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Research Article

Evaluation of the anti-diabetic potential of aqueous extract of *Clerodendrum infortunatum* L. *in vivo* in streptozotocin-induced diabetic Wistar rats

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Abstract

Diabetes Mellitus, the metabolic syndrome where the body either fails to produce or effectively utilize insulin, is associated with chronic morbidity. While a definitive cure for the disease is lacking, with the modern medicine offering mainly the means to control the extent of the disease, Complementary and Alternative Medicine (CAMs) offers additional/alternate means to tackle the disease. On the other hand, the lack of evidenced medical practices is a lacuna in most of the traditional medical applications. *Clerodendrum infortunatum*.L (Lamiaceae family), a perennial shrub found in the tropics, has been known for its numerous pharmacological properties and is found as a constituent in many Ayurvedic and Siddha drugs, especially for skin and respiratory ailments. The plant has a noted potential as anti-hyperglycemic and has been found to be used in traditional medicine for the treatment of diabetes. However, evidence based evaluations have not been conducted on the anti-hyperglycemic effect of the plant, especially with respect to the general mode of intake, *i.e.*, the aqueous form. In the current study, the aqueous extract of *C. infortunatum* (CI), was scientifically assessed for its effect on streptozotocin induced diabetes in Wistar albino rats. The diabetic rats were divided into 5 groups of 6 animals each. For testing the efficacy of extracts, two groups were intra-orally provided with dosages of 200 mg/Kg and 400 mg/Kg of body weight of animals, respectively, of aqueous extracts of CI. Control groups were maintained for evaluation, which included vehicle control as well as with Glibenclamide, a standard anti-diabetic drug. The extracts at a dose of 400 mg/Kg body weight was found to be associated with significant amelioration of many of the diabetes induced conditions, suggesting that the plant extract could be a strong potential CAM candidate for therapeutic management of diabetes.

Keywords

Complementary and alternative medicine; *Clerodendrum infortunatum*; diabetes; streptozotocin

Citation

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Introduction

Diabetes mellitus (simply referred diabetes), a metabolic disorder in which the body fails either to produce or to respond to insulin and thus resulting in faulty glucose metabolism, is a chronic disease condition with a prevalence of 8.5% of the world adult population (1). Diabetes type II is the most common, responsible for 90% of diabetes, arises due to dysfunctional pancreatic beta cells or impairment to insulin response by the body cells. The disorder is associated with morbidities and reduction in the quality of life and in addition, also could shorten the lifespan due to complications such as cardiovascular diseases (2). There are no curative therapies yet identified for diabetes, while management of the blood glucose levels by restriction of sugar and calorie intake (especially low glycemic index foods), regular monitoring of blood sugar parameters, and more importantly by adoption of healthy lifestyle (3), in an optimal manner have been shown to improve the quality of life near normal. In chronic cases, the effective management of type 2 diabetes involves insulin therapy. However, the administration of the same is fraught with difficulties such as the requirement of a trained person for administration, pain and relative cost. Other medicines taken orally including sulfonylureas and biguanides have limited utility and are also fraught with side effects.

Complementary and Alternative Medicine (CAM) offers some attractive means of managing diabetes, some of which uses herbal/plant based treatments (4). CAM has an added advantage that some of the most populous countries in Asia and Africa have a ready acceptability for traditional therapeutic systems. Many of the CAM regimens involve components of plant origin which are known to be more bio tolerable than chemically synthesized agents. In addition, most of the CAMs are relatively cheap compared to often proprietary synthetic compounds, increasing the acceptability. However, a major drawback with most CAM is that while many of them are renowned, the efficacy, mode of action, dosage and side effects has not been extensively evaluated when compared to the pharmaceutical agents of modern medicine. In the current study, the anti-hyperglycemic properties of a shrub, *Clerodendrum infortunatum*, was evaluated.

