Clitoria ternatea, a herb from Indian folklore, improves streptozotocin-induced diabetes and diabetes-induced cognitive decline in rats

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OBJECTIVE: To study the anti diabetic, neurochemical-antioxidant and cognition protective effects of Clitoria ternatea leaves on a rat model of diabetic cognitive decline.

METHODS: Antidiabetic activity was evaluated by serum glucose and body weight estimation in ethanol extract of Clitoria ternatea (EECT)-treated diabetic rats. Effects of EECT on spatial working memory (SWM) and spatial reference memory (SRM) were evaluated by Y-maze and Morris water maze tests respectively. Neurochemical-antioxidant effects of EECT were studied by acetylcholinesterase assay, and measurements of thiobarbituric acid reactive substances (TBARSs), superoxide dismutase (SOD) and catalase (CAT) levels in diabetic rats.

RESULTS: The 200 and 400 mg/kg of EECT showed a significant anti diabetic activity by decreasing serum glucose level (P<0.05, P<0.01), and there was a significant increase in the body weight in 400 mg/kg of EECT-treated diabetic rats (P<0.01). EECT was found to cause significant increases in SWM and SRM in retention trials on Y-maze and Morris water maze respectively (P<0.05, P<0.01). Significant decreases in acetylcholinesterase activity and TBARS level, and significant increase in CAT level were observed in rats treated with 200 and 400 mg/kg of EECT compared with rats in the diabetic control group (P<0.05 or P<0.01). Significant increase was also found in SOD in rats treated with 400 mg/kg of EECT.

CONCLUSION: Clitoria ternatea exhibits anti diabetic and antioxidant activities, offers the protection against diabetes-induced cognitive decline, and warrants the need for further studies to elucidate its mode of action.

KEYWORDS: diabetes mellitus; hypoglycemic agents; plants, medicinal; neurobehavioral manifestations; rats, Sprague-Dawley
Diabetes mellitus (DM) is a devastating disease throughout the world. Cognitive dysfunction, Alzheimer's disease and dementia are some of well recognized complications of DM though less addressed\(^4\). Cognitive dysfunction itself represents a serious problem and is rising in prevalence worldwide, especially among the elderly diabetics. Hyperglycemia, oxidative stress and cholinergic decline play a pivotal role in cognitive impairment in diabetic encephalopathy\(^4,5\). Antidiabetic and psychotropic drugs are associated with several adverse effects, hence in recent years, there has been a gradual revival of interest concerning the use of medicinal plants worldwide, even their biologically active compounds are unknown, because plant-derived drugs have been reported to be safe and without side effects\(^4,7\).

Hyperglycemia produces reactive oxygen species (ROS) as a result of glucose auto-oxidation, metabolism and the development of advanced glycosylation end products. In fact, diabetes is typically associated with increased generation of free radicals and impaired antioxidant defense qualifications, representing a central contribution for ROS in the onset, progression, and pathological consequences of DM, namely, vascular complications, nephropathy and cognitive impairment or dementia\(^6,9\). This oxidative stress became evident by increased lipid peroxide and decreased superoxide dismutase (SOD) and catalase (CAT) levels\(^10\).

Neurotransmitter functions which are altered in DM include decreased acetylcholine production, serotonin turnover and dopamine activity, and increased norepinephrine\(^11\). Considerable experimental evidence exists for a relation between the decline in cholinergic functions and dementia\(^5\).

In Ayurveda, the roots, seeds and leaves of *Clitoria ternatea* have been widely used as a brain tonic and are believed to promote memory and intelligence\(^12\). *C. ternatea* is reported to have antidepressant, anticonvulsant\(^13\), anti-inflammatory, analgesic, antiplatelet\(^10\), local anesthetic\(^12\), purgative\(^12\) and antidiabetic\(^17\) activities. It is also used for snakebite and scorpion sting in India\(^18\). The current study was designed to explore the antidiabetic, neurochemical-antioxidant and cognition protective activities of ethanol extract of *Clitoria ternatea* (EECT) leaves in an experimental rat model for DM-induced cognitive decline. This study may be a holistic approach towards developing a novel drug from Indian folklore for diabetic dementia.

