

## ORIGINAL ARTICLE

# Antidiabetic effect of *Cephalandra indica* Q in diabetic rats

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### ABSTRACT

**Background:** Diabetes mellitus is an emerging global epidemic, taking its toll in developing countries as India. Presently, the treatment for diabetes using allopathic drugs gives only a temporary relief. A combination of right medicine and potency, on other hand has given Homoeopathy a high success rate in the treatment of this disease.

**Objective:** The aim of this study was to investigate the effects of *Cephalandra indica* mother tincture and potencies on blood glucose level, cholesterol level, body weight, and beta-cells of pancreatic islets of Langerhans, in streptozotocin (STZ)-induced diabetic Wistar rats.

**Material and Methods:** Glucose uptake was monitored in mother tincture-treated mouse fibroblast cell line. Diabetes mellitus was induced by intraperitoneal injection of STZ (55 mg/kg body weight) in adult male rats. After three days of injection, diabetic rats received mother tincture orally (750 µL/kg body weight) daily for three weeks.

**Results:** There was a significant reduction of blood glucose level, regain of body weight, and regeneration of beta-cells in the pancreas of the mother tincture-treated rats. Mother tincture-treated 3T3 cells also showed reduced uptake of glucose in comparison to normal cells.

**Conclusion:** The present study clearly indicates a significant antidiabetic effect of *Cephalandra indica* and lends support for its usage as a homoeopathic medicine.

**Keywords:** Antidiabetic, *Cephalandra indica* mother tincture, Diabetes mellitus, Wistar rat

### INTRODUCTION

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycaemia arising as a consequence of relative or absolute deficiency of insulin secretion, resistance to insulin action, or both. The International Diabetes Federation has estimated that the total number of cases of diabetes would be about 350 million by 2030.<sup>[1]</sup> Hyperglycaemic conditions result in increased glycosylation. This leads further to biochemical abnormalities due to altered protein structure which over a period of time develops into diabetic complications such as nephropathy,

retinopathy, neuropathy, and cardiomyopathy.<sup>[2]</sup> Pharmacotherapies for the treatment of diabetics include oral hypoglycaemic agents and insulin. These are however, not free from side effects.<sup>[3]</sup> The World Health Organization (WHO) has recommended the evaluation of traditional plants used for the treatment of diabetics as they are effective, nontoxic with fewer or no side effects, and are considered to be excellent candidates for oral therapy.<sup>[4]</sup> There are also several reviews on medicinal plants possessing antidiabetic activity that have been used as traditional medicines.<sup>[5,6]</sup>

The present study was carried out to evaluate

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the antidiabetic activity of *Cephalandra indica* [mother tincture (MT) and potencies] belonging to the family of Cucurbitaceae. The medicine is used in biliousness, bilious complaints, poisonous boils, abscesses, carbuncles, blood poisoning, inflammation, and glandular swelling of the neck. *Cephalandra indica* has been acknowledged for its wound healing ability.<sup>[7]</sup> It also finds use in the traditional system of Ayurveda to treat diabetes, jaundice and dysentery.

## MATERIAL AND METHODS

### Sources of Reagents, Medicines, Model Animals, and Cell Lines

All chemicals and reagents were purchased from Sigma-Aldrich, unless mentioned otherwise. *Cephalandra indica* MT and potencies (6C, 24X, 12C and 30C) were brought from Hahnemann Publishing Company Private Ltd. (HAPCO), Kolkata. Healthy male Wistar rats were procured from M/s SC Ghosh, Kolkata. The rats were housed in polypropylene cages and were maintained in ventilated caging (Citizen Industries Ltd., India) conditions (temperature:  $22 \pm 2^\circ \text{C}$ , relative humidity:  $57 \pm 10\%$ , and 12:12 light: dark cycle) at the Indian Institute of Technology, Kharagpur. The rats were put on a standard diet and had free access to water. The experiments were performed after approval of the protocol by the Institutional Animal Ethics Committee (IAEC) and were carried out in accordance with the current guidelines for the care of laboratory animals. Mouse fibroblast 3T3 cells were purchased from the National Centre for Cell Science, Pune, India. The cells were maintained at the Indian Institute of Technology, Kharagpur. The experiments were also conducted in the Indian Institute of Technology, Kharagpur from September 2009 to December 2011.

### Design of Animal Experiments

#### Induction of diabetes mellitus

Diabetes mellitus was induced in healthy male Wistar albino rats by a single intraperitoneal injection of freshly prepared streptozotocin (STZ; in 0.1 M citrate buffer pH 4.5) at a dose of 55 mg/kg body weight. After three days of STZ injection, animals with fasting blood glucose above 400 mg/dL were considered as diabetic and included in the study. After three days of injection, diabetic rats received MT/potencies orally (750  $\mu\text{L}/\text{kg}$  body weight) daily for three weeks.