Clerodendrum infortunatum.L. is a perennial shrub, with wide distribution noted in the tropical regions of Asia, specifically of south Asia (5). The English common name of the plant is 'Hill glory bower'. Various parts of the plant find uses in traditional medicine (6). Routine uses include use as styptic and as anti-ascarid (7) while the tribes of North-East India use the plant's extract for subsiding fever and intestinal diseases. Some of the closely related species, *C. serratum* (8) referred in texts of Ayurveda as an antidiabetic (Madhumehagna), and *C. phlomoides* shown to

reduce fasting blood sugar in human subjects (9), have been known to have potent anti-hyperglycemic properties. While anti-hyperglycemic effects of solvent extracts have been shown for *C. infortunatum* (10), the traditional route of intake, aqueous decoction, has not been evaluated. The objective of this study was to assess the anti-diabetic activity of the aqueous extracts of *Clerodendrum infortunatum*. L. in streptozotocin induced diabetes in rats.

Materials and methods

Plant material

C. infortunatum was collected and identified as correct species from its natural habitat at Pandalam, Kerala, India. The species was verified by a specimen submitted to Jawharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Thiruvananthapuram, Kerala.

The plant body (stem and leaves) were shade dried in such a way that the weight of the dried item remained same over 3 consecutive days. 50 grams of the dried material was weighed and was finely ground in a mortar with the help of pestle. The aqueous extract of CI was prepared by boiling 50 grams of dry CI powder in a clean round bottom flask by boiling for 2 hours, followed by sieving the extract first with a sterile mesh cloth and subsequently through a coarse filter paper. The extract, devoid of visible solids, were allowed to evaporate by gentle heating to leave thick slurry in the end. The slurry was then suspended in distilled water to get desired concentration as per the dose (mg/kg body weight). Formulation of the test item was prepared shortly before dosing. The homogeneity of the test formulation was maintained by continuous stirring of the glass rod during dosing.

Animal Studies

The animal studies were conducted after obtaining ethics committee approval and were conducted at department of toxicology, CArE Keralam (Thrissur, India). Wistar Albino rats (procured from Veterinary University, Thrissur, India) were used. 30 male rats were used during the conduct of the study. Animals weighing between 200-250 grams were selected for the treatment at the age of between 12 to 14 weeks post birth.

Induction of diabetes and subsequent treatment

Animals, after acclimatization (5 days) in the animal house, were randomly divided into five groups of six animals. Diabetes was induced using streptozotocin (STZ) (Sigma-Aldrich Inc, Missouri, USA), dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5) and administered as intraperitoneal. Rats previously fasted for 16 h were given single intraperitoneal injection of 120 mg/kg Nicotinamide 30 minutes prior to

administration of 40 mg/kg body weight. Animals with fasting blood glucose between 200 -250 mg/100 mL, three days after STZ administration were further selected for the study. Glibenclamide (Sigma-Aldrich Inc, Missouri, USA) was used as the standard antidiabetic drug and was administered peri-orally at a concentration of 600 µg/Kg body weight.

The groups were treated (Table 1) and were monitored for 40 days. At the end of study all the animals were sacrificed by cervical dislocation. Wherever applicable, distilled water was used as control.

Table 1. The stratification of the animals into groups and respective treatments

Category	Treatment
Group-1	Vehicle control
Group-2	STZ only
Group-3	STZ + Glibenclamide 600 µg/Kg body weight
Group-4	STZ + CIE 200 mg/Kg body weight
Group-5	STZ + CIE 400 mg/Kg body weight

Administration of CI extract

Acute oral toxicity studies were conducted to determine the toxicity against 500mg/Kg, 1gram/Kg and 2gram/Kg body weight. No toxicity or adverse responses were noted. Test doses were fixed at 200 and 400 grams (10% and 20% of upper test dose, respectively). The extract, as well as the standard anti-diabetic drug glibenclamide, was administered orally by gavage to each rat as a single dose (as mentioned in Table 1), once daily, for a period of 40 days. The dosage volume administered to individual rat was adjusted according to its body weight recorded on the day of dosing. The dose volume was 1 ml/100 g body weight for all animals. Food was offered 3-4 hours after dosing.

