AMELIORATIVE EFFECT OF ETHANOLIC EXTRACT OF *COPTIS TEETA* WALL. ROOTS IN ALLOXAN INDUCED DIABETIC RATS

Bhagyabhumi P. Shah[a], Dipen R. Patel[b] and Samir K. Shah[c]*

ABSTRACT

The antidiabetic activity of *Coptis teeta* extract (CE) was investigated in a diabetic rat model. Alloxan (150 mg kg^{-1}, i.p.) was used to induce hyperglycemia in rats. Oral administration of CE at the dose of 250 and 500 mg kg^{-1} for 28 days lowered the blood glucose level. Various physical parameters (body weight, food and fluid intake) and biochemical parameters like serum glucose, insulin level and kidney functions were measured. Results revealed that rats treated with CE forbade the decrease in body weight, and showed a significant ($p < 0.05$) reduction in food and fluid intake, serum glucose, serum urea, serum creatinine, and a significant increase in insulin level and serum protein as compared to diabetic rats. Histopathological study of kidneys supported the data and CE reduced the damage to the glomerulus and bowman capsule produced by alloxan. The study clearly shows that the ethanolic extract of *C. teeta* possesses potent antidiabetic activity.

Keywords: Diabetes, *Coptis teeta*, alloxan, glucose, antidiabetic activity

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by a rise in blood glucose level which affects a large population across the world[1]. Due to abnormalities in fat, carbohydrate and protein metabolism, some common symptoms like polydipsia, polyurea, and ketonuria are observed in diabetes patient[2]. Diabetes mellitus is associated with many life-threatening complications which can be controlled through various approaches including diet changes, light exercise and pharmacological treatment[3]. Allopathic drugs used in treating diabetes do possess pharmacological action, but all of them vary in reducing hyperglycemia and few drugs are even not efficacious in people with other comorbidities[3,4]. Allopathic drugs have various side effects and there is a need for safer and more effective hypoglycemic agents[5]. Due to this WHO had recommended the use of medicinal plants and emphasized their importance in reducing blood glucose levels in diabetic patients[6]. Herbal medicines have been used for treating diabetes mellitus due to their high acceptability, compatibility, and fewer side effects. In the last few years, ayurvedic plants have shown their preventive role in diabetes and its progression[7]. According to recent research data, many herbal medicines have shown a beneficial effect in treating diabetes mellitus[8,9].

Coptis teeta Wall (family - *Rananculace*) is a Chinese medicinal plant commonly found in Bhutan, China, Nepal and India[10,11]. Different parts of this plant have been used for years for various ailments[12]. Traditionally, rhizomes of *C. teeta* are used in the treatment of malaria and insect bite. It is also used in treating gastrointestinal disorders like diarrhea and stomach ache[13] and also used in treating various inflammatory disorders, skin diseases, and cancer[14]. The anti-inflammatory and anti-microbial activity of *C. teeta* is due to alkaloidal phytoconstituents such as isoberberine, coptisine, berberine and palmatine present in the rhizomes[14]. *C. teeta* also contains various alkaloids like jatrorrhizine, columbamine and epiberberine. It comprises other secondary metabolites, lignans, phenylpropanoids, flavonoids, phenolic acids, saccharides and steroids[15]. However, there are no scientific reports on the antidiabetic activity of *C. teeta* roots on diabetes recorded in the literature so far. In the

[a] Department of Pharmacology, Ramanbhai Patel College of Pharmacy, CHARUSAT Campus, Changa - 388 421, Gujarat, India

[b] Department of Pharmacology, Sardar Patel College of Pharmacy, Bakrol - 388 315, Gujarat, India

[c] Department of Pharmacology, SAL Institute of Pharmacy, Ahmedabad - 380 060, Gujarat, India

*For Correspondence: E-mail: samirkshah77@gmail.com

light of the above observations, the present study was aimed to evaluate the antidiabetic activity of the *C. teeta* wall extract using experimental animals.

Procurement and authentification of plant materials

The fresh roots of *C. teeta* were collected from VHCA Herbals, Haryana, and were authenticated by Professor G.C. Jadeja, Botany Department of Agricultural University, Anand, Gujarat. The herbarium sheet was preserved at the institute.