#### Recording of body weight, blood glucose, and cholesterol

The animals were randomly assigned into following groups and given the following treatments:

Group I: Normal control with citrate buffer

Group II: Placebo control (40% ethanol)

Group III: Normal rats with MT

Group IV: Diabetic control with 40% ethanol

Group V: Diabetic negative control without medicine

Group VI: Diabetic positive control with glibenclamide

Group VII: Diabetic rat with MT (75  $\mu\text{L}/100 \text{ g}$  body weight)

Group VIII: Diabetic rat with 6C ('C' = Centesimal Potency)

Group IX: Diabetic rat with 24X ('X' = Decimal Potency)

Group X: Diabetic rat with 12C

Group XI: Diabetic rat with 30C

Doses: Glibenclamide - 1mg/ kg body weight. 40% ethanol, MT, 6C, 24X, 12C and 30C - 75  $\mu\text{L}/100 \text{ g}$  body weight. Data were recorded at the end of each week for four weeks.

MT, placebo, and glibenclamide were administered orally. Body weight, cholesterol, and blood glucose analyses were done weekly on overnight fasted animals. At the end of the experimental period, the animals were sacrificed by cervical decapitation. Organs like liver and pancreas were dissected, immediately rinsed in liquid nitrogen, and stored at  $-80^\circ \text{C}$  for further studies.

#### Determination of blood glucose and cholesterol

Blood samples were taken from the tail vein [according to Guideline 9 (3/10/99) of the Institutional Animal Care and Use Committee (IACUC)] before oral administration of MT or the vehicle. Twenty-two microliters of blood were used for each assay; the glucose concentration was measured in plasma serum with a glucometer (Accu-Chek Compact, Roche). Cholesterol estimations were done spectrophotometrically (Chemito Technologies, India) using standard kits (Merck, India). Additional analyses were done for cross-checking using Transasia semiautomated analyzer.

#### Liver and pancreatic biopsy

Tissue specimens of the liver and pancreas of STZ-induced treated and untreated rats (stored at  $-80^\circ \text{C}$ ) were fixed in Bouin's fluid for 24 hours and dehydrated through alcohol gradient series up to absolute ethanol. These were transferred to xylene and saturated with wax overnight for block preparation.

Microsections were cut and mounted on clean slides at 37°C. The sections were deparaffinized by two changes in xylene, 10 minutes each time. The sections were rehydrated by two changes in absolute ethanol (5 minutes each) followed by passage through 95% and 70% ethanol for two minutes each time. Finally, these were washed in distilled water. The sections were stained in Mayer's hematoxylin solution (0.1%) for eight minutes. The stained sections were washed in running tap water and finally rinsed in distilled water. They were counterstained in 1% Eosin Y solution for 45 seconds. The double-stained sections were finally mounted in di-n-butylphthalate-polystyrene-xylene (DPX) mounting medium after following reverse dehydration up to 100% ethanol. The sections were photographed by an Olympus microscope, with magnification ×400.

#### Glucose uptake assay

The 3T3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, USA) with 15 mM HEPES [HEPES: (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)] buffer supplemented with 10% heat-inactivated (30 minutes, 56°C) fetal bovine serum (FBS) (Invitrogen) and antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin, HiMedia). They were incubated in a 5% carbon dioxide (CO<sub>2</sub>) atmosphere incubator (Heracell 150i, Thermo Fisher Scientific Inc., USA) at 37°C. Subculturing was done after the cells reached confluence level by detaching cells from flasks using 0.25% trypsin-0.02% ethylenediaminetetraacetic acid (EDTA) solution in a 1:4 splitting ratio. The cell viability was measured using trypan blue (0.4%) dye exclusion method and cells with >95% viability were used for further experiments.

The 3T3 cells were incubated in serum-free medium for a short period and then incubated in phosphate-buffered saline (PBS)-containing insulin. After washing, the cells were incubated in PBS containing 2-deoxyglucose and 2 mCi/mL 2-deoxy-D-[3H/14C] glucose. 3H-glucose uptake was detected using liquid scintillation counter (Beckman Coulter, USA). About 5 × 10<sup>6</sup> cells were taken in microcentrifuge tubes. The cells were centrifuged at 250 g for five minutes. The supernatant was discarded and 1 mL assay buffer (37°C) was added and recentrifuged to wash the cells. This step was repeated thrice. Then 14C glucose was added into the reaction mixture and incubated for 20 minutes at 37°C. The reaction mixture was vacuum-filtered with

0.1 µm porosity nitrocellulose filter membrane disc. Then, 3 mL of ice cold assay buffer, 1 mL at a time, was added to wash by vacuum filtration. Thereafter, 3 mL ice-cold 5% trichloroacetic acid (TCA), 1 mL at a time, was added to the filtration device and filtered. After the liquid from the disc was totally filtrated, the filter disc was dried in room temperature. The filter discs were put in a liquid scintillation counting (LSC) vial containing 3 mL of LSC cocktail and the β-count was taken.

#### Statistical Analysis

Each data point represents the mean and standard deviation of three samples. Analysis was performed using a Student's *t*-test designed by GraphPad Software QuickCalcs. The significance and *P* values are indicated in data tables.