### 1 Materials and methods

#### 1.1 Plant materials

The leaves of *Clitoria ternatea* were collected in November 2009 from the local areas of Pune, Maharashtra, India. The plant material was authenticated at Botanical Survey of India, Pune, and the voucher specimen BB680518 was deposited. The leaves were dried under shade and later pulverized in a mechanical grinder. The 100 g of powder was then extracted with 95% ethanol for one week. The mixture was then filtered and the filtrate was concentrated under reduced pressure to yield semisolid 3.70% (weight ratio) extract. The extract was preserved in a refrigerator till further use.

#### 1.2 Selection and maintenance of animals

Adult Sprague-Dawley rats of either sex, weighing between 150 to 250 g were used for the study. The animals were housed in standard polypropylene cages at room temperature and provided with standard diet and water *ad libitum*. The experimental protocol has been approved by the Institutional Animal Ethics Committee (SCOP/IAEC/Approval/2009-10/10). The animals were shifted to the laboratory 1 h prior to the experiment.

#### 1.3 Drugs and chemicals

Metformin was obtained from the Cipla Pharmaceuticals Ltd, India. Piracetam (Noortropil, 80 mg tablet); streptozotocin (Sigma Aldrich, USA); diagnostic kits (Biolab, India) for detecting malondialdehyde (MDA), SOD, CAT (Sigma Aldrich, USA) were purchased from local market. Other chemicals used were of analytical grade and obtained from Qualigens, India.

#### 1.4 Phytochemical study

*Clitoria ternatea* was evaporated to residue and diluted hydrogen chloride...
was added into it. After shaking, the extract was filtered and filtrate tests were performed for detection of various constituents using conventional protocol of Wagner’s, Hagné’s, and Dragendorff’s tests for alkaloids; Baljet’s, Borntrager’s and Legal’s tests for glycosides; foam, bromine water and hemolytic tests for saponins; Salkowski’s and Lieberman-Burchard tests for steroids and triterpenoids; gelatin, potassium dichromate, ferric chloride and lead acetate tests for tannins; ferric chloride, alkaline reagent, lead acetate solution and Shinoda tests for flavonoids; Molisch’s test and Benedict’s test for carbohydrates; Millon’s and ninhydrin tests for proteins and amino acids respectively.

1.5 Acute toxicity study Acute toxicity study was carried out according to the Organization for Economic Co-Operation and Development (OECD) Guidelines No. 423[26]. Three animals were used for each step. The dose level to be used as the starting dose was selected from one of four fixed levels, namely, 5, 50, 300, and 2000 mg/kg body weight per oral. As per the OECD recommendations, we used 300 mg/kg of the extract as the starting dose.

1.6 Antidiabetic study

1.6.1 Animal grouping and administration All the animals were fasted overnight before the administration of streptozotocin (STZ), an antibiotic that can cause pancreatic β-cell destruction, which is widely used experimentally as an agent capable of inducing insulin-dependent DM, namely type 1 DM[27]. STZ was prepared in cold citrate buffer (pH 4.45, 0.1 mol/L) and was injected intraperitoneally in the dose of 55 mg/kg to different experimental groups of animals, while the normal control animals were injected only with citrate buffer (pH 4.45, 0.1 mol/L). After 48 h of STZ injection, blood samples were collected, and serum glucose level was estimated. Animals with serum glucose more than 2.5 g/L were considered as diabetic and used for further study[27].

After eight weeks of STZ injection to establish the diabetic rat model, and citrate buffer injection to the normal controls, animals were divided into six groups with six in each as follows, namely, normal control group received 5 mL/(kg · d) of 2% gum acacia per oral; diabetic control group received 5 mL/(kg · d) of 2% gum acacia per oral; 200 mg/kg of EECT group received 200 mg/(kg · d) of EECT per oral for two weeks; 400 mg/kg of EECT group received 400 mg/(kg · d) of EECT per oral for two weeks; metformin group received 200 mg/(kg · d) of metformin per oral for two weeks; piracetam group received 200 mg/(kg · d) of piracetam per oral for two weeks.

