Phytochemical Screening and In-vivo Evaluation of Hypoglycemic Activity of *Clerodendrum indicum* Leaf Extract

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Abstract

The aim of the present study was to evaluate in vivo hypoglycemic activity of the ethanolic leaf extract of Clerodendrum indicum using experimental animal model of diabetes mellitus. The hypoglycemic activity of the extract was investigated at two test doses (500 mg/kg BW and 250 mg/kg BW) for 7 days treatment period in streptozotocin induced diabetic rat model. In addition, preliminary phytochemical screening of the extract was done by standard protocol. The results showed that C. indicum leaf extract significantly (P<0.01) reduced blood glucose level of the experimental rats compared to diabetic control in a dose dependent manner. The greater hypoglycemic activity was obtained at 500 mg/kg test dose where the blood glucose level lowers from 8.73 ± 0.75 mmol/l to 4.85 ± 0.31 mmol/l during the study period. Moreover, the phytochemical analysis was found to be positive for alkaloid, phenol, flavonoid, tannin, glycoside and steroid. These findings suggested the prospect of C. indicum leaf extract as a natural hypoglycemic agent in the treatment of diabetes mellitus.

Keywords: *Clerodendrum indicum,* Streptozotocin, Hypoglycemic, Diabetes, Phytochemical.

Introduction

Diabetes mellitus is an emergent public health concern throughout the world. Around 200 million people of the world are currently suffering from it. Diabetes is characterized by hyperglycemia arising from absolute or relative inadequacy of insulin and is accompanied by characteristic long-term renal, hepatic, cardiovascular and neurological disorders (Donovan, 2004; Nathan, 1993). Apart from the modern medicines, phytotherapies have been used for years in the treatment of diabetes mellitus considering their folkloric medicinal uses. Most of these therapies usually have less or no side-effects. That is why the demand for natural products is increasing day by day (Nasri et al., 2015). Consequently, numerous biological investigations have been carrying out to isolate, identify and analyze the active phytochemical compounds and to find out potent biological activities of plant derived drugs.

Clerodendrum indicum (family Verbenaceae), a promising medicinal plant, is locally known as Bamanhati in Bengali. It is widely distributed in many countries throughout the Southeast and Southern region of Asia (Manandhar, 2002). The plant has folkloric use in infectious diseases such as scrofula, venereal infections and also aid in the treatment of inflammatory diseases (Rehman et al., 1997; Shrivastava & Patel, 2007). Furthermore, a number of pharmacological activities including antimicrobial, anxiolytic, antiasthmatic, antirheumatic, antinociceptive, and antidiarrheal activities are attributed to C. *indicum* (Majumder et al., 2019; Pal et al., 2012; Shrivastava & Patel, 2007). Although some other species of *Clerodendrum* genus were reported for having hypoglycemic activity (Adeneye et al., 2008; Kar et al., 2014; Ly et al., 2019), C. indicum is yet to explore such activity

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till date. Therefore, the present investigation was aimed to evaluate in vivo hypoglycemic activity of the ethanolic leaf extract of *C. indicum* using streptozotocin induced diabetic rat model.

Materials and Methods

Chemicals

Streptozotocin was purchased from Wako Pure Chemical Industries, Ltd. (Japan) and Metformin was obtained from Square Pharmaceuticals Ltd. (Bangladesh). The other procured chemicals were of analytical grade and accessible locally.

Collection of Plant Material

The fresh leaves of *C. indicum* were collected from Dhaka, Bangladesh. The plant was identified taxonomically from National Herbarium of Bangladesh (Accession number of *C. indicum:* 45999).

Preparation of Plant Extract

The green leaves of the plant were shed-dried, then crushed into coarse powder and subjected to extraction. In cold extraction process, the chemical constituents were isolated from crude leaves by the solvent ethanol with occasional stirring. After that, the crude extract was filtered with Whatman filter paper and the ethanol was evaporated from the extract at room temperature to yield semi-solid extract.

Phytochemical Screening

The preliminary phytochemical investigation of the leaf extract was done by standard methods (Braca et al. 2001) to identify the presence of different phytochemical constituents. This preliminary screening of the extract used several qualitative phytochemical tests including the tests for alkaloid, glycoside, tannin, flavonoid, steroids, phenol and saponin.

Test for Hypoglycemic Activity

Selection of Animals

Healthy male Long-Evans rats were obtained from the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B). All of the experimental rats were housed under standard laboratory condition and fed standard rodent food and water.