Biochemical analyses

Blood samples were collected by the tail- vein method and blood glucose levels were measured immediately by using glucose oxidase- peroxidase reactive strips and a glucometer. Blood samples were collected on days 0, 10, 20, 30 and 40, respectively, for this purpose. Additionally blood samples were collected on the 41st day of administration and were allowed to clot for 30 minutes. After that, the animals were sacrificed by cervical dislocation for visceral inspection as well as necropsy analysis. Serum separated from the blood samples were used for the estimation of biochemical parameters including Alkaline phosphatase (ALP), Aspartate amino transferase (AST), Alanine amino transferase (ALT), Bilirubin, Serum creatinine, total protein and plasma insulin. The biochemical parameters were tested

using commercially available kits and were done as per manufacturer's protocols. Plasma insulin was determined using Chemiluminescent Microparticle Immuno Assay.

Statistical Analysis

The data of all the parameters were analyzed using Analysis of variance (ANOVA). One way ANOVA followed by Dunnet's test was performed in which the diabetic control was compared with all the other groups and the values are expressed in Mean \pm SD. Statistical analyses were conducted using SPSS statistics version 13 software (IBM inc., USA). P-value less than 0.05 was considered to be statistically significant.

Results

In order to identify the effect of *C. infortunatum* on the glucose level of the animals, periodic monitoring, on an interval of 10 days, of blood glucose was performed (Fig. 1). The reduction of the blood glucose levels was discernable in diabetes induced rats from the tenth day of the treatment (in both CI extracts and in Glibenclamide treatment). Significant reduction of the glucose levels were seen in *C. infortunatum* at 400 mg/kg dose ($p < 0.001$), although not abrupt as that of the anti-diabetic drug glibenclamide at 0.6mg/kg dose. However, at lower doses of CI (200 mg/kg), the reduction though noted, was not highly statistically significant. The reduction in the elevated blood sugar in the diabetic rats was relatively slow in case of the extracts treated group (in both doses). Glibenclamide, on the other hand had a relatively fast paced action, wherein by the thirtieth day of the study, the glibenclamide treated diabetic rats had a closer-to-normal blood glucose level. The CI extract at 400 mg dose was able to bring down the glucose in a gradual manner, with only a modest reduction noted till the 30th day (at 215 ± 12.88 mg/dL Vs. 126.67 ± 7.71 mg/dL in case of glibenclamide). However, a stronger reduction in blood sugar level was noted in the CI extract at 400 mg dose by day 40 (blood sugar level at 175.67 ± 13.5 mg/dL). On the other hand the low dose (CIE 200) treatment was not very effective in bringing down the sugar levels, even at the end of the study (blood sugar level at 231.33 ± 12.48 mg/dL on the 40th day).

Similarly, there was significant elevation in the serum insulin levels of the diabetic rats when treated with CI extracts at 400 mg/kg body weight ($p < 0.01$) (Fig. 2). However, at lower dose of the extract, the inducements of insulin in diabetic rats were only modest and were not significant. While the normal (un-diabetic) rats had an average serum insulin of 3.37 ± 0.56 µIU/mL, a drastic reduction in serum insulin was noted in the diabetic rats (serum insulin 0.18 ± 0.08 µIU/mL) indicating the extensive destruction of pancreatic beta cells by streptozotocin. As expected,

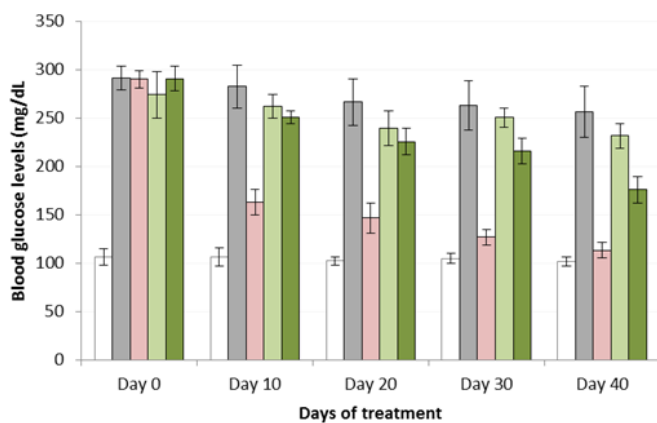


Fig. 1. Reduction in blood glucose levels in various test groups over the period of study (40 days). Glibenclamide and CIE400 both significantly reduced the blood sugar levels ($p < 0.001$).

