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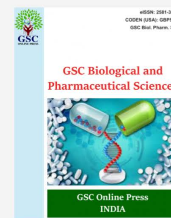

(Research Article)

Anti-diabetic activity of *Clerodendrum paniculatum* leaves by *In-vitro, In-vivo* and *Ex-vivo* methods

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Abstract

The present study has been undertaken to evaluate the *in-vitro, in-vivo and ex-vivo* anti-diabetic activity of leaves of chloroform extract *Clerodendrum paniculatum* (CECP). The extract was prepared by soxhlet extraction. Phytochemical screening indicates the presence of flavonoids, phenols, carbohydrates etc. The anti-diabetic activity of extract was studied by *in-vitro* (alpha amylase inhibition and alpha glucosidase inhibition assay), *in-vivo* (streptozotocin induced diabetes) and *ex-vivo* (glucose uptake by rat hemi - diaphragm method). For *in-vitro* studies, the inhibitory action of CECP was compared with standard drug Acarbose. The IC_{50} values of CECP for alpha amylase and alpha glucosidase was found to be 158.396 µg/ml and 113.122 µg/ml respectively and the extract shows significant anti-diabetic activity. For *in-vivo* and *ex-vivo* studies Glibenclamide was used as a standard drug to compare the blood glucose level and uptake of glucose was calculated. The results obtained from the study indicate that both 200 mg/kg and 400 mg/kg of CECP showed significant anti-diabetic activity. The 400 mg/kg of CECP showed better activity when compared to 200 mg/kg of the extract. The glucose uptake study was performed by isolated rat hemidiaphragm method. The hemi diaphragm obtained from the rats treated with both the doses of CECP showed significant glucose uptake.

Keywords: *Clerodendrum paniculatum; In-vitro; In-vivo and Ex-vivo*; Anti-diabetic activity

1. Introduction

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic disorder characterized by hyperglycaemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometimes ketonaemia. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced [1]. The worldwide prevalence of diabetes mellitus has risen dramatically over the past two decades. Based on current trends, the international diabetes federation projects that 592 million individuals will have diabetes by the year 2035 [2]. Medicinal plants and their bioactive constituents are used for the treatment of diabetes throughout the world and popular as nutraceutical. Many indigenous medicinal plants have been found to be useful to successfully manage diabetes. The synthetic drugs are either too expensive or have undesirable side effects or contraindications. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an area of active research [3]. *Clerodendrum paniculatum* is plant belonging to the family Lamiaceae. It has been used for traditional medicine in India, China and Japan in the treatment of rheumatism, neuralgia, ulcer, inflammation, and healing wounds [4].

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2. Material and methods

2.1. Chemicals and Instruments

- Drug for induction of diabetes: Streptozotocin.
- Drug for *in-vivo* and *ex-vivo*: Glibeclamide,
- Drug for *in-vitro*: Acarbose (Sigma Aldrich Chemicals Private Limited, Bengaluru).
- Alpha amylase (Sigma Aldrich Chemicals Private Limited, Bengaluru).
- Alpha glucosidase (SISCO RESEARCH).
- All other reagents used were of analytical grade.
- Instruments used: UV VISIBLE Spectrophotometer.

2.2. Plant Material and Extraction Procedure

Leaves of *Clerodendrum paniculatum* were collected from Kozhikode district, Kerala, India, (Figure 1). The plant specimen (No: 148221) was authenticated by Dr. A. K. Pradeep, Department of Botany, University of Calicut. The specimen voucher was deposited in the Department of Botany, University of Calicut, itself. The dried leaves were ground to coarse powder by grinder. Chloroform extract of *Clerodendrum paniculatum* was produced by soxhlet extraction. The percentage yield of extract was found to be 12 % w/w.

Figure 1 *Clerodendrum paniculatum* Leaves

2.3. Preliminary Phytochemical Analysis

The Chloroform extract of *Clerodendrum paniculatum* was subjected to preliminary phytochemical screening by using standard procedures [5].