1.6.2 Serum glucose estimation Serum glucose was estimated before and after the STZ injection in diabetic controls and 200 and 400 mg/kg of EECT-treated animals. For blood glucose level measurement, animals of all groups were anesthetized with anesthetic ether and the blood was withdrawn by puncturing retro-orbital plexus by using fine glass capillary and collected in epindorff tubes. The blood was allowed to clot at room temperature and the serum was separated by centrifugation at 3000 × g for 10 min and was used for estimation of serum glucose by glucose oxidase and peroxidase oxidase enzymatic method[28].

1.6.3 Body weight measurement All animals were weighed before STZ injection and then on the 57th (pre-treatment) and 71st days (post-treatment) after STZ injection. Animals were weighed gravimetrically by using electronic digital balance[24].

1.7 Neurochemical and antioxidant study

1.7.1 Obtaining supernatant Post-treatment animals were sacrificed by cervical dislocation and the brain was isolated and weighed. Whole brain was rinsed with ice cold saline (0.9% sodium chloride) and homogenized in chilled phosphate buffer (pH 7.4) to adjust the concentration to 20 mg/mL. The homogenates were centrifuged at 800 × g for 5 min, and then the nuclear debris was separated at 4 °C. The supernatant obtained was centrifuged at 10500 × g for 20 min at 4 °C. Such obtained supernatant was then used for neurochemical and antioxidant studies[25,26].

1.7.2 Acetylcholinesterase assay For estimation of acetylcholinesterase activity, Ellman’s method named after George Ellman was used[21]. A total of 0.4 mL supernatant was added to a cuvette containing 2.6 mL of phosphate buffer (0.1 mol/L, pH 8) and 100 μL of 5,5′-dithiobis-(2-nitrobenzoic acid). The contents of the cuvette were mixed thoroughly by bubbling air and the absorbance was measured at 412 nm by a spectrophotometer. When the absorbance reaches a stable value, it was recorded as the basal reading. The substrate of 20 μL of acetylthiocholine iodide was added and the changes in absorbance were recorded for a period of 10 min at intervals of 2 min. Change in the absorbance per minute was thus determined.

The mean change in absorbance was considered for calculation using the following formula and the acetylcholinesterase activity was measured as micromole per liter per minute per gram tissue[25]:

$$R = 5.74 \times 10^{-4} \times \frac{\Delta A}{Co}$$

In the above formula, R means the rate, in moles substrate hydrolyzed per minute per gram tissue; ΔA means the change in absorbance per minute, and Co is the original concentration of tissue (20 mg/mL).

1.7.3 Lipid peroxide levels The thiobarbituric acid reactive substance (TBARS) level was measured as an index of MDA production, an end product of lipid peroxidation. MDA reacts with thiobarbituric acid to form a red colored complex. The measurement of MDA levels by thiobarbituric acid reactivity is the most widely used method for assessing lipid peroxidation. And then 0.5 mL of Tris hydrochloric
acid was added in 0.5 mL of supernatant and incubated at 37 °C for 2 h. After incubation, 1 mL of 10% trichloroacetic acid was added and centrifuged at 3,000×g for 10 min, and then 1 mL of 0.67% thiobarbituric acid was added to 1 mL of the supernatant. The tubes were kept in boiling water for 10 min. After cooling, 1 mL of double distilled water was added and the absorbance was measured at 532 nm. The MDA concentration of the samples was derived from the standard curve prepared using known amounts of MDA and expressed as nanomolar MDA per milligram protein\(^{21}\).

1.7.4 SOD level One milliliter of sodium carbonate (1.06 g sodium carbonate in 100 mL water), 0.4 mL of 24 mmol/L nitroblue tetrazolium (NBT) and 0.2 mL of ethylenediaminetetra-acetic acid (EDTA, 37 mg EDTA in 100 mL water) were added to 100 μL of supernatant, and 0 min reading was taken at 560 nm. Reaction was initiated by addition of 0.4 mL of 1 mmol/L hydroxyquinoline hydrochloride, then incubated at 25 °C for 5 min and the reduction of NBT was measured at 560 nm. The SOD activity of the samples was derived from the standard curve prepared using known amounts of SOD and expressed as milligram per microgram protein\(^{27}\).

