterile polypropylene cages with six rats per cage under standard conditions of temperature (25–30°C), relative humidity (45–55%), and light/dark cycle (12/12 hours). Chemicals: Loba Chemie's streptozocin. Aventis Pharma's standard Glibenclamide (Daonil). Analysis-grade ethanol and a 5% dextrose solution [7].

Experimental design

Over 14 days, five groups of six rats each received the following treatment regimen.

Group I: Normal control (10 ml/kg, p.o.) by normal saline

Group II: The control group administered streptozotocin (100 mg/kg, i.p.)

Group III: Standard medication Glibenclamide (5 mg/kg, p.o.) combined with streptozotocin (100 mg/kg, i.p.). Streptotozocin (100 mg/kg, intraperitoneally) + Strobilanthes Cuspidata (HCB)

Group IV: Pet. Ether extract (200 mg/kg, intraperitoneally) makes up [8].

Group V: Streptotozocin (100 mg/kg, i.p.) + HCB Pet. Ether extract (400 mg/kg, p.o.) makeup. Group VI: Streptotozocin (100 mg/kg, i.p.) and HCB Ethyl Acetate (400 mg/kg, p.o.) make up.

Group VII: HCB Ethyl Acetate + Streptotozocin (100 mg/kg, i.p.). (200 mg/kg, intraperitoneally) Group VIII: HCB Ethanolic Extract (400 mg/kg, p.o.) + Streptotozocin (100 mg/kg, i.p.) Group IX: HCB Ethanolic Extract (200 mg/kg, p.o.) + Streptotozocin (100 mg/kg, i.p.) Group X: HCB Water Extract (400 mg/kg, p.o.) + Streptotozocin (100 mg/kg, i.p.) Group XI: HCB Water Extract (200 mg/kg, p.o.) + Streptotozocin (100 mg/kg, i.p.) [9] Standard medication, plant leaf extract, and regular saline were given using an oral feeding needle. Received extracts and the usual drug Glibenclamide (5 mg/kg, p.o.) for 14 consecutive days [10].

Collection of blood samples

Rats had their retro-orbital punctures performed every week until the completion of the trial on days 1, 7, 14, and 21 to obtain fasting blood samples. Calculating biochemical parameter values Blood samples from fasting periods of 1, 7, 14, and 21 days were obtained, and serum was separated and its glucose content examined [11].

Serum blood glucose

The serum blood glucose test measures the amount of glucose in the blood sample obtained from the animals. Typically, the test is carried out to check for elevated blood glucose levels, which may indicate insulin resistance or diabetes [12].

RESULTS AND DISCUSSION

***In-vitro* antidiabetic activity:**

The results showed that the highest percentage of Inhibition was found in Ethyl acetate extract (91 µg/ml), followed by water (80 µg/ml), petroleum ether (75 µg/ml), and acarbose (positive control) (61 µg/ml). All of these groups had alfa amylase inhibition properties. Still, the lowest percentage of Inhibition was found in ethyl acetate, similar to the percentage in positive control. This suggests that Strobilanthes Cuspidata ethanolic extract contains active antioxidants.

***In vivo* anti-diabetic activity:**

Compared to normal standard levels, the body weight of diabetic control rats significantly decreased. Comparing the diabetic control group to the diabetic rat treated with extract, no discernible changes in body weight occurred. Rats with diabetes who received Strobilanthes Cuspidata pet ether extract showed only slight weight changes compared to the diabetic control group. Strobilanthes Cuspidata ethyl acetate-treated diabetic rats exhibited slight weight changes compared to the control group. Diabetic rats exhibit a substantial increase in blood glucose levels. When compared to diabetic control rats, Strobilanthes Cuspidata petroleum ether and ethyl acetate treatment resulted in a slight decrease in blood glucose levels.

In comparison to the diabetic organized group, diabetic rats treated with Pet Ether Extract showed a significant drop in blood glucose levels in the efficacy studies. Additionally, it was discovered that the diabetic rats treated with ethyl acetate had significantly lower blood glucose levels, which were somewhat comparable to those of the group treated with Glibenclamide (standard). These findings unequivocally show that pet ether and ethyl acetate are effective oral treatments for diabetes management and patient compliance with lower doses and dosing incidence.