Plant extraction[16]

The roots of *C. teeta* were shade dried, powdered, and extracted with 200 mL of 50 % ethanol. The mixture was filtered and the same process was repeated till the filtrate became colorless. All filtrates were pooled and the solvent was evaporated. The yellowish-brown colored powder extract was obtained (% yield= 7.5 % w/V). The ethanolic extract of *C. teeta* was coded as CE and used for further studies. A preliminary phytochemical investigation of CE was carried out[17,18].

Animal selection[19]

In this experimental study, 30 Wistar rats of either sex weighing 180 to 220 g were selected and housed in clean polypropylene cages. The lids of the cages were made of strong steel mesh, and were designed to contain a feed hopper and accommodation to hold drinking water bottles. The rats were maintained at 23 °C ± 2 °C under a 12/12 h/light/dark cycle. They were fed with standard rat feed and water *ad libitum*. The husk in the cages was renewed daily to ensure hygiene and provide maximum comfort to animals. The study protocol was approved by the Institutional Animal Ethical Committee and studies were carried out according to CPCSEA guidelines.

Diabetes induction and animal grouping[19]

Diabetes was induced in the overnight fasted rats by a single intraperitoneal injection of alloxan monohydrate (150 mg kg^{-1} body weight, dissolved in normal saline). Normal control animals received an equal volume of normal saline. Alloxan-injected animals were given 5 % w/V glucose in drinking water for 24 h. Hyperglycemia was confirmed by elevated serum glucose levels after 72 h of alloxan administration by the glucose oxidase-peroxidase method (GOD-POD) estimation method. The animals with serum glucose values >200 mg dL^{-1} were considered diabetic and included in the study. Rats were assigned into five groups comprising 6 rats in each. Rats in the normal control (NC) group and diabetic control (DC) received only a vehicle. Diabetic rats in (STD M) group was treated with standard metformin, 250 mg kg^{-1} (p.o.), and diabetic rats were treated with CE 250 mg kg^{-1} (p.o.) and 500 mg kg^{-1} (p.o.) in (CE 1) and (CE 2) groups, respectively. All the rats received treatment for 28 days. Several physical parameters like body weight were measured on 0, 7th, 14th, 21st, and 28th day, and food intake and fluid intake were measured on daily basis. Blood samples were withdrawn from the retro orbital` route at 0, 7th, 14th, 21st and 28th day[20]. Serum was separated and was used for the estimation of glucose[21] and insulin level was carried out[22]. Insulin was determined by RIA (Radio immuno assay). Serum separated on the 28th day was used to measure kidney parameters such as serum urea[23], serum creatinine[24] and serum protein[25].

Histopathology of kidney

At the end of 28 days of the study, the blood samples were collected and various serological parameters were measured. The rats were sacrificed and kidneys were removed and stored in 10 % formalin solution for histopathological examination. The kidney sections were stained with hematoxylin and eosin for histological studies.

Statistical analysis

All values are expressed as mean ± S.E.M. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using GRAPH PAD PRISM software version 8. The results were considered statistically significant at $p < 0.05$.

RESULTS

Preliminary phytochemical investigation

Results of preliminary phytochemical investigation of ethanolic extract of *C. teeta* showed the presence of carbohydrates, glycosides, alkaloids, saponins and flavonoids.

Effect of various treatments on physical parameters

Effect on change in body weight of rats

The changes in body weight were measured on 0, the 7th, 14th, 21st, and 28th day of the study. The body weight of diabetic rats in the DC group was significantly ($p < 0.05$) decreased as compared to the NC group. The diabetic rats treated with STD M, CE 1, and CE 2 significantly ($p < 0.05$) prevented the fall in body weight from the 21st day of treatment (Fig. 1a).

Table I: Effect of various treatments on fluid intake and food intake

GROUP	FLUID INTAKE (mL)	FOOD INTAKE (g)
NC	16.53±1.15	12.88±2.59
DC	36.48±2.30#	28.79±2.67#
STD M (250 mg kg⁻¹, p.o.)	18.43±2.07*	14.93±1.49*
CE 1 (250 mg kg⁻¹, p.o.)	25.18 ±2.3	23.01 ± 3.12
CE 2 (500 mg kg⁻¹, p.o.)	21.90 ±1.56*	19.23 ± 2.52*

NC: Normal control; DC: Diabetic control (Alloxan monohydrate 150 mg kg⁻¹, i.p.); STD M (Metformin 250 mg kg⁻¹, p.o.); CE 1 (C. teeta ethanolic extract 250 mg kg⁻¹, p.o.); CE 2 (C. teeta ethanolic extract 500 mg kg⁻¹, p.o.)