1.7.5 CAT level The ability of CAT to decompose the hydrogen peroxide to water and oxygen was estimated by determining the decomposition of hydrogen peroxide at 240 nm. CAT activity was assayed by the method of Claiborne. One milliliter of 0.019 mol/L hydrogen peroxide and 0.05 mL of 10% supernatant were added to 1.95 mL of phosphate buffers (0.05 mol/L, pH 7.0) to make a final volume of 3 mL. Changes in absorbance were recorded at 240 nm and expressed as milligram per microgram protein. The CAT activity of the samples was derived from the standard curve prepared using known amounts of CAT and expressed as milligram per microgram protein\(^{28}\).

1.8 Cognitive study

1.8.1 Animal grouping and administration The EECT was tested for cognitive activity using rats as per the method suggested by Hritcu \textit{et al}\(^{29}\), Morris \textit{et al}\(^{30}\) and Tuzcu \textit{et al}\(^{31}\). The selected animals were divided into six groups with six in each. The normal and diabetic control groups received 5 mL/(kg ∙ d) of 2% gum acacia per oral. The standard groups received metformin and piracetam at the dose of 200 mg/(kg ∙ d) and the test groups received the EECT at the doses of 200 and 400 mg/(kg ∙ d) per oral.

1.8.2 Y-maze test Short-term spatial working memory (SWM) was assessed by spontaneous alternation behavior in the Y-maze task. The Y-maze used in the present study consisted of three arms (35 cm in length, 25 cm in height and 10 cm in width), and an equilateral triangular central area. Animal was placed at the end of one arm and allowed to move freely through the maze. Time limit in Y-maze test was fixed to 8 min hence every session ended after 8 min. An arm entry was counted when the hind paws of the rat were completely within the arm. Spontaneous alternation behavior was defined as entry into all three arms on consecutive choices. The number of maximum spontaneous alternation behaviors was then the total number of arms entered minus two. Percent spontaneous alternation was calculated as (actual alternations/maximum alternations) × 100\(^{21}\). The training was given for 5 d and percent spontaneous alternation was measured on the 71st and 75th days.

1.8.3 Morris water maze test On the 71st day, rats’ spatial reference memory (SRM) was tested in a spatial version of the Morris water maze. The apparatus consisted of a circular water tank (180 cm in diameter and 60 cm in height). A platform (12.5 cm in diameter and 30 cm in height) invisible to the animals was set inside the tank and filled with water maintained at (28 ± 2) °C at a height of 40 cm. The tank was located in a large room where there were several brightly colored cues external to the maze: these were visible from the pool and could be used by the animals for spatial orientation. The position of the cues was kept unchanged throughout the experiment. The water maze task was carried out for five consecutive days after treatment. The animals received daily training trials for four of these five consecutive days, with each 90 s trial and a trial interval of approximately 30 s. For each trial, each animal was put into the water at one of four starting positions, the sequence of which being selected randomly. During test trials, animals were placed into the tank at the same starting point, with their heads facing the wall. The animal had to swim until it climbed onto the platform submerged underneath the water. After climbing onto the platform, the animal allowed to remain there for 20 s before the commencement of the next trial. The escape platform was kept in the same position relative to the distal cues. If the animal failed to reach the escape platform within the maximally allowed time of 90 s, it was gently placed on the platform and allowed to remain there for the same time. The time to reach the platform (latency in seconds) was measured. A probe trial was performed wherein the extent of memory consolidation was assessed. In the probe trial, the animal was placed into the pool as in the training trial, except that the hidden platform was removed from the pool. The transfer latency (TL) to reach platform was measured on the 75th day to study SRM\(^{30,31}\).