Compared to the standard control group, the diabetic control rats' bodies showed a marked decline. Comparing the diabetic control group to the diabetic rat treated with a pure substance, no discernible changes in body weight were seen. Diabetic rats exhibit a substantial increase in blood glucose levels. Compared to diabetic control rats, the efficacy investigation found that diabetic rats treated with pure chemical and water extract had a slightly lower blood glucose level. Compared to the diabetic group and the group treated with the standard drugs, diabetic rats treated with ethanolic extract showed a significant drop in blood glucose levels in the efficacy experiments. According to these findings, Strobilanthes Cuspidata extracts provide an effective oral treatment for diabetes management with a reduced dose incidence that is also patient-compliant. The results of the oral glucose tolerance, streptozocin-induced diabetic mellitus,

and hypoglycemic investigations are tabulated below.

Because the ethanolic extract contains more anti-diabetic bioactive components than the other extract, our final report showed that the ethanolic extract had more anti-diabetic efficacy.

Table 1 Inhibition of Ethyl Acetate Extract by α-Amylase

Concentration (µg/ml)	Percentage Inhibition (%)
0	0
25	29
50	33
75	42
100	53
125	56

Table 2 Inhibition of Water Extract by α-Amylase

Concentration (µg/ml)	Percentage Inhibition (%)
0	0
25	34
50	41
75	48
100	56
125	60

Table 3 Petroleum Ether Inhibition by α-Amylase

Concentration (µg/ml)	Percentage Inhibition (%)
0	0
25	35
50	42
75	45
100	57
125	62

Table 4 F4 Inhibition by α-Amylase

Concentration (µg/ml)	Percentage Inhibition (%)
0	0
25	25
50	39
75	42
100	51
125	65

Table 5 The Inhibition of acarbose by α-amylase (positive control)

Concentration (µg/ml)	Percentage Inhibition (%)
0	0
25	26
50	46
75	55
100	58
125	66