#### Metabolite Profiling

Phenolics and terpenoids were isolated from MT as mentioned elsewhere.<sup>[8]</sup> MT was extracted with methanol (1:4 w/v). The extract was filtered and dried to concentrate. The extract was then acidified with 2N H<sub>2</sub>SO<sub>4</sub> and further extracted with three volumes of chloroform. The chloroform extract was dried and used for analysis by High Performance Thin Layer Chromatography (HPTLC) and liquid chromatography–mass spectrometry (LC-MS).

#### Identification of Phenolics by HPTLC

The extracts were subjected to HPTLC<sup>[9]</sup> on a Linomat 5 (Camag, Switzerland) system using toluene: ethyl acetate: acetic acid (3:1:1) as the mobile phase. TLC silica gel 60 F254 plates (Merck, Germany) with fluorescent indicator (254 nm) were used for normal-phase HPTLC and R<sub>f</sub> value determination. Each time, 20 µl of the sample was spotted on the silica gel-coated plates.

#### LC-MS/MS analyses of extracted phenolics

LC-MS/MS analyses were performed<sup>[10]</sup> using a Waters 2695 separation module coupled with Quattro microTM API mass spectrometer (Waters, USA). The liquid chromatographic (LC) system equipped with quaternary pump, on-line vacuum degasser, autosampler and thermostatic column compartment was connected to a photo diode array (PDA) detector (model no: Waters 2998). Data acquisition and analyses were carried out in Waters MassLynx 4.1 software. The sample (10 µL) was injected into the LC system by the autosampler and separation was performed on a XTerra MS C18 reversed-phase column (2.1 × 100 mm

Internal Diameter (ID), 5  $\mu$ m particle size, Waters). The mobile phase comprised 0.1% aqueous formic acid and acetonitrile 0.3 mL/minute, and column temperature was maintained at 25°C. All the compounds were detected within the range of 230-360 nm in the PDA detector.

The LC-eluted samples were introduced into the electrospray ionization (ESI) source in a postcolumn splitting ratio of 3:1. An ES source with negative ionization mode (source block temperature: 130°C, desolvation temperature: 300°C, capillary voltage: 3 kV, cone voltage: 30 V) was used for mass analysis. The desolvation and cone gas were 650 and 50 L h<sup>-1</sup>, respectively. The data were recorded in the MS scanning mode with a scan range of 100-600 (m/z); the scan time was 0.5 seconds, and interscan delay time was 0.1 seconds.

## RESULTS

### Evaluation of Blood Glucose Levels

At the end of four weeks, blood glucose level for the MT-treated animals was found to be 269 mg/dL, whereas rats treated with glibenclamide showed a blood glucose concentration of 278 mg/dL [Table 1]. In comparison to diabetic control animals (blood glucose level 486 mg/dL), this is a significant reduction. MT therefore compares very well with glibenclamide in therapeutic effect. For all the potencies, the blood glucose levels remained significantly higher (in the range of 390-485 mg/dL) and were therefore not effective antidiabetically.

### Variations in Body Weight

The body weight of the rats were measured every week up to four weeks. Variations in body weight

**Table 1: Measure of blood glucose level in diabetic rats**

Group (#)	Before treatment (mg/dL)	1 <sup>st</sup> week (mg/dL)	2 <sup>nd</sup> week (mg/dL)	3 <sup>rd</sup> week (mg/dL)	4 <sup>th</sup> week (mg/dL)
Normal control	86±4.2	88±3.3	86±4.4	88±2.1	87±4.2
Placebo control	78±3.3	81±4.2	79±3.4	85±5.2	86±2.1
Normal rat with MT	89±2.2	90±3.2	89±5.5	91±4.2	91±4.2
Diabetic control with ethanol	95±7.2	487±11.2	486±13.2	398±10.3	486±10.7
Diabetic control without medicine	93±11.1	487±18.7*	483±13.8*	482±16.2*	479±13.1*
Diabetic positive control with glibenclamide	90±5.2	369±11.6	272±15.6*	276±13.1*	278±10.2*
Diabetic with MT	91±6.2	216±11.5*	227±11.4*	264±9.2*	269±9.4*
Diabetic with 6C	87±2.2	385±21.3	384±11.2	481±11.9	485±10.2
Diabetic with 24X	91±1.9	389±15.2	387±12.5	392±17.2	384±13.2
Diabetic with 12C	92±1.7	391±21.4	387±12.2	389±12.4	390±14.7
Diabetic with 30C	94±4.3	387±13.4	382±14.2	401±11.8	397±17.2

(#) Each group with six rats as replica, Values have been rounded to the nearest integer; Data are mean of six replicates±standard error (SE), \*The result obtained from the above test for MT and glibenclamide were found to be statistically significant ( $P<0.05$ ) compared to that of diabetic control without medicine, MT: Mother tincture