2.4. Pharmacological studies

2.4.1. In-vitro methods

Alpha amylase inhibition assay

Different concentrations of plant extract were prepared in distilled water. A total of 500 µl of plant extract and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing alpha amylase solution (0.5 mg/ ml) were incubated for 10 minutes at 37°C. After pre incubation 500 µl of 1 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube. This reaction mixture was the incubated for 10 minutes at 37°C. 1 ml of DNSA colour reagent was added to stop the reaction. These test tubes were then incubated in a boiling water bath for 5 minutes and cooled to room temperature. Finally, this reaction mixture was again diluted by adding 10 ml distilled water following which absorbance was measured at 540 nm. This procedure was repeated for the standard drug Acarbose. The percentage alpha amylase inhibition of both extract and standard drug were calculated by the following formula [6].

$$\% \text{ inhibition} = (\text{Abs of control} - \text{Abs of test}) / \text{Abs of control} \times 100$$

From the percentage inhibition value, IC_{50} value of extract was determined by using Instat 7 software.

Alpha glucosidase inhibition assay

The enzyme solution is prepared by dissolving 0.5 mg α-glucosidase in 10ml phosphate buffer (pH 7) containing 20 mg BSA. It is diluted further to 1:10 with phosphate buffer. Different concentrations of samples are prepared and 5µl each of the solution or blank is then added to 250 µl of 20 mM p-nitrophenyl-α-D-glucopyranoside and 490 µl of 100 mM phosphate buffer. It is then pre-incubated at 37°C for 5 minute and the reaction started by addition of 250 µl of enzyme solution and incubated at 37°C for 15 minutes 250 µl of phosphate buffer is added instead of enzyme for blank. The reaction is then stopped by addition of 1000 µl of 200 mM sodium carbonate solution and the amount of p-nitrophenol released is measured by reading the absorbance of sample against a blank at 400 nm. This procedure was repeated for the standard drug Acarbose. The percentage inhibition of both extract and standard drug were calculated by the following formula [7].

$$\% \text{ inhibition} = (\text{Abs of control} - \text{Abs of test}) / \text{Abs of control} \times 100$$

From the percentage inhibition value, IC_{50} value of extract was determined by using Instat 7 software.

2.4.2. In -vivo methods

Animals

Institutional Animal Ethics Committee (IAEC) approval number is DAMCOP/IAEC/039**.** Male Wistar albino rats of weight 150-200 g were used for the study. All animals were maintained under standard laboratory conditions [temperature (22±2°C) and humidity (45±5%)] with 12 hours day: 12 hours night cycle. The animals were acclimatized to laboratory condition for 15 days before commencement of experiments. All the procedure were performed in strict accordance with institutional animal ethics committee guidelines for the care and use of laboratory animals.

Streptozotocin induced diabetes mellitus in rats

Diabetes was induced in overnight fasted rats by single intra-peritoneal injection of 40 mg/kg of STZ, dissolve in 0.1 M cold citrate buffer, pH 4.5. To prevent the STZ induced hypoglycaemia, rats were provided with 5% dextrose solution after 6 hours of STZ administration for next 24 hours. Induction of diabetes was verified after 72 hours. The animals having a blood glucose level higher than 200 mg/dl was considered diabetic and used for the experiments.

- Group 1**:** Vehicle control (0.2% CMC).
- Group 2**:** Diabetic control (Sterptozotocin 40 mg/kg)
- Group 3**:** Glibenclamide (5 mg/kg) is given to the diabetic rats once a day for 28 days (orally).
- Group 4**:** 200 mg/kg of CECP is given to the diabetic rats once a day for 28 days (orally).
- Group 5**:** 400 mg/kg of CECP is given to the diabetic rats once a day for 28 days (orally).

Blood was collected from the tip of rat tail and blood glucose level were estimated at 0, 7th, 14th, 21st, and 28th days of treatment using Glucometer. Measured the body weight initially and during the treatment period. On the 29th day blood was collected by retro orbital puncture from the inner canthus of the eye under mild anaesthesia with Thiopentone sodium (40 mg/kg i.p). Then serum was separated by centrifugation of blood at 5000 rpm for 10 minutes and the biochemical parameters (Glucose, Total cholesterol, HDL, LDL, VLDL, and Triglycerides) were analysed.