1.9 Statistical analysis Data were expressed as mean ± standard error of mean. Statistical analysis was performed using one-way analysis of variance followed by Dunnett’s test. When \(P<0.05\), it was considered to have significant statistical difference. All statistical analysis was performed with OpenEpi software.
2 Results

2.1 Phytochemical analysis The total of extract yield was 3.7% (weight ratio). Phytochemical screening of the extract determined presence of alkaloids, glycosides, steroids and flavonoids as major constituents, while tannins, triterpenoids, saponins, carbohydrates, proteins and amino acids were found absent.

2.2 Acute toxicity study The results of acute toxicity study showed no clinical signs of toxicity and mortality in the EECT-treated rats even after administration of 2,000 mg/kg dose. Hence, as per OECD guidelines, lethal dose was assigned to be more than 2,000 mg/kg. One-tenth and one-fifth of this lethal dose, namely, 200 and 400 mg/kg were taken as effective doses for the study.

2.3 Antidiabetic study

2.3.1 Serum glucose estimation After induction of diabetes on the 7th day, the serum glucose level was highly elevated in the diabetic animals (P < 0.01). After treated with 200 and 400 mg/kg of EECT, the serum glucose level was dose dependently reduced compared to that of the diabetic controls (P < 0.05, P < 0.01). And 200 mg/kg of metformin significantly decreased the serum glucose level (P < 0.01), while 200 mg/kg of piracetam showed statistically insignificant effect on serum glucose level in diabetic rats. See Table 1.

Table 2 Effect of two-week treatment of EECT on body weight in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>6</td>
<td>206.00±5.78</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>6</td>
<td>150.00±7.07**</td>
</tr>
<tr>
<td>EECT (200 mg/kg)</td>
<td>6</td>
<td>164.00±9.27</td>
</tr>
<tr>
<td>EECT (400 mg/kg)</td>
<td>6</td>
<td>207.00±11.58△△</td>
</tr>
<tr>
<td>Metformin (200 mg/kg)</td>
<td>6</td>
<td>204.00±5.99△△</td>
</tr>
<tr>
<td>Piracetam (200 mg/kg)</td>
<td>6</td>
<td>160.00±8.94</td>
</tr>
</tbody>
</table>

* * P<0.01 vs normal control group; △△ P<0.01 vs diabetic control group. EECT: ethanol extract of C. terntana.

Table 3 Effect of EECT on acetylcholinesterase activity in rats’ brain

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Acetylcholinesterase (μmol/L·min·g) of tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>6</td>
<td>3.67±0.47</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>6</td>
<td>6.36±0.30**</td>
</tr>
<tr>
<td>EECT (200 mg/kg)</td>
<td>6</td>
<td>5.45±0.25△</td>
</tr>
<tr>
<td>EECT (400 mg/kg)</td>
<td>6</td>
<td>4.07±0.42△</td>
</tr>
<tr>
<td>Metformin (200 mg/kg)</td>
<td>6</td>
<td>6.65±0.44</td>
</tr>
<tr>
<td>Piracetam (200 mg/kg)</td>
<td>6</td>
<td>3.15±0.20△△</td>
</tr>
</tbody>
</table>

* * P<0.01 vs normal control group; △△ P<0.01 vs diabetic control group. EECT: ethanol extract of C. terntana.

2.3.2 Body weight measurement STZ-induced diabetes significantly reduced body weight in the diabetic animals as compared with the normal controls (P < 0.01). After two weeks of treatment with 400 mg/kg of EECT and 200 mg/kg of metformin, there was a significant increase in body weight of the rats in the treatment groups compared to that of the diabetic control rats (P < 0.01), while piracetam and 200 mg/kg of EECT could not produce any significant effect on body weight. See Table 2.

2.4 Neurochemical-antioxidant study

2.4.1 Acetylcholinesterase assay Acetylcholinesterase activity was significantly increased in diabetic controls when compared with the normal controls (P<0.01). Two weeks of repeated treatment with 200 and 400 mg/kg of EECT showed a significant decrease in acetylcholinesterase activity in diabetic animals (P<0.05, P<0.01), and 200 mg/kg of piracetam-treated group showed a significant decrease in acetylcholinesterase activity (P<0.01), whereas 200 mg/kg of metformin-treated group did not show the significant effect in this regard. See Table 3.