**Table 2: Measure of body weight in diabetic rats**

Group (#)	Before treatment (g)	1 <sup>st</sup> week (g)	2 <sup>nd</sup> week (g)	3 <sup>rd</sup> week (g)	4 <sup>th</sup> week (g)
Normal control	116±1.2	118±1.3	116±1.4	118±1.1	117±1.2
Placebo control	112±1.3	113±1.2	112±1.4	112±1.2	116±1.1
Normal rat with MT	109±1.2	110±1.2	109±1.5	111±1.2	111±1.2
Diabetic control with ethanol	115±1.2	87±1.2	86±1.2	85±1.3	86±1.7
Diabetic control without medicine	113±1.1	87±1.7	83±1.8	82±1.9	79±1.9
Diabetic positive control with glibenclamide	110±1.2	69±1.6*	72±1.6*	76±1.2*	78±1.2*
Diabetic with MT	111±1.2	116±1.5*	117±1.4*	114±1.2*	119±1.4*
Diabetic with 6C	110±1.2	85±1.3	84±1.2	81±1.9	85±1.2
Diabetic with 24X	111±1.2	89±1.2	87±1.5	92±1.2	84±1.2
Diabetic with 12C	112±1.2	91±1.4	87±1.2	89±1.4	90±1.7
Diabetic with 30C	114±1.2	87±1.4	88±1.2	91±1.8	97±1.2

(#) Each group with six rats as replica, Values have been rounded to nearest integer; data are mean of six replicates±standard error (SE), \*The result obtained from the above test for MT was found to be statistically significant ( $P<0.05$ ) compared to that of diabetic positive control with glibenclamide, MT: Mother tincture

are presented in Table 2. In the treated animals, MT resulted in regain in body weight to normalcy. The glibenclamide-treated animals lost body weight to the extent of 30%.

### Evaluation of Serum Cholesterol

The serum cholesterol levels were not altered significantly between treatment groups and control. They all ranged within 183-234 mg/dL [Table 3].

### Liver and Pancreatic Biopsy of Normal, Diabetic, and MT-Treated Rats

Tissue biopsy of treated and untreated rats showed partial recovery of liver and islets of Langerhans for MT-treated STZ-induced diabetic rats. Figure 1 shows the liver of normal rat with a prominent central vein and symmetrical spoke-wheel appearance of other veins. Figure 2 shows deformed liver of STZ-induced

diabetic rats. However, liver biopsies of MT-treated diabetic rats showed a partial recovery [Figure 3]. Histopathology of normal rat pancreas is shown in Figure 4. Pancreatic biopsy showed no recovery of beta cells of the islets of Langerhans and interlobular duct in untreated diabetic animals [Figure 5] whereas MT-treated diabetic rats [Figure 6] showed maximum recovery.

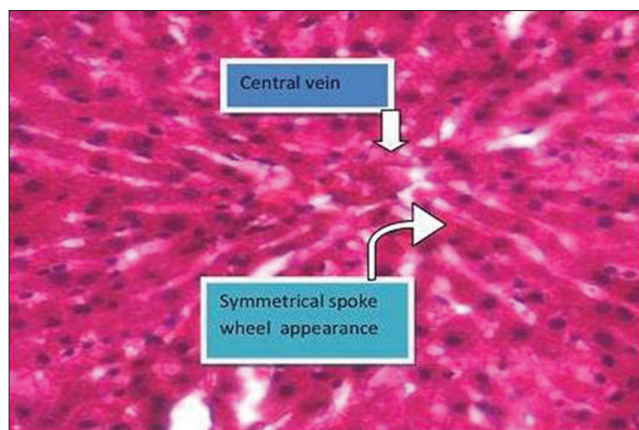
### Inhibition of Glucose Uptake in MT-Treated 3T3 Cells

Experiments with radiolabeled glucose in cultured 3T3 cell line are presented in Table 4 and Figures 7-9 show that both glibenclamide and MT showed reduced count per minute (CPM), indicating reduced glucose uptake in comparison to dimethyl sulfoxide (DMSO)-treated control.

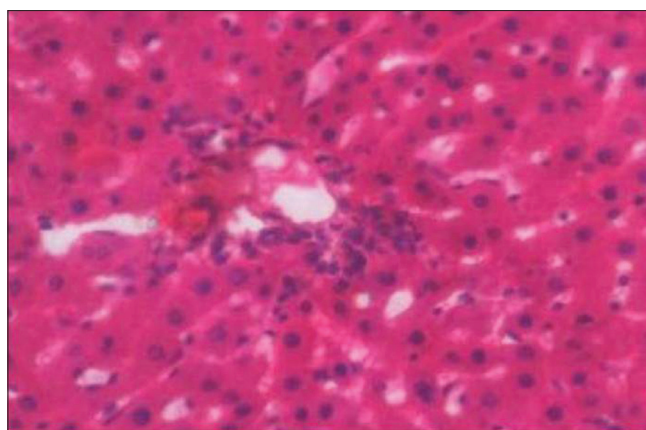
**Table 3: Measure of blood cholesterol level in diabetic rats**