Parameters are measured on various days (day 0, day 7, day 14, day 21, and day 28). All values are expressed as Mean ± S.E.M (n=6). Statistical analysis was performed by using One Way ANOVA followed by the Dunnett t-test test.

* (P<0.05) as compared to the diabetic control group.

 # (P<0.05) as compared to the normal control group.

Effect on food intake and fluid intake of rats

Food intake and fluid intake were significantly (p<0.05) increased in the DC group as compared to the NC group. Treatment with STD M and CE 1 and CE 2 reduced the fluid intake and food intake compared to DC group animals (Table I).

Effect on blood glucose level

A marked elevation in blood glucose level was observed in DC control rats as compared to NC rats. DC rats treated with STD M, CE 1, and CE 2 exhibited a significant (p< 0.05) reduction in blood glucose levels on the 7th, 14th, 21st, and 28th day after treatment as compared to rats of the DC group (Fig. 1b).

Effect on serum insulin level

Serum insulin levels were measured in NC and all the DC groups on the 7th, 14th, 21st, and 28th day of treatment. The serum insulin level in the DC group was significantly lower (p<0.05) as compared to the NC group. DC rats treated with STD M, CE 1, and CE 2 produced a significant increase in serum insulin level as compared to diabetic animals (Fig. 1c).
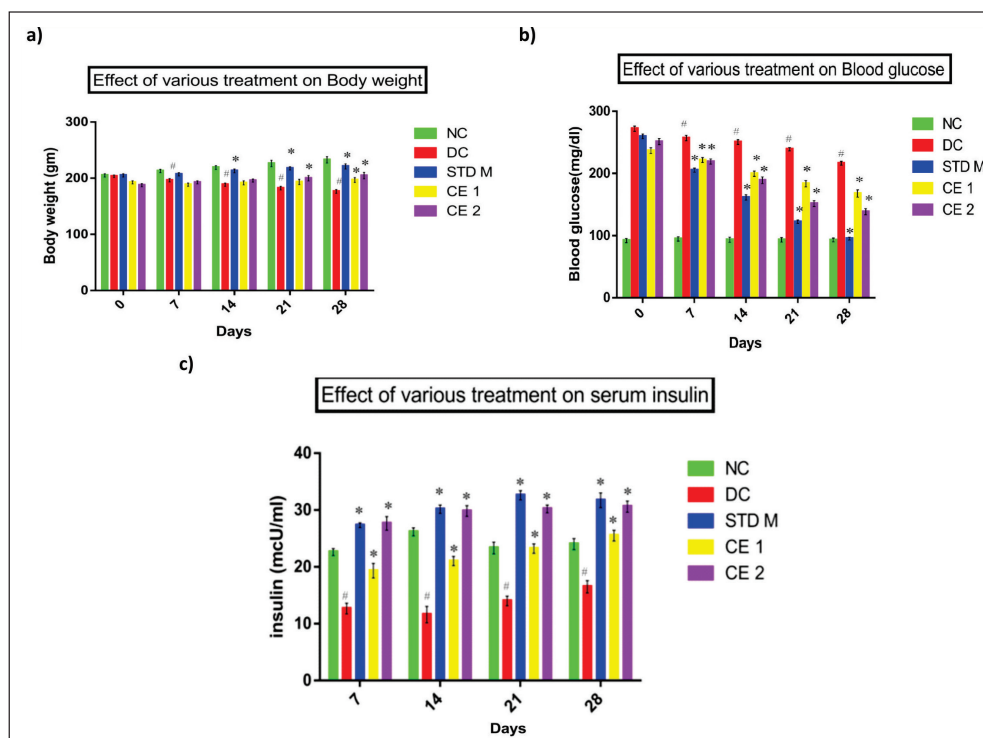
Serum kidney function parameters

CE 250 mg kg⁻¹ (p.o.) treated groups did not produce a significant change in serum urea and serum protein but serum creatinine was significantly decreased as compared to diabetic animals. A significant decrease in serum urea and creatinine levels and a significant increase in protein level was observed in animals treated with CE 500 mg kg⁻¹ and metformin (Fig. 2 a,b,c).

