



## Pharmacological Study

# Antihyperglycemic and antioxidant activity of *Clitorea ternatea* Linn. on streptozotocin-induced diabetic rats

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### Abstract

Ethanol extract of *Clitorea ternatea* Linn. (EECT) was evaluated for its antihyperglycemic and antioxidative activity in normal and streptozotocin-induced diabetic rats. Antihyperglycemic activity of EECT was studied in normal fasted and glucose fed hyperglycemic and epinephrine induced hyperglycemic rats by estimating fasting serum glucose (FSG) by glucose oxidase or peroxidase enzymatic method. Antioxidant activity of EECT was studied by assaying lipid peroxide/Thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), total nitric oxide, catalase (CAT) and glutathione levels in diabetic rats. The EECT (200 and 400 mg/kg) showed significant antihyperglycemic activity by decreasing FSG in all hyperglycemic models except epinephrine induced hyperglycemic rats; in which improvement in FSG was observed only with EECT in 400 mg/kg dose, whereas significant decrease in TBARS ( $P < 0.001$ ), nitric oxide ( $P < 0.001$ ) and significant increase in SOD ( $P < 0.001$ ), CAT ( $P < 0.01$ ) and reduced glutathione levels ( $P < 0.001$ ) was observed in animals treated with EECT (200 and 400 mg/kg) compared to diabetic control group. The results indicated that EECT has remedial effects on hyperglycemia and oxidative stress in diabetic rats.

**Key words:** Antioxidant, *Clitorea ternatea*, epinephrine, streptozotocin models

## Introduction

Diabetes mellitus (DM) is a syndrome characterized by hyperglycemia and changed metabolism of carbohydrates, lipids, and proteins resulting from absolute or relative lack of insulin or malfunction of insulin action.<sup>[1-3]</sup> Over the past two decades, there is an explosive increase in the number of people diagnosed with DM worldwide.<sup>[4]</sup> It appears to be one of the main threats to human health in the 21<sup>st</sup> century.<sup>[5]</sup> As per statistical reports, in India the number of diabetics will rise from 15 million in 1995 to 57 million in the year 2025, the highest number of diabetics in the world. Pronounced changes in the life style, urbanization, consumption of energy-rich diet, obesity, etc., are considered to be the cause for this alarming rise in DM.<sup>[6]</sup> Chronic hyperglycemia brings about an increased production of free radicals by glucose oxidation, nonenzymatic glycation of proteins and lowering of the antioxidant enzyme activities.<sup>[7-9]</sup> Experimental as well as

clinical studies have shown that oxidative stress plays a major role in the pathogenesis of DM.<sup>[10]</sup>

Presently DM is treated with different oral antidiabetic agents that include insulin secretors, sensitizers,  $\alpha$ -glycosidase inhibitors and insulin therapy. However, repeated injections of insulin and synthetic drugs used for the treatment of diabetes have several undesirable adverse effects and they are ineffective against chronic diabetic complications.<sup>[11]</sup> Hence there is a need for alternative oral hypoglycemic agents with improved safety and efficacy.

More than 400 plant species with a hypoglycemic activity have been mentioned in the literature. Most of the plants containing glycosides, alkaloids, terpenoids, flavonoids, carotenoids etc., are found to have an antidiabetic effect.<sup>[12,13]</sup> On the same grounds, the present study was designed to explore antihyperglycemic and antioxidant activities of *Clitorea ternatea* on normal and streptozotocin (STZ)-induced diabetic rats and to identify functional groups present in the plant.

In Ayurveda, the roots, seeds, and leaves of *C.ternatea* have long been widely used as a brain tonic and is believed to promote memory and intelligence.<sup>[14]</sup> It is reported to have antidepressant, anticonvulsant,<sup>[15]</sup> anti-inflammatory, analgesic and antipyretic,<sup>[16]</sup> local anesthetic,<sup>[17]</sup> purgative<sup>[18]</sup> and antidiabetic<sup>[19,20]</sup> activity. It is also used for snakebite and scorpion sting in India.<sup>[21]</sup>

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## Materials and Methods

### Collection of plant material and preparation of extract

The leaves of *C. ternatea* were collected in the month of November 2009 from the local areas and was authenticated at Botanical Survey of India; Pune (voucher specimen: BB680518). The leaves were dried under shade and later pulverized in a mechanical grinder, and 100 g of powder was extracted with ethanol (95%) for a week. The mixture was then filtered and the filtrate was concentrated under reduced pressure to yield semisolid (3.70% w/w) extract.

### Preliminary phytochemical study

Freshly prepared Ethanol extract of *C. ternatea* (EECT) was subjected to standard phytochemical screening tests for the qualitative detection of various functional groups like alkaloids, glycosides, saponins, steroids/triterpenoids, tannins, flavonoids, carbohydrates, proteins and amino acids.<sup>[22]</sup>

### High performance thin layer chromatogram profile

EECT 29.2 mg was dissolved in 29.2 ml of methanol and sample of 5 µl, 10 µl and 20 µl were applied as 8 mm wide bands, under a continuous flow of nitrogen, using CAMAG LINOMATE V automatic sample applicator. Sample was applied with a 100 µl syringe (Hamilton, Bonaduz, Switzerland) at a constant application rate of 0.1 µl/s and the distance between adjacent bands was 15 mm. The plate was developed using the solvent system Chloroform: Toluene: Methanol [4:4:1 (v/v)] and scanned by a densitometer (CAMAG) at 650 nm.