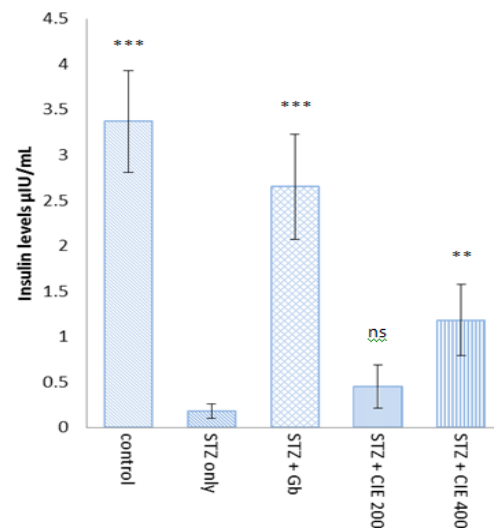


Fig. 2. Plasma Insulin level measured on day 40. Significant enhancement in insulin production was observed in Glibenclamide ($p < 0.001$) and CIE400 ($p < 0.01$). ns – not significant, STZ- streptozotocin, Gb –glibenclamide, CIE200 and CIE400 – cleorodendrum infortunatum extract 200 mg and 400 mg/kg doses respectively.

Table 2. Biochemical parameters relevant to liver function. ($*p < 0.1$, $**p < 0.01$, $***p < 0.001$)

Group	Serum Creatinine (mg/dL)	Serum Urea (mg/dL)	Total protein (mg/dL)
I - Vehicle control	0.457±0.132***	32.900±2.407***	8.873±0.563***
II - STZ only	1.537±0.120	64.367±4.030	4.343±0.429
III - STZ + Glibenclamide	0.613±0.109***	41.133±5.673***	7.617±0.514***
IV - STZ + CI extract 200 mg/kg	1.452±0.284ns	60.733±7.184ns	4.723±0.695n
V - STZ + CI extract 400 mg/kg	1.130±0.215**	50.950±7.074**	5.498±0.677**

glibenclamide induced the elevation of insulin to the maximal levels among the treatment group, with the insulin production measured at 2.65 ± 0.58 $\mu\text{IU/mL}$ by the 40th day. The CI extracts at 400 mg/Kg dose could also improve the insulin levels; however, the levels were not as much as that of glibenclamide (1.18 ± 0.39 $\mu\text{IU/mL}$ in 400 mg dose on 40th day). At the lower dose of 200 mg/Kg the production of insulin could only be boosted to 0.45 ± 0.24 $\mu\text{IU/mL}$ and was not significant.

Next, we evaluated the liver function parameters of the animals to ascertain the aftermath of anti-hyperglycemic effect of the CI extracts on liver since liver function abnormalities are usual in diabetes (especially with relevance to the gluconeogenesis and ketone body synthesis). We ascertained the liver function by evaluating the major biochemical indicators of liver functions, viz. ALT, AST, ALP, creatinine and bilirubin. As expected, the inductions of diabetes by STZ lead to significant elevation of the indicators when compared to the vehicle control animals (Fig. 3). In all the tested parameters, the liver functions of the diabetes induced rats could be significantly improved by CI extracts. CI extracts at 400 mg/kg dose could significantly

lower the levels of the elevated liver parameters ($p < 0.01$ in all the tested parameters, Fig. 3 and Table 2), although lower doses could not exhibit such a significant reduction. As expected, glibenclamide treatment could also significantly lower the levels of the indicators ($p < 0.01$, Fig. 3).

Discussion

The usual effects of diabetes and its allied pathophysiology are widespread. This involves not only changes in the overall sugar metabolism, but also effects on the functional and structural integrity of the major organs including liver and pancreas (11). For management of diabetes the foremost importance is given to the upkeep of the blood sugar levels within or close to the normal physiological levels. For therapeutic management, blood-sugar lowering drugs such as metformin, which mainly acts by decreasing the glucose production by liver, are used (3). Some of the drugs also increase the insulin sensitivity. Similarly, insulin inducing drugs (as secretagogue) and drugs which enhance the insulin production from beta-cells are also used. Exogenous insulin is