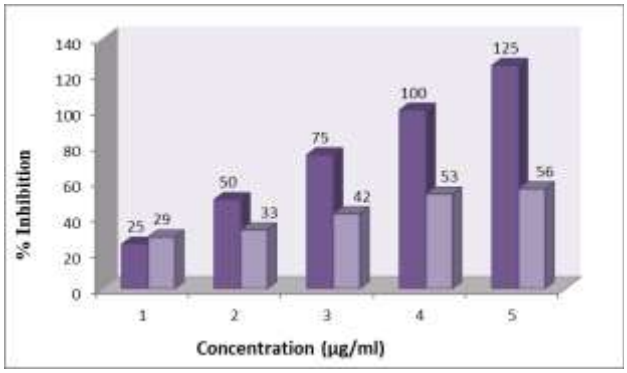

Figure 1 Ethyl Acetate Extract by α-Amylase

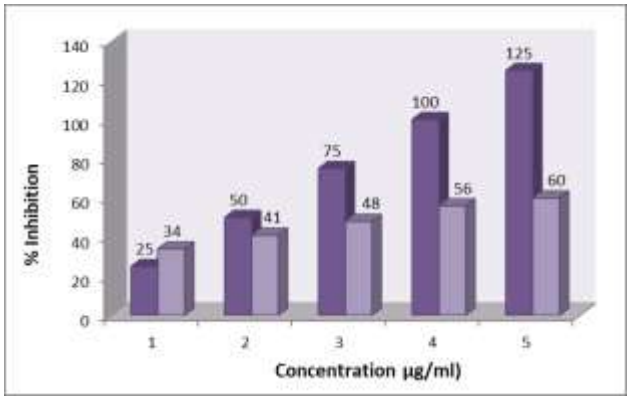

Figure 2 Water Extract by α-Amylase

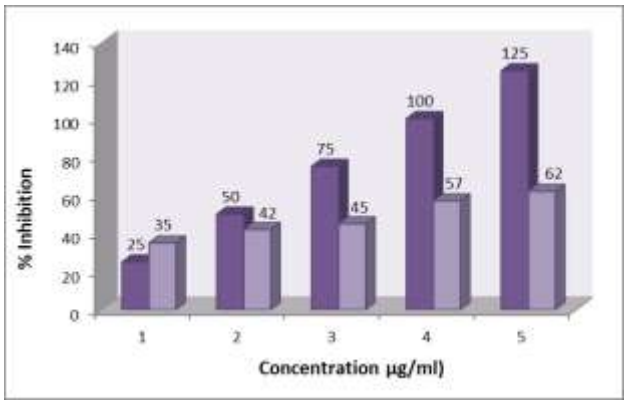

Figure 3 Petroleum Ether Inhibition by α-Amylase

Table 6 Test for oral glucose tolerance at various intervals

Treatment	Dose mg/kg	Blood Glucose Level (mg/dl) in hr						
		0	0.5	1	1.5	2	2.5	3
Control CMC)	0.5	66.65±0.1	138.8±0.5	168.4±0.2	164.2±0.2	155.4±0.3	146.6±0.3	134.7±0.4
Gliben clamide	0.2	66.31±0.2	101.7±0.2	102.7±0.2	98.83±0.2	94.82±0.4	92.66±0.4	82.68±0.4
EA	400	66.82±0.2	107.0±0	117.2±0.3	112.0±0.2	102.6±0.4	98.82±0.4	93.68±0.4
EA	200	66.65±0.1	122.2±0.	126.4±0.1	125.3±0.5	118.7±0.3	112.5±0.4	105.4±0.4
Petroleum Ether	400	68.31±0.2	101.3±0.2	108.2±0.4	103.7±0.5	98.32±0.2	92.65±0.5	85.32±0.4
Petroleum Ether	200	66.15±0.2	115.0±0.1	113.1±0.4	105.5±0.3	102.2±0.2	98.82±0.4	95.51±0.4
ET	400	66.00±0.2	98.4±0.2	96.15±0.3	94.33±0.2	93.14±0.2	85.82±0.5	82.51±0.3
ET	200	66.15±0.2	100.0±0.2	113 ±0.2	105.5±0.3	103.1±0.3	98.82±0.4	95.51±0.2
Wat	400	66.65±0.1	113.7±0.4	136.4±0.2	132.2±0.2	134.3±0.3	126.6±0.3	104.7±0.2
Wat	200	66.65±0.1	117.7±0.4	147.4±0.2	142.2±0.2	154.3±0.3	136.6±0.3	114.7±0.2

Table 7 Diabetes Mellitus Induced by Streptozotocin

Treatment	Dose mg/kg	Blood Glucose Level (mg/dl) in hr				
		0	3	7	14	21
Control CMC)	0.5	78.82±0.308	302.4±0.332	284.5±0.563	269.3±0.475	297.0±0.364
Gliben clamide	0.2	71.32±0.212	272.6±0.222	126.0±0.364	115.3±0.306	119.0±0.259
EA	400	82.16±0.403	290.6±0.342	144.3±0.954	129.6±0.422	122.0±0.259
EA	200	85.16±0.476	294.6±0.222	232.0±0.682	173.3±0.602	199.6±0.422
Petroleum Ether	400	75.51±0.427	272.6±0.564	132.2±0.476	118.4±0.495	112.3±0.704
Petroleum Ether	200	83.82±0.30	285.4±0.212	186.2±0.402	158.1±0.684	178.4±0.342
ET	400	68.51±0.427	262.6±0.342	125.0±0.306	115.2±0.334	115.3±1.015
ET	200	66.51±0.327	265.6±0.242	225.0±0.408	215.2±0.434	195.3±1.025
Wat	400	85.16±0.476	282.6±0.24	135.0±0.686	172.2±0.63	189.6±0.424
Wat	200	75.51±0.427	293.6±0.564	232.2±0.476	212.2±0.496	214.3±0.704