Group (#)	Before treatment (mg/dL)	1 <sup>st</sup> week (mg/dL)	2 <sup>nd</sup> week (mg/dL)	3 <sup>rd</sup> week (mg/dL)	4 <sup>th</sup> week (mg/dL)
Normal control	186±4.2	188±3.3	186±4.4	158±2.1	187±4.2
Placebo	178±8.3	181±4.2	179±3.4	185±5.2	186±2.1
Normal rat with MT	189±5.2	190±3.2	189±5.5	191±4.2	201±4.2
Diabetic control with ethanol	195±7.2	207±11.2	186±13.2	198±10.3	196±10.7
Diabetic control without medicine	193±11.1	197±18.7	193±13.1	182±16.2	199±13.1
Diabetic positive control with glibenclamide	190±5.2	219±11.6*	202±11.6*	196±13.1	198±10.2
Diabetic with MT	211±6.2	196±11.5*	187±11.4*	184±9.2	199±9.4
Diabetic with 6C	187±5.2	215±11.3	214±11.2	211±11.9	215±14.2
Diabetic with 24X	191±7.9	199±15.4	217±17.5	212±17.2	214±13.4
Diabetic with 12C	202±8.7	225±21.4	213±12.6	219±12.4	190±15.7
Diabetic with 30C	194±6.2	197±12.4	208±16.2	211±11.1	217±17.2

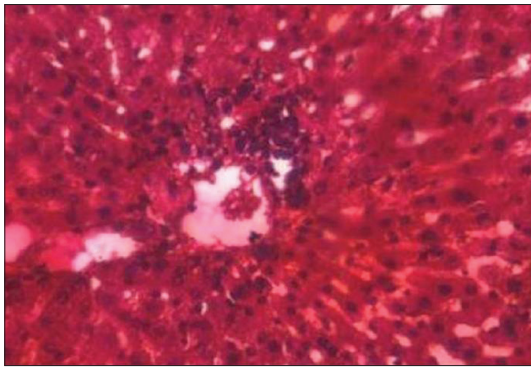
(#) Each group with six rats as replica, Values have been rounded to nearest integer; data are mean of six replicates±standard error (SE), \*The result obtained from the above test for MT was found to be statistically significant ( $P<0.05$ ) compared to that of glibenclamide, MT: Mother tincture



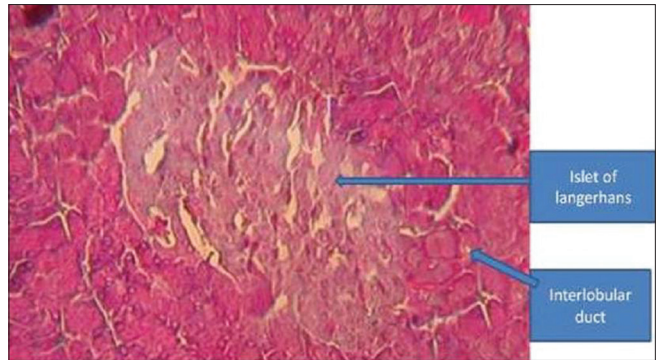
**Figure 1:** Simple microscopic image (×400) of rat liver control, stained with eosin



**Figure 2:** Simple microscopic image (×400) of streptozotocin-treated diabetic rat liver; central vein and spoke-wheel appearance altered



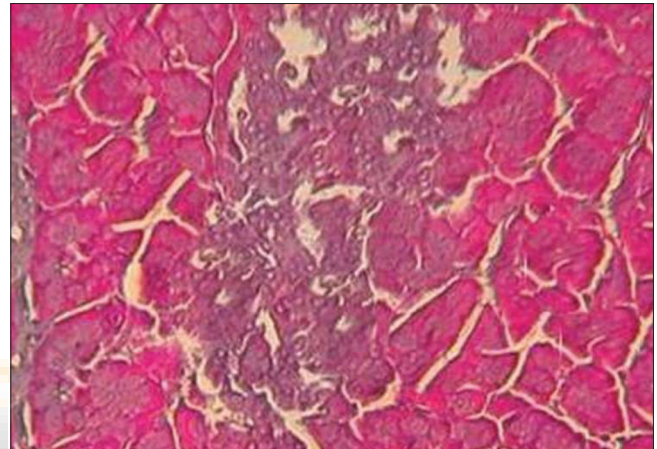
**Figure 3:** Simple microscopic image ( $\times 400$ ) of MT-treated streptozotocin-induced diabetic rat liver, showing partial recovery



**Figure 4:** Histopathology of normal rat pancreas



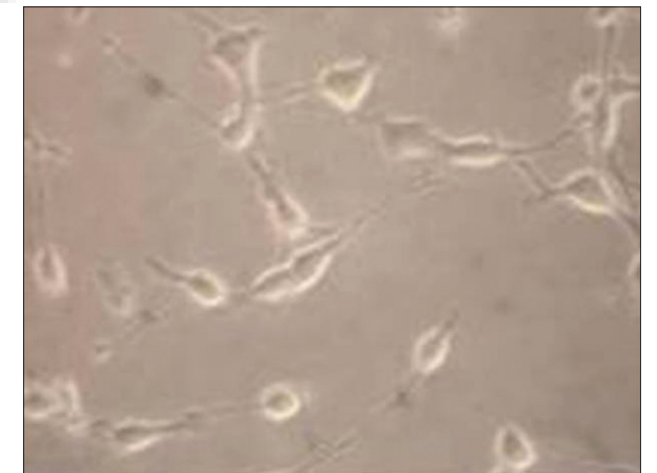
**Figure 5:** Histopathology of pancreas of streptozotocin-treated diabetic rats showing altered islets of Langerhans and interlobular duct