Histopathology of kidney

The histopathology of the renal glomerulus in the normal control group (Fig. 3a) shows the normal size of the glomerulus and Bowman capsule. The proximal convoluted tubules, macula densa and juxtaglomerular cells are identified in the histology. Tubular epithelial
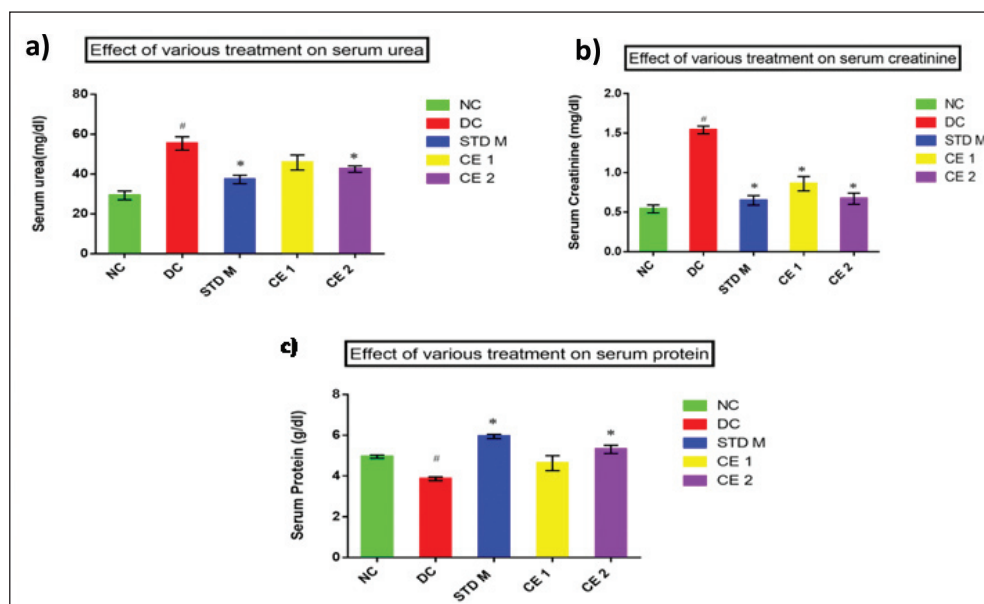

Fig. 1: Analysis of various parameters a) Change in body weight, b) Blood glucose level, c) Serum insulin level

NC: Normal control; DC: Diabetic Control (Alloxan monohydrate 150 mg kg⁻¹, i.p.); STD M (Metformin 250 mg kg⁻¹, p.o.); CE1 (C. teeta ethanolic extract 250 mg kg⁻¹, p.o.); CE 2 (C. teeta ethanolic extract 500 mg kg⁻¹, p.o.). Parameters are measured on various days (day 0, day 7, day 14, day 21, and day 28).

All Values are expressed as Mean ± S.E.M (n=6). Statistical analysis was performed by using One Way ANOVA followed by the Dunnett t-test test. * (P<0.05) as compared to the diabetic control group. # (P<0.05) as compared to the normal control group

Fig. 2: Effect of treatment on various serum parameters. a) Serum urea level b) Serum creatinine level c) Serum protein level

NC: Normal control; DC: Diabetic control (Alloxan monohydrate 150 mg kg^{-1}, i.p.); STD M (Metformin 250 mg kg^{-1}, p.o.); CE1 (C. teeta ethanolic extract 250 mg kg^{-1}, p.o.); CE 2 (C. teeta ethanolic extract 500 mg kg^{-1}, p.o.).