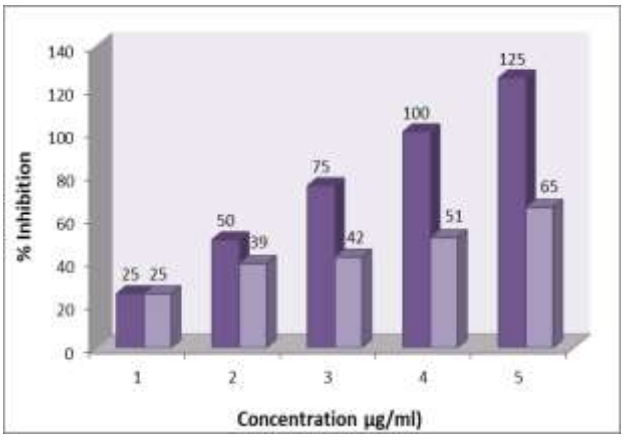

Figure 4 F4 Inhibition by α-Amylase

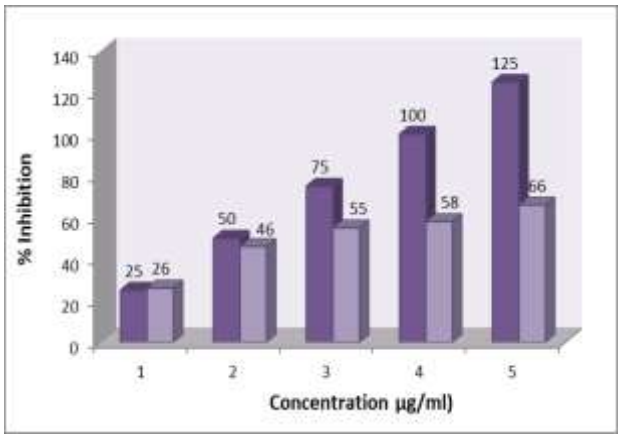

Figure 5 Inhibition of acarbose by α-amylase

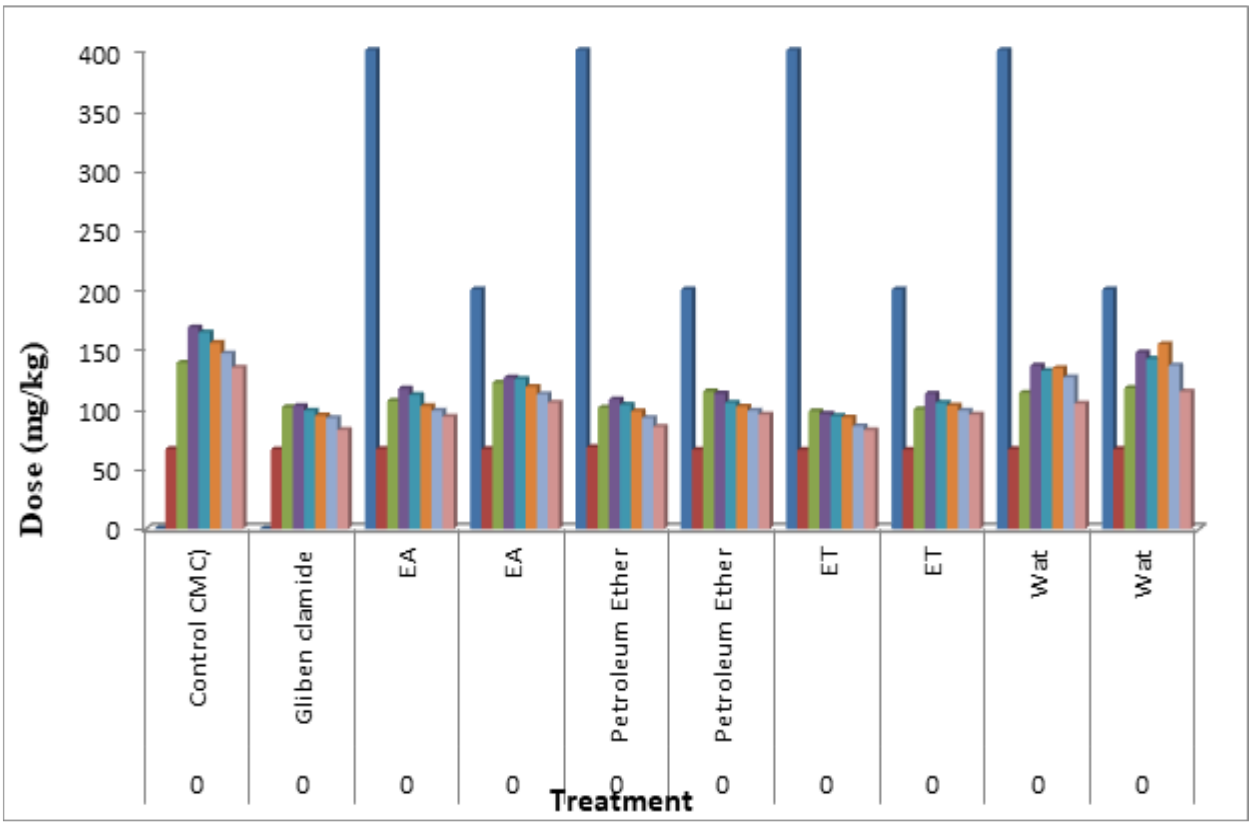

Figure 6 Oral glucose tolerance at various intervals

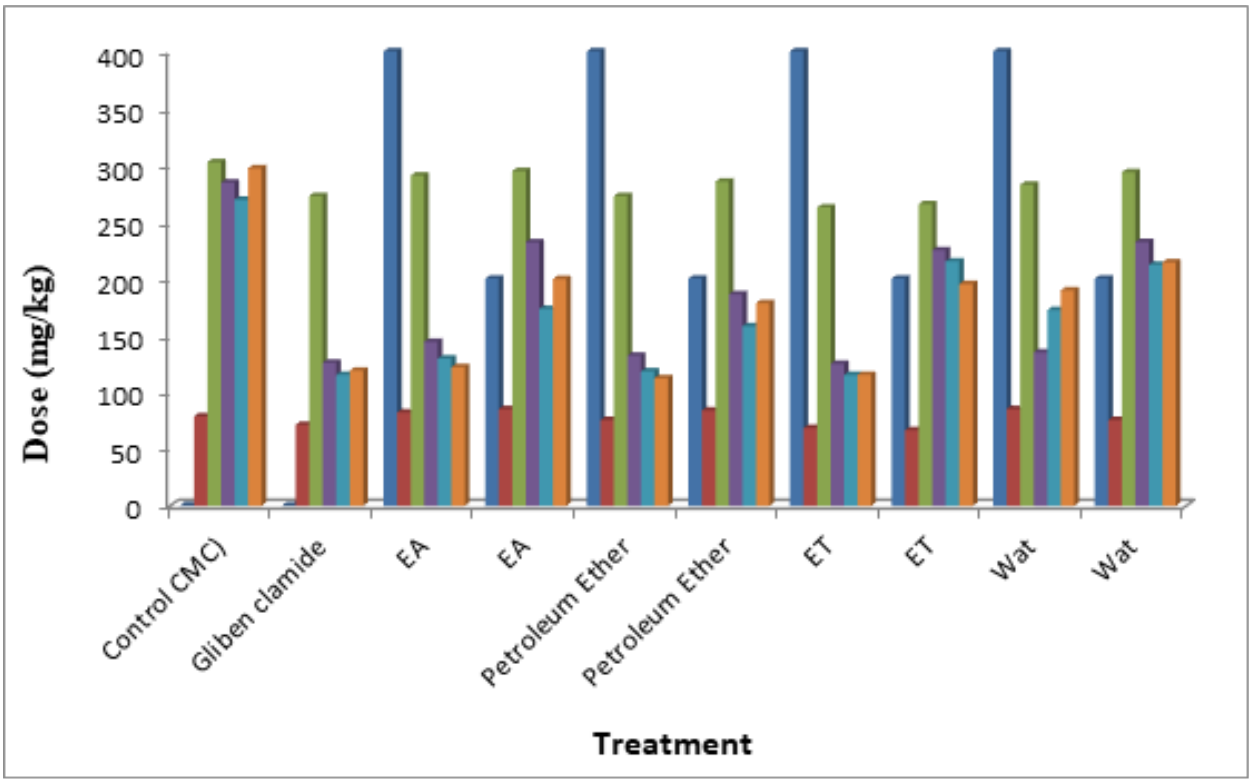

Figure 7 Diabetes Mellitus Induced by Streptozotocin

Table 8 Hypoglycemic Test

Treatment	Dose mg/kg	Blood glucose level (mg/dl)		
		0 hr	0.5 hr	1 hr
Control CMC)	0.5	67.51±0.224	67.33±0.333	70.32±0.556
Gliben clamide	0.2	67.32±0.334	51.33±0.557	26.51±0.501
EA	400	67.16±0.308	61.17±0.4014	42.02±0.517
EA	200	67.32±0.422	67.17±0.401	54.51±0.429
Petroleum Ether	400	67.01±0.259	55.00±0.365	32.84±0.655
Petroleum Ether	200	66.82±0.306	63.83±0.401	42.51±0.502