**Figure 6:** Pancreas of streptozotocin-treated rats also treated with MT; picture shows partial recovery of the islets of Langerhans and interlobular duct



**Figure 7:** Blank 3T3 cells treated with radioactive glucose



**Figure 8:** 3T3 cells treated with glibenclamide with radioactive glucose

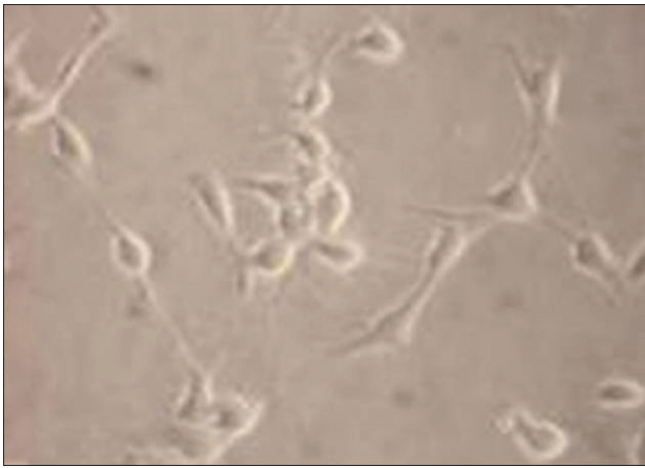
### Metabolite Profiling

The HPTLC and LC-MS/MS-based phytochemical analyses revealed [Figures 10-12] the presence of phenolics protocatechuic acid (154.9), gallic acid (171.1), syringic acid (198.3), p-coumaric acid derivatives (205.1), and gallocatechin (305.1), with their

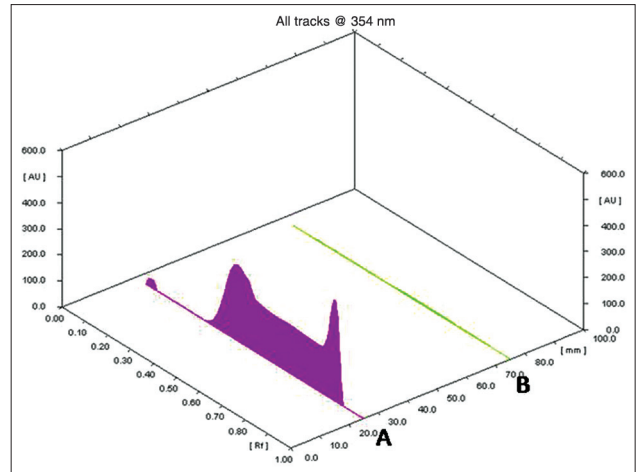
corresponding mass, as indicated within parentheses, in MT but not detectable in the potencies.

LC-MS/MS-analyzed phenolics in MT (m/z values in parentheses) are as follows:

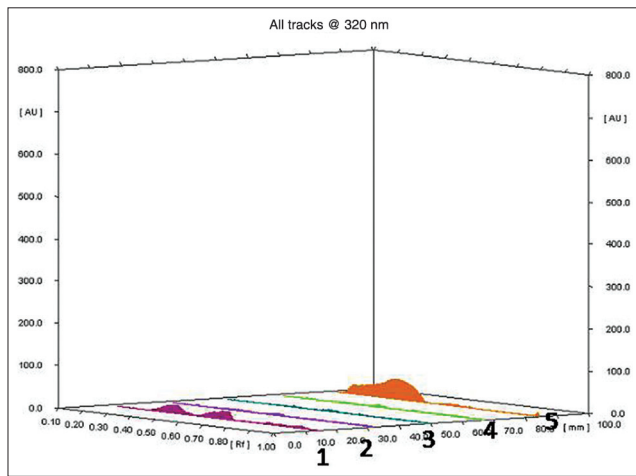
Protocatechuic acid (154.9)  
Gallic acid (171.1)



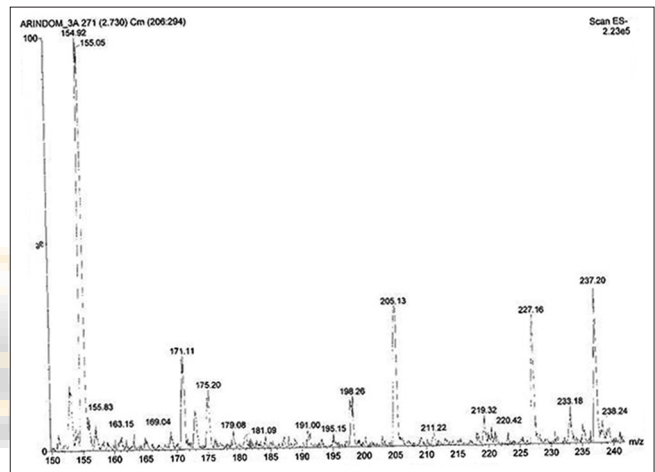
**Figure 9:** 3T3 cells treated with mother tincture with radioactive glucose



**Figure 10:** High-performance thin-layer chromatography (HPTLC) profile of A) mother tincture and B) ×24 dilution



**Figure 11:** High-performance thin-layer chromatography (HPTLC) of *Cephalandra indica* mother tincture (1), 6C (2), 12C (3), 30C (4), and standard phenolics (5)



**Figure 12:** Liquid chromatography–mass spectrometry (LC-MS) profile of high-performance thin-layer chromatography (HPTLC) separated phenolics from mother tincture.