2.4.2 TBARS, SOD and CAT levels The results showed a significant increase in TBARS level, and significant decreases in SOD and CAT levels in the diabetic controls compared to those of the normal controls (P<0.01). Two weeks of treatment with 400 mg/kg of EECT showed a significant decrease in TBARS level and improvement in SOD and CAT levels (P<0.01), whereas 200 mg/kg of EECT caused a significant reduction in TBARS level (P<0.01), a significant increase in CAT (P<0.05) and no significant increase in SOD level in the diabetic animals. After treated with 200 mg/kg of metformin and piracetam, it showed a significant reduction (P<0.01) in TBARS level while a significant increase in SOD level in the diabetic animals (P<0.01). See Table 4.

2.5 Cognitive studies

2.5.1 Y-maze test The percent of spontaneous alternation of the diabetic controls was significantly decreased in retention trial as compared to the normal controls (P<0.05). The 200 and 400 mg/kg of EECT-, 200 mg/kg of metformin- and piracetam-treated diabetic animals showed significant increases in the percentage of spontaneous alternations in retention trial as compared to that of the diabetic controls (P<0.05, P<0.01). See Table 5.
### Table 4 Effects of EECT on TBARS, SOD, and CAT levels in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>TBARS (MDA nmol/mg of protein)</th>
<th>SOD (µg/mg of protein)</th>
<th>CAT (µg/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>6</td>
<td>23.83±0.57</td>
<td>108.40±0.37</td>
<td>4.33±0.05</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>6</td>
<td>52.70±2.36**</td>
<td>47.85±2.98**</td>
<td>2.19±0.26**</td>
</tr>
<tr>
<td>EECT (200 mg/kg)</td>
<td>6</td>
<td>35.50±2.09&lt;Δ</td>
<td>52.35±7.48</td>
<td>3.87±0.65Δ</td>
</tr>
<tr>
<td>EECT (400 mg/kg)</td>
<td>6</td>
<td>25.14±0.27&lt;Δ&lt;ΔΔ</td>
<td>86.61±1.09&lt;Δ&lt;ΔΔ</td>
<td>3.46±0.28&lt;Δ&lt;ΔΔ</td>
</tr>
<tr>
<td>Metformin (200 mg/kg)</td>
<td>6</td>
<td>39.88±0.38&lt;ΔΔΔ</td>
<td>83.99±1.05&lt;ΔΔΔ</td>
<td>3.72±0.05&lt;ΔΔΔ</td>
</tr>
<tr>
<td>Piracetam (200 mg/kg)</td>
<td>6</td>
<td>25.80±0.32&lt;ΔΔΔ</td>
<td>61.37±0.50&lt;ΔΔΔ</td>
<td>2.64±0.34</td>
</tr>
</tbody>
</table>

* *P<0.01, vs normal control group; Δ P<0.05, ΔΔ P<0.01, vs diabetic control group. EECT: ethanol extract of *Citorea ternatea*. TBARS: thiobarbituric acid reactive substance; SOD: superoxide dismutase; CAT: catalase; MDA: malondialdehyde.

### Table 5 Effects of EECT on spontaneous alternations tested by Y-maze test in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Spontaneous alternations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>6</td>
<td>50.40±10.50</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>6</td>
<td>34.27±2.52&lt;Δ&lt;ΔΔ</td>
</tr>
<tr>
<td>EECT (200 mg/kg)</td>
<td>6</td>
<td>52.50±5.83&lt;Δ&lt;ΔΔ</td>
</tr>
<tr>
<td>EECT (400 mg/kg)</td>
<td>6</td>
<td>58.98±6.77&lt;ΔΔΔ</td>
</tr>
<tr>
<td>Metformin (200 mg/kg)</td>
<td>6</td>
<td>56.24±9.10&lt;ΔΔΔ</td>
</tr>
<tr>
<td>Piracetam (200 mg/kg)</td>
<td>6</td>
<td>61.76±1.57&lt;ΔΔΔ</td>
</tr>
</tbody>
</table>

* P<0.05, vs normal control group; Δ P<0.05, ΔΔ P<0.01, vs diabetic control group. EECT: ethanol extract of *Citorea ternatea*.