*All values are expressed as Mean ± S.E.M (n=6). Statistical analysis was performed by using One Way ANOVA followed by the Dunnett t-test test. * (P<0.05) as compared to the model control group. # (P<0.05) as compared to the normal control group.*
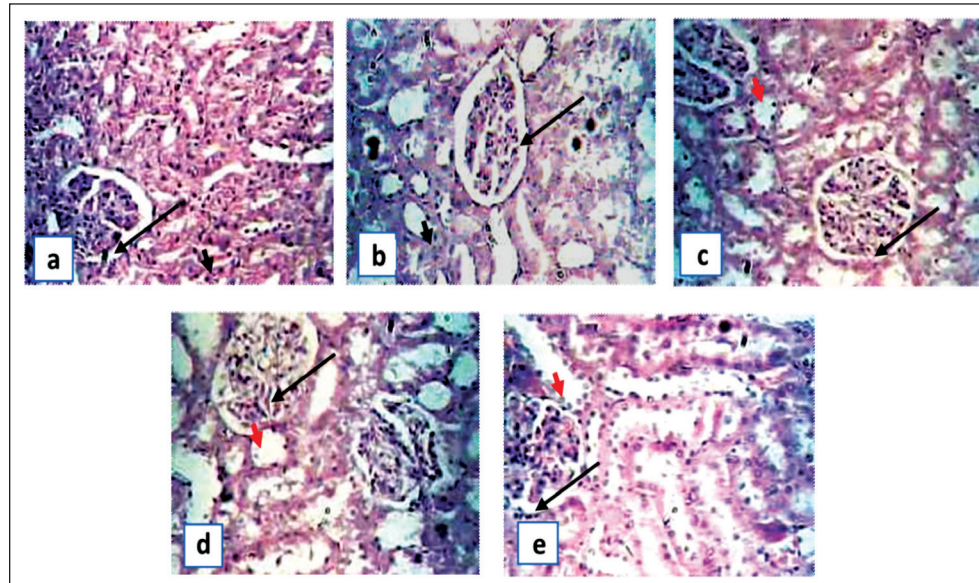

Fig. 3: Histopathology of the kidney

a= NC: Normal Glomerulus (long arrow) and intact tubules (short black arrows). b=DC: Shows damaged glomerulus (long arrow) and tubular showing necrosis (short black arrows). c= STD M: Glomerulus, podocytes, and juxtaglomerular cells are seen. Macula densa has been seen intact (short red arrow). d and e= CE 1 and CE 2 groups show the repaired damaged glomerulus (Long arrow) and Bowman capsule as compared to the diabetic group. Macula densa (short red arrow) and cells with nuclei are observed showing the efficacy of CE in treating diabetes mellitus.

NC: Normal control; DC: Diabetic Control (Alloxan monohydrate 150 mg kg^{-1}, i.p.); STD M (Metformin 250 mg kg^{-1}, p.o.); CE 1 (C. teeta ethanolic extract 250 mg kg^{-1}, p.o.); CE 2 (C. teeta ethanolic extract 500 mg kg^{-1}, p.o.).

cells are seen with clear cell nuclei. Alloxan-induced diabetic rats show damaged glomerulus and Bowman capsules (Fig. 3b). The necrosis of cells and tubules at some places is observed in the kidneys of diabetic rats. The standard metformin improved the damaged glomerulus, tubules and showed improvement in cell and tissue architecture (Fig. 3c). Treatment with 250 mg kg^{-1} and 500 mg kg^{-1} of CE dose-dependently improved the damaged glomerulus, Bowman capsules, and tubules. The cells with centered nuclei are seen which can be seen in the normal control group. Macula densa and juxtaglomerular cells are observed in the CE 1 and CE 2 groups (Fig 3d and 3e). CE 1 and CE 2 repaired the kidney damage showing their positive role in treating diabetes.

DISCUSSION

Diabetes mellitus is not a single disorder but it is a group of metabolic disorders and its prevalence is increasing. Hyperglycemia is mainly due to less secretion of insulin from the pancreas[26]. Alloxan is used in inducing Type 1 diabetes in this study. The mechanism behind causing diabetes is the accumulation of alloxan in the β-cells of the pancreas and is toxic to those cells, leading to inadequate secretion of insulin and causing hyperglycemia. Alloxan is a glucose analog and it is toxic to β-cells due to the production of ROS[27].

Alloxan is accumulated in β-cells of the pancreas

through the uptake by the GLUT2 glucose transporter and further breakdown of fatty acids to ketone bodies[28]. The destruction of β-cells leads to an imbalance in glucose metabolism producing glycogenolysis, gluconeogenesis, lipolysis and a rise in all of this leads to weight loss[29]. Results of the present study supported the fact that diabetic rats showed a decrease in body weight. Oral administration of STD M, CE 1, and CE 2 to the diabetic rats significantly (p<0.05) forbade the fall in body weight. This indicates that STD M, CE 1, and CE 2 prevented muscle tissue damage in diabetes. Elevated body weight can be related to the improvement in insulin secretion and glycemic control. A similar kind of effect i.e. body weight gain, was reported with many other plants, such as *Aavaraiyathi churnam,* well known for their antidiabetic activity[30].