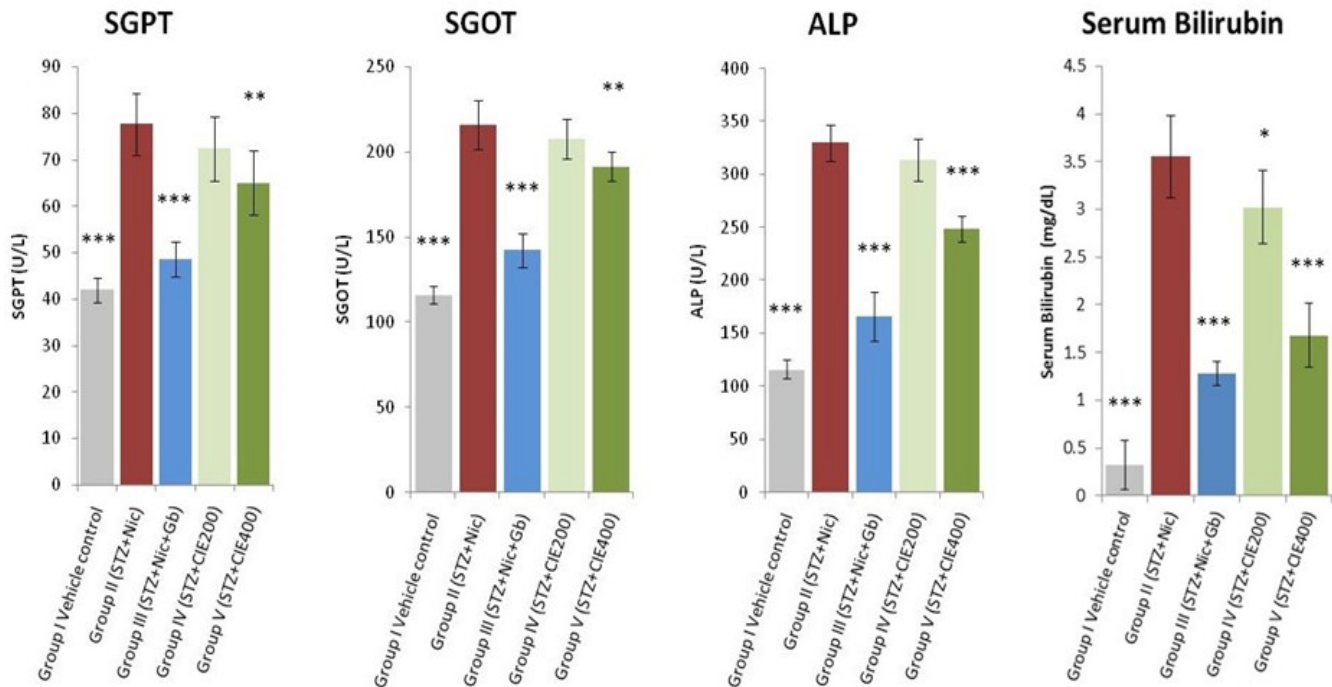


Fig. 3. Liver function parameters among the various test groups (* $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$)

also given as hypodermic injection depending on the severity of the disease.

Complementary and Alternate Medicine (CAM) also offer anti-diabetic medications, especially some of them, with components such as fenugreek, being in effective use for many centuries (12). Some of the herbal medicines are also made as formulations of multiple individual components, however, even when the effects of the herbal drugs and their formulations have known physiological impact, details regarding the mechanism of action and dosage are often left subjective (13). Furthermore, there is the need to further explore alternative remedies which could offer more potent or more bio-tolerant medications.

In the study, aqueous CI extracts could lower the blood glucose in multitude of ways, which may perhaps be due to the synergistic effects exerted by individual phytochemical components (14). The improvement of glucose metabolism, as seen in the progressive improvement in the blood glucose of the animals over the period of study (Fig. 1), points to the anti-hyperglycemic potential of the plant extracts. However, when compared to the standard drug glibenclamide, the effects were more gradual. While glibenclamide could bring the blood glucose levels in the diabetic animals near normal within 30 days (at 126.67 ± 7.71 mg/dL on 30th day), the blood glucose levels (even though significantly reduced) in animals treated with CI extracts at 400 mg/kg was still at an elevated level on 40th day (at 175.67 ± 13.5 mg/dL on 40th day) (Table S1). This could be due to the moderation due to the synergistic effects of multiple components (14), unlike single agent therapy where the dose is optimized for rapid