Induction of Diabetes in Animals

In experimental rats, diabetes was induced by streptozotocin (STZ) in single dose (100mg/kg, BW) intraperitoneal injection and the fasting blood glucose level was measured after 72 hours. The rats weighting between 98 and 132 gm with fasting blood glucose levels greater than 7.0 mmol/L were chosen for further investigation.

Experiment Design

The rats were allotted randomly into Group I to V, as per Normal control, Diabetic control, Metformin, ELCI 250 and ELCI 500 respectively. The Group I was non-diabetic and received saline water. In contrary, Group II-V were diabetic; whereas, Group II received saline water, Group III was treated with Metformin (100 mg/kg, BW) and Groups IV & V were treated orally with the plant extract having doses of 250 mg/kg, BW and 500 mg/kg, BW respectively.

Blood Sample Collection

Fasting blood samples were obtained from the experimental rats on day 0, day 3, day 5 and day 7 using glucometer to measure the blood glucose level.

Statistical Analysis

The data were presented as Mean ± Standard Error of Mean (SEM). The statistical analysis was carried out by one way analysis of variance (ANOVA) using SPSS 20 software, wherever the difference between groups were chosen to be significant at a level of *p< 0.01.

Results

Phytochemical Screening

The preliminary phytochemical investigation on the extract revealed the presence of alkaloid, glycoside, flavonoid, phenol, steroid and tannin shown in Table 1.

Plant Extract	Phytochemicals						
	Alkaloid	Glycoside	Tannin	Flavonoid	Steroid	Phenol	Saponin
ELCI	+	+	+	+	+	+	-

Table 1. Results of phytochemical screening of ethanolic leaf extract of *C. indicum* (ELCI)
'+' and '–' denotes for present and absent respectively