Alloxan (150 mg kg^{-1}, i.p.) in adult rats produces some Type 1 diabetes symptoms like loss of body weight, polydipsia, and polyphagia. The body weight loss in diabetic animals could be related to insulin deficiency. Insulin deficiency not only affects glucose metabolism but also affects protein and fat metabolism[31]. Ethanolic extract of mulberry fruit abolished the increase in water intake and food intake showing its action against polyphagia and polydipsia produced by alloxan[32]. Similar results are observed in the present study, data suggested a significant increase in fluid intake and food intake in alloxan-induced diabetic rats as compared to normal control rats. Treatment with STD M, CE 1 and CE 2 significantly reversed this condition. Thus, treatment with STD M, CE 1 and CE 2 has a beneficial effect in improving polydipsia and polyphagia in diabetic rats.

Alloxan destroys pancreatic β-cells, leading to insufficient secretion of insulin and causing increased serum glucose level and reduced serum insulin level[33]. Previous studies involving herbal drugs indicated that the antidiabetic activity could be due to an increase in the secretion of insulin from β-cells, increased regeneration of β-cells, and or increased utilization of glucose in the peripheral tissue. Studies conducted in the past indicated that herbs such as *Viscum album* and *Morinda lucida* can exert an insulin-like glucose utilization effect in the peripheral tissue[34,35]. In the present study, serum glucose was increased in rats in the 2nd, 3rd, 4th and 5th groups because of alloxan administration that was significantly higher than in the normal group. On the 28th day, this level was significantly decreased in treated groups. Metformin (250 mg kg^{-1}) brought the values to normal. Reduction in serum glucose level and rise in serum insulin level may be due to the regeneration of β-cells or enhanced insulin secretion and action. Alloxan lowered the serum insulin level significantly. Similar results

are obtained in the recent studies on DPP-4 enzyme inhibition with naringin (a naturally occurring compound present in orange peel) and *Berberis aristata* root extract, suggesting that inhibition of the DPP-4 enzyme increases the serum insulin level[16,36].

A significant rise in serum urea and creatinine levels and a significant decline in protein levels indicated impaired renal function in diabetic animals. The result of the present study indicated that CE (500 mg kg^{-1}, p.o) extract significantly reduced the levels of serum urea and creatinine and elevated the levels of serum protein, showing the protective effect of CE on kidney, liver, and pancreas, thereby reducing the caution of diabetes in experimental animals. Thus, treatment with CE prevented an increase in serum urea and creatinine levels and increased serum protein levels, indicating its beneficial effect on kidney function in diabetic conditions.

Alloxan destroys the pancreatic β-cells producing insulin and it also damages the kidney and shows damaged glomerulus and Bowman capsule in histopathology of the kidney. The alloxan administration destroys cell architecture in kidneys are stated by some earlier research work by Ghosh et al[37]. Similar kind of results was observed in alloxan-induced diabetes in rats in the DC group. Raut et al. stated that polyurea is a common symptom observed in diabetes mellitus and that is due to the structural changes in renal tubules and glomerulus[38]. The recovery in renal function can be observed by decreasing the renal damage and in the present study, CE 1 and CE 2 improved the renal function and can be histologically proven by the intact architecture of glomerulus, Bowman capsule, macula densa and juxtaglomerular cells. Similar results are observed by the administration of seed powder of *Trigonella foenum graecum*[39]. The recent research work showed the improvement in renal function depicting its efficacy in treating diabetes and proving its antidiabetic activity.

Plants contain various phytoconstituents like alkaloids, terpenoids, flavonoids, glycosides, etc. which have an anti-diabetic effect. Plants like *Berberis aristata,* and *Cocsnium fenestratum,* which have berberine as a major chemical constituent was reported to have antihyperglycemic activity[16,40]. Even *C. teeta* contains various phytoconstituents like berberine, isoberberine coptisine, palmatine, etc and it may be possible that the antidiabetic activity of CE may be due to all these phytoconstituents.

CONCLUSION

The findings of the present study show that ethanolic extract of *C. teeta* (CE) possesses antidiabetic properties as it lowers blood glucose levels and improved symptoms of Type 1 diabetes like polyphagia and polydipsia. We also showed that CE also protected the kidney and pancreas in alloxan-induced diabetes. However, a further detailed investigation is necessary to determine the exact phytoconstituents, which are responsible for the antidiabetic effect.

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