2.4.3. Ex - vivo methods

Study of glucose uptake by rat hemi - diaphragm methods

The animals were sacrificed and diaphragms were dissected, divided into two halves and weighed. The hemi-diaphragms were then rinsed in cold Kreb's Ringer bicarbonate buffer (without glucose) and placed in small culture tubes containing Kreb's Ringer bicarbonate buffer with 5.55 mM glucose and incubated for 30 minutes at 37°C. Following incubation, the hemi-diaphragms was taken out. The glucose content of the incubated medium was measured by GOD-POD method. The uptake of glucose was calculated in mg/g tissue. Glucose uptake per gram of tissue was calculated as the difference between the initial and final glucose content in the incubated medium.

2.5. Histopathological studies

The animals were sacrificed by cervical dislocation. Dissect the pancreas, store in 10% formalin. Then observed for histopathological studies.

2.6. Statistical analysis

The data are expressed as Mean ± SEM statistically analysed using Instat and Graph Pad Prism softwares. The difference between groups (P value) are considered significant at P<0.05.

3. Results and discussion

3.1. Preliminary Phytochemical Analysis

Preliminary Phytochemical Analysis shows the presence of flavonoids, glycosides carbohydrates, alkaloids, tannins, phenolic compounds, steroids, terpenoids and amino acids.

3.2. Pharmacological studies

3.2.1. In-vitro methods

Alpha amylase inhibition assay

Alpha amylase inhibitory activity of CECP was evaluated and the IC_{50} value was found to be 158.396 µg/ml. The results from the study showed that CECP has significant alpha amylase inhibitory activity. (Table 1).

Table 1 Alpha amylase inhibition assay

Sr. No	Concentration (µg/ml)	Percentage inhibition	
		Acarbose	CECP
1	25	27.88±0.23***	19.76±0.11***
2	50	33.47±0.08***	24.21±0.19***
3	75	39.02±0.24***	33.11±0.36***
4	100	45.25±0.31***	36.62±0.21***
5	125	51.97 ± 0.50***	43.09±0.19***
6	150	57.81±0.19***	49.21±0.39***
7	175	65.24±0.10***	52.83±0.15***
8	200	72.05± 0.48***	59.05±0.16***
IC_{50}		116.113(µg/ml)	158.396(µg/ml)

Values are expressed as mean ± SEM. One way ANOVA comparison of control with standard and test (Tukey's Method). The data are considered significant if *p<0.05, **p<0.01, ***p< 0.001, ns-not significant.

3.3. Alpha glucosidase inhibition assay

Table 2 Alpha glucosidase inhibition assay

Sr. No	Concentration (µg/ml)	Percentage inhibition	
		Acarbose	CECP
1	20	18.98±0.12***	10.31±0.32***
2	40	28.99±0.46***	17.58±0.16***
3	60	38.34±0.36***	29.26±0.11***
4	80	45.20±0.25***	38.45±0.23***
5	100	56.3±0.29***	46.53±0.18***
6	120	62.32±0.39***	55.24±0.31***
7	140	70.81±0.52***	58.89±0.22***
8	160	75.12±0.43***	67.47±0.25***
IC_{50}		91.208 (µg/ml)	113.122(µg/ml)

Values are expressed as mean ± SEM. One way ANOVA comparison of control with standard and test (Tukey's Method). The data are considered significant if *p<0.05, **p<0.01, ***p< 0.001, ns-not significant.

Alpha glucosidase inhibitory activity of CECP was evaluated and the IC_{50} value was found to be 113. 122 µg/ml. The results from the study showed that CECP has significant alpha glucosidase inhibitory activity. (Table 2)

3.4. *In-vivo* methods

3.4.1. Streptozotocin induced diabetes in rats

Changes in body weight (g)

The CECP treated rats showed significant glucose lowering activity when compared to the diabetic control rats and also showed significant improvement in body weight during the treatment period. (Table 3).