### 2.5.2 Morris water maze test

There was no significant statistical difference observed in TL in animals during the training. On the 75th day, TL was found to be significantly increased in the diabetic controls as compared with the normal controls (P<0.01). After two weeks of repeated treatment with 200 and 400 mg/kg of EECT and 200 mg/kg of piracetam, TL was significantly decreased in retention trial as compared with that of the diabetic controls (P<0.05, P<0.01), and this effect was found insignificant in 200 mg/kg of metformin-treated animals (P>0.05). See Table 6.

### Table 6 Effects of EECT on transfer latency in Morris water maze test in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Transfer latency (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>6</td>
<td>54.2±4.54</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>6</td>
<td>77.8±1.42**</td>
</tr>
<tr>
<td>EECT (200 mg/kg)</td>
<td>6</td>
<td>63.2±6.00&lt;ΔΔΔ</td>
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<tr>
<td>EECT (400 mg/kg)</td>
<td>6</td>
<td>56.4±1.28&lt;ΔΔΔ</td>
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<tr>
<td>Metformin (200 mg/kg)</td>
<td>6</td>
<td>68.8±3.83</td>
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<tr>
<td>Piracetam (200 mg/kg)</td>
<td>6</td>
<td>52.6±2.65&lt;ΔΔΔ</td>
</tr>
</tbody>
</table>

* *P<0.01, vs normal control group; Δ P<0.05, ΔΔ P<0.01, vs diabetic control group. EECT: ethanol extract of *Citorea ternatea*.

### 3 Discussion

The incidence of cognitive dysfunction or dementia appears to be doubled in elderly subjects with DM[12,31]. Hyperglycemia in DM results in overproduction of oxygen free radicals, which contributes to the progression of diabetes. The development of complications during diabetes is also associated with oxidative stress. The neurodegenerative disease as Alzheimer’s disease or dementia has also been related to oxidative stress during DM[34]. The neurochemical changes causing cerebrovascular alterations are also considered as potential mechanism for diabetic cognitive decline[35,37].

Antidiabetic, psychotropic drugs, antioxidants like vitamins A, C, and E and SOD, CAT enzymes have been found to prevent the progression of diabetes and the occurrence of complications resulted from DM[31]. However these drugs are associated with several adverse effects which have provoked research in field of traditional systems of medicine to deduce the drugs with less toxicity and better tolerability.

From the vast array of materia medica of the indigenous system, many plants have been reported to have activity against DM and central nervous system disorders thus act as very useful remedies for the alleviation of human suffering. *Citorea ternatea* is one of them and the effects of *Citorea ternatea* leaves have been reported to decrease the levels of blood glucose and glycosylated hemoglobin significantly and increase the serum insulin to normal level in alloxan-induced diabetic animals[37]. The methanolic extract of *Citorea ternatea* has been studied for its effect on cognitive behavior, anxiety, depression, stress and convulsions induced by pentylenetetrazol and maximum electroshock. The effect of *Citorea ternatea* was also studied on behavior mediated by dopamine, noradrenaline, serotonin and acetylcholine[31]. Hence it was thought worth to investigate the effects of *Citorea ternatea* on diabetes-induced cognitive decline in experimental animals along with its role in oxidative stress and acetylcholinesterase activity. The cognitive decline study was focused on SWM- and SRM-based mazes.