**Table 4: Reduced glucose uptake in MT-treated 3T3 cells**

Experiment details	CPM
Only DMSO+ <sup>14</sup> C glucose	5755±171
Glibenclamide (100 µM) in DMSO+ <sup>14</sup> C glucose	663±33
10 µL MT+ <sup>14</sup> C glucose	4110±89

Data are mean of 3 replicates±SE, MT: Mother tincture; DMSO: Dimethyl sulfoxide, CPM: Count per minute

Syringic acid (198.3)  
 p-coumaric acid derivative (205.1)  
 Galocatechin (305.1)  
 Unidentified mass values 140.8; 386.9; 227.1  
 The fresh leaf extract had many more numbers of known and unidentified phenolics (data not presented).

**DISCUSSION**

This investigation revealed that *Cephalandra indica*

MT is a potential antidiabetic medicine. The testing of biological activities *in vivo* in rats and *in vitro* in 3T3 cell lines has indicated that *Cephalandra indica* MT reduced the blood sugar level in diabetic rats significantly and inhibited the uptake of radioactive glucose, respectively. The partial recovery of altered islets of Langerhans and interlobular ducts in the pancreas of STZ and *Cephalandra indica* MT-treated rats also support this observation.

Evaluation of antidiabetic activity of *Cephalandra indica* MT was conducted in STZ-induced diabetic rats. The blood glucose level for diabetic rats untreated with any drug reached up to ~485 mg/dL at the end of the fourth week, whereas diabetic rats treated with glibenclamide at a concentration of 1mg/kg body weight showed a blood glucose concentration of ~278 mg/dL. The oral administration of MT for 28

days (75 µL/100 g) showed a much lower glucose (~269 mg/dL) at the end of the fourth week. The characteristic loss of body weight associated with diabetes is due to increased muscle wasting or loss of muscle proteins due to hyperglycaemia.<sup>[11,12]</sup>

The body weight of diabetic rats (secondary complication resulting from diabetes) treated with MT also did not show much variation, whereas those of untreated and glibenclamide-treated rats decreased considerably, suggesting that MT has a protective role against muscle wasting. The body weight of diabetic untreated rats decreased from ~115 to ~86 g, whereas those of glibenclamide-treated rats decreased from ~110 to ~78 g. Reports on the change in body weight in diabetic rats treated with glibenclamide and the extracts of antihyperglycaemic herbs varied widely. In sandalwood oil-treated rats, the weight gain was 59.2% above normal in rats treated with vehicles, whereas glibenclamide-treated rats recovered weight to the extent of 3.5% below normal.<sup>[13]</sup> In an investigation, diabetic rats treated with *Momordica charantia* (bitter melon, karela) showed an increase of 4–6% of body weight over control but no net gain of body weight was observed for glibenclamide-treated ones.<sup>[14]</sup> While comparing hypoglycemic activities of *Syzygium cumini* (jamun) and *Tinospora cordifolia* (giloi) to those of glibenclamide, all animals regained body weight to the extent of 12% above normal rats.<sup>[15,16]</sup> In fenugreek-treated rats, there was no change in body weight.<sup>[17]</sup>

Liver and pancreatic biopsy of the MT-treated rats showed partial recovery in comparison to untreated and glibenclamide-treated rats. Glucose uptake assay also confirmed that MT-treated 3T3 cells uptake less glucose in comparison to untreated cells. In conclusion, the present study supported the use of *Cephalandra indica* in the treatment of diabetes. *Cephalandra indica* MT could reverse the effect of STZ-induced glucose levels indicating antidiabetic activity. The antidiabetic activity may be due to the restoration of liver and pancreatic tissue, thus reducing the risk of secondary complications associated with diabetes. Further detailed activity of isolated molecules of *Cephalandra indica* MT may unveil the constituents responsible for the antidiabetic activity. The active constituents of *Cephalandra indica* were identified by HPTLC and LC-MS. There were no terpenoids identified in MT, whereas all other dilutions (6 to 30C) were found

to have nondetectable levels of active constituents. Although specific molecules responsible for weight increment in MT-treated rats were not identified, it may be suggested that as MT is rich in phenolics [Figures 10-12]; these molecules may have a contributory role, possibly by preventing protein and lipid catabolism.

This is the first ever study on *Cephalandra indica* MT, identifying several molecules in it. The results will induce further research in exploring the usefulness of specific molecules responsible for antidiabetic effects. A systematic review on 108 clinical studies<sup>[17]</sup> on well-known herbs (used commonly in glycemic control) such as ivy gourd (*Cephalandra indica*), ginseng, garlic, tulsi, fenugreek, gurmar, karela, and aloe indicated that all these herbs have efficacies similar to conventional drugs. These investigations, however, lacked uniformity with regard to experimental design, methods, molecular constituents, and doses. The evidence was therefore termed as preliminary and warranted in-depth study.