Table 3 Changes in body weight (g)

Days	Control	Diabetic control	Standard Glibenclamide (5mg/kg)	CECP (200 mg/kg)	CECP (400 mg/kg)
0	181.6±4.89	182.16±3.62	179.66±4.65	177±5.41	177.66±5.90
7	189.6±4.82	166±3.76*	173.5±4.81 ns	162.66±5.45 ns	168.66±6.25 ns
14	197.66±5.14	145.33±8.066 **	171.166±12.60 ns	155.16±5.50 ns	164±6.15 ns
21	204.2±5.21	128.5±2.93***	174.66±5.47***	155.5±5.35**	167±6.51***
28	214.75±6.38	108±4.06***	177.8±5.84***	160.6±5.59***	173.6±7.51***

Values are expressed as mean ± SEM. One way ANOVA comparison between negative group and control group and between negative group and treatment groups (Tukey's Method). The data are considered significant if *$p<0.05$, **$p<0.01$, ***$p< 0.001$, ns - not significant.

3.5. Changes in blood glucose level (mg/dl)

The administration of Streptozotocin produces selective pancreatic beta cell cytotoxicity and induces diabetic condition. The results obtained from the study indicate that both 200 mg/kg and 400 mg/kg of CECP showed significant anti-diabetic activity. The 400 mg/kg of CECP showed better activity when compared to 200 mg/kg of the extract. (Table 4).

Table 4 Changes in blood glucose level (mg/dl)

Days	Control	Diabetic control	Standard Glibenclamide (5mg/kg)	CECP (200 mg/kg)	CECP (400 mg/kg)
0	92.2±3.48	264.0±32.56	280.83±21.24	294.16±25.97	288.83±15.50
7	95.0±5.51	322.0±36.29***	234.33±18.3 ns	264.5±20.25 ns	248.66±13.36*
14	91.16±3.18	407.66±39.41***	200.83±6.50***	249.0±13.67***	233.16±8.22***
21	93.0±4.83	478.83±32.65***	161.0±11.45***	202.5±8.09***	187.0±5.58***
28	96.2±5.03	535.8±26.46***	118.2±7.88***	183.6±7.80***	156.0±4.60***

Values are expressed as mean ± SEM. One way ANOVA comparison between negative group and control group and between negative group and treatment groups (Tukey's Method). The data are considered significant if *$p<0.05$, ***$p< 0.001$, ns - not significant.

3.6. Changes in lipid profile (mg/dl)

The CECP produced a significant decrease in triglycerides, total cholesterol, LDL, VLDL, and an increase in HDL levels of diabetic rats (Table 5).

Table 5 Changes in lipid profile (mg/dl)

Parameters	Control	Diabetic control	Standard Glibenclamide (5 mg/kg)	CECP (200 mg/kg)	CECP (400 mg/kg)
Total cholesterol	116.4±6.46	219±10.36 ***	138.6±3.35 ***	197.0±7.19 ns	Total cholesterol
Triglycerides	109.8±6.30	174.8±6.42 ***	122.8±3.15 ***	162.6±4.97 ns	Triglycerides
HDL	34.0±1.58	16.0±0.70 ***	29.0±0.70 ***	18.8±0.86 ns	HDL
LDL	60.44±6.34	168±9.52 ***	85.04±3.67 ***	145.68±7.55 ns	LDL

Values are expressed as mean ± SEM. One way ANOVA comparison between negative group and control group and between negative group and treatment groups (Tukey's Method). The data are considered significant if ***p< 0.001, ns – not significant.

3.7. *Ex-vivo* methods

3.7.1. Study of glucose uptake by rat hemi-diaphragm method

The glucose uptake study was performed by isolated rat hemidiaphragm method. The hemi diaphragm obtained from the rats treated with both the doses of CECP showed significant glucose uptake (Table 6).