ET	400	66.51±0.426	49.00±0.578	24.18±0.705
ET	200	67.02±0.258	55.06±0.366	32.86±0.656
Wat	400	67.51±0.224	55.34±0.334	40.34±0.558
Wat	200	67.18±0.306	64.18±0.4015	52.02±0.517

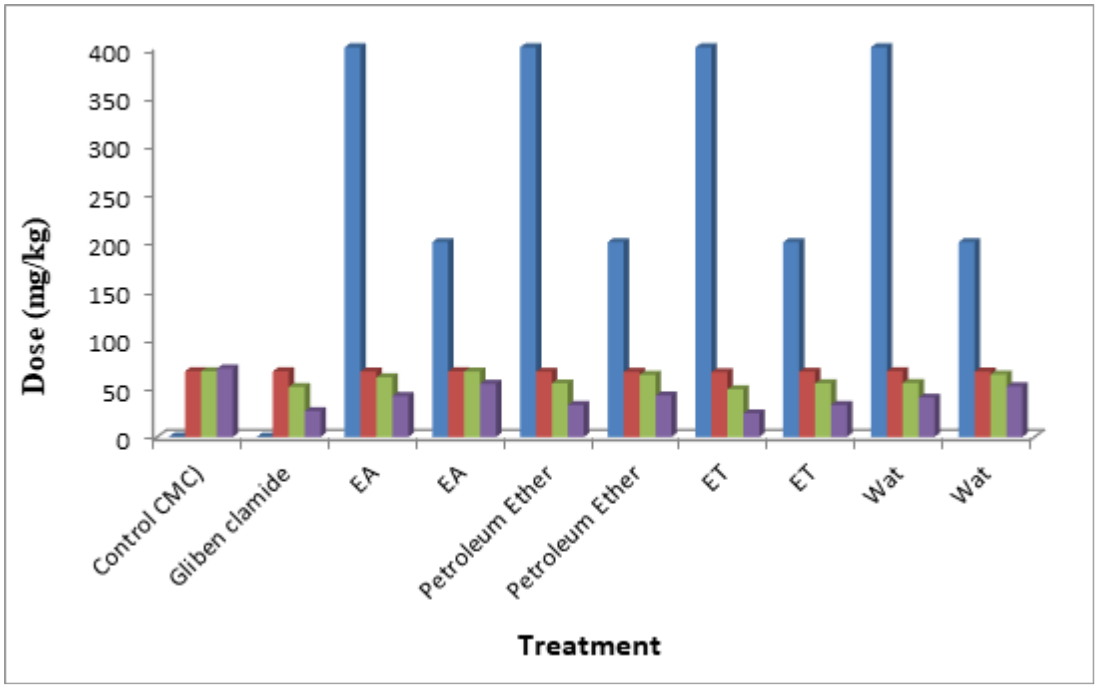

Figure 8 Hypoglycemic Test

CONCLUSION:

Alpha-amylase Inhibition may be one of the modes of action displayed by the plant extract to regulate and avoid postprandial hyperglycemia, according to the in vitro tests. According to this study, Strobilanthes cuspidata has a significant, long-lasting antidiabetic impact that is well-proven for treating diabetes. In summary, Strobilanthes Cuspidata aqueous leaf extract effectively lowers the increased blood glucose level and lipid profile of STZ-induced diabetic rats while not affect normal rats.

Thus, the assertion made by the classics of Ayurveda is justified. Therefore, the chemical components of the plant extract may help avoid complications from diabetes and could be used as a substitute for the current Strobilanthes Cuspidata of antidiabetic medications. Additional research is advised to support the plant's use as an antidiabetic.

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Author Contribution

All authors made substantial contributions to the conception, design, acquisition, analysis, or interpretation of data for the work. They were involved in drafting the manuscript or revising it critically for important intellectual content. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work, ensuring its accuracy and integrity.

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