The efficacy of *Cephalandra indica* MT as an antidiabetic homoeopathic medicine was reported<sup>[18]</sup> as early as 1988. Pancreatic beta-cell regeneration was also observed. Further progress through subsequent research was not evident in the literature. The present investigation on the same homoeopathic medicine, using both *in vivo* and *in vitro* model experiments with similar observations of reduced blood sugar levels as well as STZ treatment-altered central vein and spoke-wheel appearance in the liver as well as islets of Langerhans and interlobular duct in the pancreas, and the treatments with MT toward partial recovery of both, records a more concrete information for its therapeutic role. Hypoglycaemic herbal constituents reportedly have an insulin-secretagogue role in stimulating skeletal muscles, preventing neurological deficits, suppressing stress-activated protein kinase, regenerating beta-cells in the islets of Langerhans, and many phenolics compounds in them may modulate signal transduction pathways.<sup>[19]</sup> Several antidiabetic herbal products are available in the market globally. But the United Kingdom has issued a warning in using such medicines due to concerns over safe dosage and due to lack of information on actual bioactive compounds.

This investigation revealed that *Cephalandra indica* MT is rich in many phenolics that are important for health care. On the whole, it is reaffirmed that the efficacy of *Cephalandra indica* MT could be supported



by modern biological experiments as reported here. Glibenclamide was reported as an antidiabetic drug for type-II diabetes as early as 1966. But its newer mechanism of action and usefulness were established over the last 40 years,<sup>[20]</sup> establishing it as an irreplaceable molecule. The only other widely used alternative is metformin. It is therefore imperative that more research must be promoted for a greater market share of homoeopathic medicines for important diseases like diabetes. As homoeopathic medicines are much cheaper, this effort will come as a boon to poorer sections of the Indian population. In response to the increasing use of complementary and alternative medicines, even the rich nations like the United States (US Preventive Services Task Force; American Diabetes Association Guidelines) recommended more studies on *Coccinia indica* (*Cephalandra indica*) based on encouraging results from randomized controlled trials using *Cephalandra indica* powder displaying insulin-mimetic properties of a magnitude similar to conventional medicines.<sup>[17]</sup> *Cephalandra indica*, like *Gymnema sylvestre*, has no side effects on liver and kidney.

One prerequisite for attracting the global market for such low-cost medicine is specifying actual bioactive compounds and their doses. This is likely to boost the economic health of the manufacturers of homoeopathic medicine in India.

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**पृष्ठभूमि:** मधुमेह, एक उभरती हुई वैश्विक महामारी है जिसके रोगियों की संख्या भारत जैसे विकासशील देशों में बढ़ रही है। वर्तमान में मधुमेह के उपचार में एलोपैथिक दवाओं के प्रयोग से तो केवल एक अस्थायी राहत मिलती है। दूसरी ओर होम्योपैथी में सही दवा और पोटेन्सी के अनुकूल संयोजन से इस रोग के उपचार में उच्च सफलता दर प्राप्त हुई है।

**उद्देश्य:** इस अध्ययन का उद्देश्य स्ट्रेप्टोजोटोसिन (एसटीजेड)– उत्साही मधुमेह पीड़ित चूहों में सेफालेंडरा इंडिका (कुंदु) मदरटिंचर और उसकी पोटेन्सी का, उनके रक्त शर्करा स्तर, कोलेस्ट्रॉल स्तर, शरीर के वजन और लैंगरहेन्स के अग्न्याशय आइलेट्स की बीटा कोशिकाओं पर हुए प्रभाव की जांच करना था।

**सामग्री और पद्धतियाँ:** मदरटिंचर उपचारित फाइब्रोब्लास्ट सेल लाइन (रेशकोरक कोशिका वंश) के ग्लूकोज उद्ग्रहण पर नजर रखी गई। वयस्क नर चूहों में एसटीजेड (55 मिग्रा./किग्रा शरीर के वजन) आंतरपर्युदर्या (इंटरापेरीटोनियल) इंजेक्शन के द्वारा मधुमेह पीड़ित किया गया। इंजेक्शन देने के तीन दिनों के बाद, मधुमेही चूहों को मदरटिंचर (750 माइक्रोलि./किग्रा शरीर के वजन) तीन सप्ताह के लिए दैनिक सेवन कराया गया।

**परिणाम:** मदरटिंचर से उपचारित चूहों के रक्त शर्करा स्तर में महत्वपूर्ण कमी देखी गई, शरीर का वजन पुनः बढ़ने लगा और उनके अग्न्याशय में बीटा कोशिकाओं का पुनः उत्पादन होने लगा। मदरटिंचर उपचारित 3टी3 कोशिकाओं में भी सामान्य कोशिकाओं की तुलना में शर्करा की कम मात्रा देखी गई।

**निष्कर्ष:** वर्तमान अध्ययन से यह स्पष्ट होता है कि सी. इंडिका का मधुमेह प्रतिरोधन में महत्वपूर्ण प्रभाव होता है और एक होम्योपैथिक दवा के रूप में इसके उपयोग की संभावना है।