The preliminary phytochemical screening showed presence of alkaloids, glycosides, flavonoids, steroids in the *Citorea ternatea* leaves. Various phytochemicals like phenolic compounds anthocyanin glycosides known collectively as flavonoids, pentacyclic triterpenoids, and phytosterols have been reported from this plant[12,28]. It is well documented that the flavonoids exhibit a hypoglycemic activity and reverse the diabetic effects on TBARS, SOD and CAT enzyme levels[34,35], and these findings are in agreement with results of the current study. The
literature survey revealed cognitive impairment in STZ-induced diabetic animals in eight weeks\(^{[4]}\). Current study as well reflected the similar results in diabetic animals. Treatment with *Clitoria ternatea* was scheduled for two weeks after cognitive decline. Results of the present study showed significant improvement in SWM and SRM, suggestive of nootropic activity of EECT in diabetes-induced cognitive decline models.

The increased oxidative stress in diabetes produces oxidative damage in many regions of brain including the hippocampus\(^{[3]}\), and this oxidative damage in the brain is increased by experimentally induced hyperglycemia\(^{[3]}\). Oxidative damage to various brain regions constitutes into the long term complications, morphological abnormalities and memory impairments. In the present study, TBARS level was significantly increased \((P<0.01)\), whereas SOD and CAT levels were markedly reduced in the brain of the diabetic controls. Treatment with EECT significantly reversed these effects. Therefore, EECT might have protected DM-induced cognitive decline by reducing oxidative stress and the flavonoids detected in the EECT may be the phytoconstituent responsible for this effect.

Release of acetylcholine in the hippocampus is positively correlated with training on a working memory task\(^{[4]}\) and with good performance on a hippocampus-dependent, spontaneous alternation task\(^{[4]}\). In diabetic condition, the acetylcholinesterase level was found to be high, as this enzyme hydrolyses acetylcholine present in the brain and results in cognitive decline\(^{[4]}\). We observed a significant rise in acetylcholinesterase activity in the brain of the diabetic animals. Two-week treatment with EECT attenuated the increase in acetylcholinesterase activity in the brain of the diabetic animals. This decrease in acetylcholinesterase activity is attributed to flavonoids content of the extract\(^{[4]}\). Thus, EECT treatment for ameliorating the cognitive decline, cholinergic dysfunction, and oxidative stress in the diabetic animals may innovate the clinical application in treating neuronal deficit in the diabetic patients.

4 Competing interests

The authors declare that they have no competing interests.

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蝶豆根改善链脲霉素诱导的糖尿病模型大鼠认知减退

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目的：研究蝶豆根叶治疗糖尿病的疗效，对影响神经系统的化学物质的抗氧化作用，以及缓解因糖尿病引起的认知减退的作用。

方法：通过测定实验大鼠血清葡萄糖浓度和体质量研究蝶豆根乙醇提取物的抗糖尿病活性。通过 Y 字形迷宫以及迷宫迷宫测试分别评估蝶豆根对实验大鼠空间记忆以及空间参考记忆的影响。对影响神经系统的化学物质的抗氧化作用则通过乙酰胆碱酯酶实验以及糖尿病模型大鼠的脂类过氧化物、超氧化物歧化酶和过氧化氢酶水平来测定。

结果：服用 200 和 400 mg/kg 的蝶豆根提取物后，糖尿病模型大鼠血清葡萄糖水平明显降低（P<0.01），且服用 400 mg/kg 蝶豆根提取物的糖尿病模型大鼠的体质量有明显增加（P<0.01）。蝶豆根提取物可以显著改善实验大鼠的空间记忆以及空间参考记忆（P<0.05, P<0.01）。与糖尿病模型对照组相比，服用 200 和 400 mg/kg 蝶豆根提取物的糖尿病模型大鼠，其乙酰胆碱酯酶、脂类过氧化物显著减少（P<0.05），过氧化氢酶水平显著增加（P<0.05 或 P<0.01）；服用 400 mg/kg 蝶豆根提取物的糖尿病模型大鼠的超氧化物歧化酶水平增加（P<0.01）。

结论：蝶豆根具有抗糖尿病、抗氧化的作用，并可改善由糖尿病引起的认知减退，其机制还需要进一步的研究。

关键词：糖尿病；降血糖药；植物；药用；神经行为学表现；大鼠，Sprague-Dawley