Table 6 Study of glucose uptake by rat hemi-diaphragm method

Sr. No	Groups	Glucose uptake (Mean±SEM) (mg/g)
1	Control	8.49±0.23
2	Diabetic control	0.75±0.08***
3	Standard (Glibenclamide 5 mg/kg)	8.14±0.18***
4	CECP (200 mg/kg)	4.0±0.14***
5	CECP (400 mg/kg)	6.9±0.09***

Values are expressed as mean ± SEM. One way ANOVA comparison between negative group and control group and between negative group and treatment groups (Tukey's Method). The data are considered significant if ***p< 0.001.

3.8. Histopathological studies

The results from histopathological study indicate that the CECP has significant protective effect on pancreatic cell mass. (Figure 2)

The results obtained from the *in-vitro* studies indicate that the CECP has significant inhibitory effect on carbohydrate digesting enzymes. So, the plant extract can reduce the intestinal absorption of glucose. The results obtained from the *in-vivo* study indicate that both 200 mg/kg and 400 mg/kg of CECP showed significant anti-diabetic activity. The 400 mg/kg of CECP showed better activity when compared to 200 mg/kg of the extract. From the *ex-vivo* studies, the hemi diaphragm obtained from the rats treated with both the doses of CECP showed significant glucose uptake. The drugs from the natural source are more accepted by patients because of undesirable side effects of synthetic drugs. So, further studies are needed on the plant *Clerodendrum paniculatum* to make more scientific evidence.

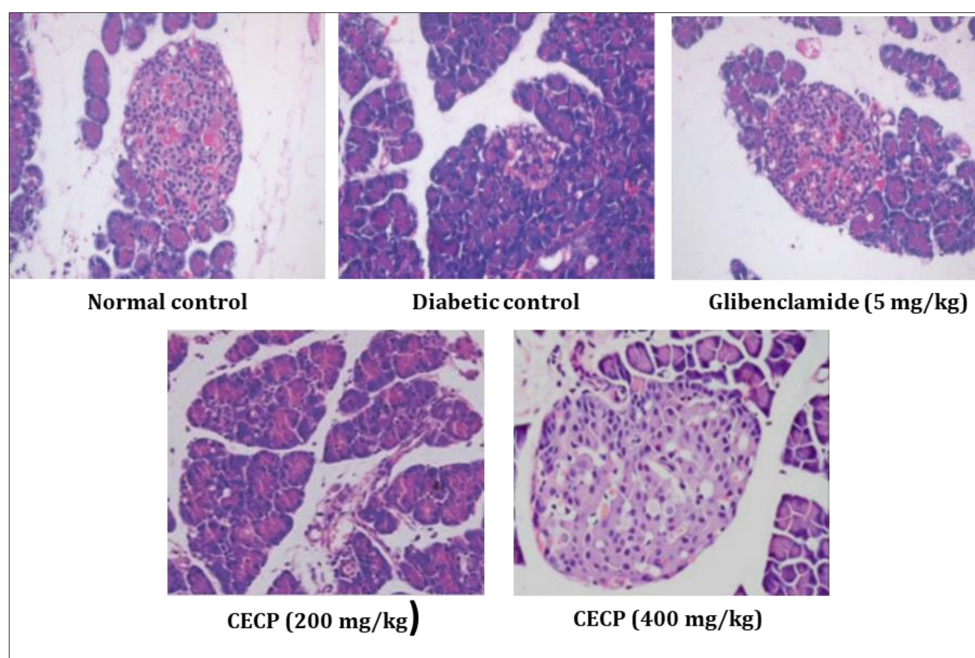

Figure 2 Histopathological examinations

4. Conclusion

The anti-diabetic potential of chloroform extract of *Clerodendrum paniculatum* leaves were studied by Streptozotocin induced diabetes method and it exhibits significant anti-diabetic activity. The phytochemical studies showed the presence of phytochemicals such as Flavonoids, Alkaloids, and Phenolic compounds, Tannins, Terpenoids, Glycosides, Saponins, Carbohydrates, Amino acids and Proteins. Hence the presence of these constituents in the extract may be responsible for the hypoglycemic activity. Further study is needed to determine the active component responsible for its activity.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

All the authors declares that they has no conflict of interest.

Statement of ethical approval

All applicable International, National and /or Institutional guidelines for the care and use of animals were followed.

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