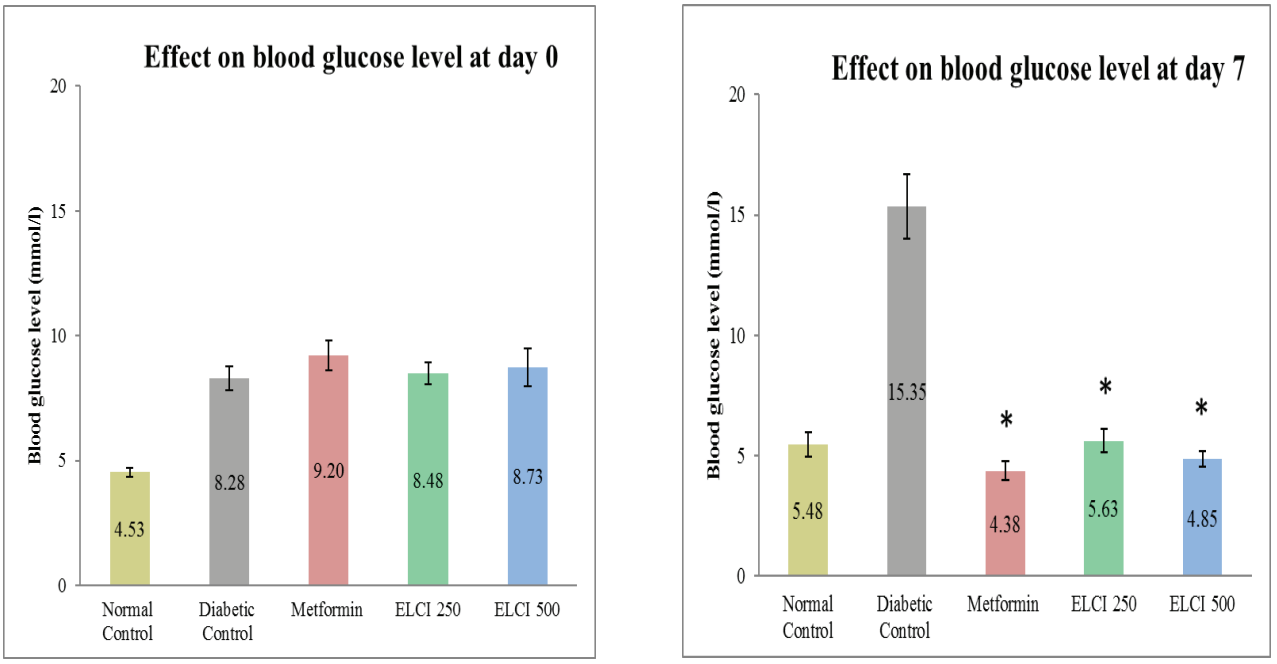
Hypoglycemic Activity Evaluation

The hypoglycemic effect of C. indicum leaf extract on fasting blood glucose was presented in Table 2. Throughout the treatment period, all the treatment groups (Metformin, ELCI 250 and ELCI 500) exhibited significant (P<0.01) decrease in fasting blood glucose level compared to Diabetic control group. Moreover, Fig 1 compared the two doses of the extract (EECI 250 and EECI 500) after 7 days of diabetes induction and also demonstrated that EECI 250 and EECI 500 lowered the mean blood glucose level in a dose-dependent manner.

Table 2. Effect of ethanolic leaf extract of *C. indicum* (ELCI) on fasting blood glucose levels in streptozotocin induced diabetic rats

Groups	Fasting blood glucose level (mmol/l)				
	At the time of grouping	Days of treatment			
		0 (day)	3 (day)	5 (day)	7 (day)
Normal Control	4.20±0.12	4.53 ± 0.18	4.90 ± 0.30	5.28 ± 0.25	5.48 ± 0.52
Diabetic Control	4.58±0.07	8.28 ± 0.48	9.38 ± 0.57	13.35 ± 0.30	15.35 ± 1.34
Metformin	5.32±0.10	9.20 ± 0.59	7.33 ± 0.79	5.38 ± 0.57*	4.38 ± 0.40*
ELCI 250	4.81±0.09	8.48 ± 0.44	7.53 ± 0.47	6.98 ± 0.22*	5.63 ± 0.50*
ELCI 500	5.03±0.07	8.73 ± 0.75	6.65 ± 0.45*	5.80 ± 0.17*	4.85 ± 0.31*

Data are presented as Mean ± SEM (n=4) and considered significant at *p <0.01 when compared with Diabetic Control. ELCI 500: Ethanolic leaf extract of C. indicum 500 mg/kg, BW & ELCI 250: Ethanolic leaf extract of *C. indicum* 250 mg/kg, BW.



(a) (b)

Figure 1. Effect of ethanolic leaf extract of *C. indicum* (ELCI) on blood glucose level at (a) day 0 and (b) day 7 in streptozotocin induced diabetic rats. Data are presented as Mean ± SEM (n=4) and considered significant at *p <0.01 when compared with Diabetic Control. ELCI 500: Ethanolic leaf extract of *C. indicum* 500 mg/kg, BW & ELCI 250: Ethanolic leaf extract of *C. indicum* 250 mg/kg, BW.

Discussion

The streptozotocin induced experimental model of diabetes mellitus is well-known, wherever the streptozotocin is recognized for its diabetes inducing effect by triggering hyperglycemia through destruction of insulin secreting pancreatic beta cells (Junod et al., 1969). In most of the cases, the single dose (70-250 mg/kg, BW) of STZ has shown to cause the entire destruction of beta cells and the multiple lower doses of STZ also partly impair those cells and activate the inflammatory process leading to insulin deficiency (Kolb, 1993; Like & Rossini, 1976). The results of the present study showed that the oral administration of *C. indicum* leaf extract for 7 days significantly (P<0.01) controlled STZ induced hyperglycemia. Hence, the observed blood glucose lowering activity of the extract was liable to the potential antidiabetic effect of it. Furthermore, the qualitative phytochemical studies of the extract showed the presence of different phytoconstituents such as tannins, flavonoids, phenols, steroids and alkaloids in it. These compounds are known to have a wide range of biological activities such as hypoglycemic, hypolipidemic, hypotensive activities, insulin-like effects and so on (Marles & Farnsworth, 1995; Olapade, 1995; Price et al., 1987). Thus, the presence of these compounds might contribute to the hypoglycemic effect of the extract as observed in the present study.

Conclusion

In light of the results, the present study suggests that the ethanolic leaf extract of *C. indicum* effectively lowers the blood glucose level in streptozotocin induced diabetes. Therefore, the extract may possibly be considered as a natural antidiabetic agent in the treatment of diabetes mellitus. However, further inquiries are required to clearly perceive the basic mechanism of the observed bioactivity and to find out the active phytochemical constituents accountable for such activities in different experimental models.

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Ethical Approval

The study was accepted by the Committee on Ethical Compliance in Research of Southeast University, Bangladesh.

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