action of one component, like the stimulated release of insulin as in the current case (15). There was significant elevation in the blood insulin levels in the animals treated at 400 mg/Kg dosage. This was comparable to the effects of glibenclamide, though not as robust as that of the latter. Glibenclamide is known to induce enhanced production of insulin from beta cells (15). When compared to non-diabetic animals and the glibenclamide treated diabetic rats, the induction of insulin by CI extract even at the maximum dose 400mg/kg was only modest (Fig. 2). Taken together, it is possible that the improvement in glucose levels seen in the extract fed rats may be due to the induction of insulin from pancreas.

Indicating the multi-target effect of the CI extracts, there was a marked improvement in the liver function in the diabetic rats, as assayed by the key liver enzymes. At 400 mg/Kg dose the improvement in liver function (as per tested biochemical parameters) was significant and was comparable to the standard drug glibenclamide. While the improvement in the liver function may be due to the lowering of blood sugar, as was the case with glibenclamide, it could be also possible that multiple phytochemical constituents may be conferring protective advantage to other vital organs such as liver (14).

In the current study, we observed a dose dependency in the administration of drugs in the amelioration of diabetic pathology. While the diabetic animals treated at lower dose (200 mg/kg) exhibited moderate levels of improvement, significant improvement was conferred by the extracts at higher dose (400 mg/Kg). Further optimization of the dose, as well as optimizing the source plant materials may be useful in improving

the efficacy of a given dose. The latter is more important as it is a well-known fact that phytochemical constituents alter in the same plant species depending on the climatic conditions, growth phase and altitude (16).

There were previous attempts in identifying the medicinal value of *C. infortunatum*. A previous study (10) using methanolic extracts of the CI (MECI) leaves on male Wistar rats identified a statistically significant improvement in the STZ induced hyperglycemia. However, aqueous extracts may be a better alternative to organic solvent based extraction in some ways. First, the traditional and folkloric use of plant based medicines predominantly utilizes raw juices, water extracts and aqueous decoctions. Being in use over centuries by the traditional medicine, they offer quick preliminary checklist to pick from while identifying alternate solutions to therapies for complex diseases. However, efficacy and dosage is often vague and rarely scientifically evidenced (16). Aqueous extracts offer high similarity with the traditional applications. Secondly, organic solvents and their by-products left over from the extracting process are at times toxic and their direct application may have unintended off-target side-effects (17). This is critical because complex diseases such as diabetes may also weaken crucial organs such as liver; the left over toxic solvents may be deleterious in such conditions, especially because anti-diabetic drugs may have to be taken regularly and lifelong.

Conclusions

There are many traditional and Ayurvedic formulations where *C. infortunatum* is a constituent. But none of them are targeted for anti-diabetic properties of the plant as the primary goal. In the current study, we evaluated the anti-diabetic properties of the aqueous extract of *C. infortunatum* on diabetes-induced Wistar rats at two doses, viz. 200mg/Kg and 400mg/Kg, and identified that 400mg/kg to have significant effect. Improvement in blood glucose levels, enhanced insulin production and improvements in liver functions were noted in extract treated diabetic rats when compared to the untreated diabetic animals. Further investigation in the medicinal properties, optimal dose and toxicology is essential so that the diabetic patients may be able to benefit from the anti-diabetic potential of this medicinal plant.

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Authors' contributions

JSP performed the experimental works, participated in design, and result analyses. RR wrote the manuscript as well participated in the result analyses. PNKG conceived the work, interpreted the results and participated in design and co-ordination. SKMG participated in co-conceiving the design and in co-ordination. RJT participated in the design, in result interpretation and in writing the manuscript. All authors read and approved the final manuscript.

Declaration on ethical use of animals

The study was reviewed and approved by Institutional Animal Ethics Committee of CARE Keralam Ltd. All the animal experiments in the study were conducted as per concerning ethical standards set by Government of India.

Competing interests

The authors declare that they have no competing interests.

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