### Experimental animals

Adult Sprague Dawley rats of either sex, weighing between 150 g and 250 g were used for the study. The animals were housed in an environmentally controlled room, maintained at uniform standard laboratory conditions. They were provided with food and water *ad libitum*. The animals were acclimatized for seven days before experiments were performed. The experimental protocol has been approved by the Institutional Animal Ethics Committee (Singhad College Of Pharmacy/IAEC/Approval/2009-10/10).

### Acute oral toxicity test

The acute oral toxicity study for EECT was carried out according to Organisation for Economic Co-operation and Development (OECD) guidelines 423.<sup>[23]</sup> Swiss albino mice were fasted overnight, water also being withheld. The EECT was administered at a dose of 2000 mg/kg. Animals were observed individually during the first 30 min and periodically during 24 h, with special attention given during the first 4 h and daily thereafter, for a total 14 days.

### Study design

- Total 72 normoglycemic animals with fasting serum glucose (FSG) level 75-85 mg/dl were included. Animals were grouped in to four with 6 animals in each group.
  - Control: Treated with 2% gum acacia (5 ml/kg, *p.o.*)
  - Standard: Treated with metformin (200 mg/kg, *p.o.*)
  - EECT 200: Treated with extract (200 mg/kg, *p.o.*)
  - EECT 400: Treated with extract (400 mg/kg, *p.o.*)
- Six normoglycemic animals (FSG level 75-85 mg/dl) and

24 STZ (55 mg/kg *i.p.*) induced diabetic animals were included in antihyperglycemic and antioxidant evaluation in STZ induced diabetic animals. 6 animals were placed in each of the below groups:

- Normal control: Treated with 2% gum acacia (5 ml/kg, *p.o.*)
- Diabetic control: Treated with 2% gum acacia (5 ml/kg, *p.o.*)
- Diabetic standard (STZ 55 mg/kg, *i.p.*): Treated with metformin (200 mg/kg, *p.o.*)
- Diabetic+EECT 200: Treated with extract (200 mg/kg, *p.o.*)
- Diabetic+EECT 400: Treated with extract (400 mg/kg, *p.o.*)

### Antidiabetic study

#### Serum glucose estimation

For blood glucose level estimation, animals of all groups were anesthetized with anesthetic ether and 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 serum was separated by centrifugation at 3000 rpm for 10 min and was used for estimation of serum glucose by glucose oxidize (GOD)/peroxidase oxidase (POD) enzymatic Method.<sup>[24]</sup>

#### Evaluation of single dose ethanol extract of *Clitorea ternatea* in normal fasted rats

Normal fasted rat model is often used for testing potential oral hypoglycemic in addition to diabetic animal models to deduce the mechanism of action. Serum glucose was estimated in these fasted animals at 0, 2, 4 and 6 h after treatment with standard drug and extract.<sup>[25]</sup>

#### Evaluation of single dose ethanol extract of *Clitorea ternatea* in glucose fed hyperglycemic rats

Two hours after the administration of standard drug and extracts all animals were administered glucose (1.5 g/kg, *p.o.*) and following the glucose load the blood glucose levels were monitored at 0, 1/2, 1 and 2 h.<sup>[26]</sup>

#### Evaluation of ethanol extract of *Clitorea ternatea* in epinephrine induced hyperglycemic rats

Epinephrine (0.8 mg/kg, *i.p.*) was administered in all animals to induce hyperglycemia 2 h after the administration of standard drug and extracts. Serum glucose level was determined at 0, 1/2, 1 and 2 h after epinephrine treatment.<sup>[27,28]</sup>

#### Evaluation of ethanol extract of *Clitorea ternatea* in streptozotocin induced diabetic rats

STZ is an antibiotic that can cause pancreatic β-cell destruction, so it is widely used as an experimental agent capable of inducing insulin-dependent diabetes mellitus (IDDM).<sup>[29]</sup> It was dissolved in a 0.1 M citrate buffer (pH 4.45) and administered intraperitoneally to overnight fasted rats at a dose of 55 mg/kg to induce diabetes. After 48 h of STZ injection DM was confirmed by FSG level estimation. The animals were observed for seven consecutive days to confirm the stability of the diabetic state considering the FSG level as one of the biosensors. Only animals with FSG higher than 250 mg/dl were considered as diabetic and used for the study.<sup>[30]</sup> Effects of EECT were studied on single and repeated dosing.

## Antioxidant study

Animals were sacrificed by cervical dislocation and brain was isolated and weighed. Whole brain was rinsed with ice cold saline (0.9% sodium chloride) and homogenized by making 20 mg of the tissue per ml in chilled phosphate buffer (pH 7.4). The homogenates were centrifuged at 800 g for 5 min at 4°C to separate the nuclear debris. The supernatant thus obtained was centrifuged at 10500 g for 20 min at 4°C to get the supernatant. Such obtained supernatant was then used for antioxidant study.<sup>[31-35]</sup>

### Lipid peroxide levels

0.5 ml of Tris HCl 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 3000 rpm for 10 min. 1 ml of 0.67% thiobarbituric acid was added to 1 ml of supernatant, and the tubes were kept in boiling water for 10 min. After cooling 1 ml double distilled water was added and absorbance was measured at 532 nm. The malondialdehyde (MDA) levels were calculated using the standard curve of MDA and its level expressed as nmol/mg of protein.<sup>[31]</sup>

### Total nitric oxide levels

To 100 µl of supernatant, 500 µl of Greiss reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% naphthylamine diamine dihydrochloric acid in water) was added and absorbance was measured at 546 nm. Nitrite concentration was calculated using a standard curve for sodium nitrite and expressed as ng/mg of protein.<sup>[32]</sup>

### Superoxide dismutase levels

To 100 µl of supernatant, 1 ml of sodium carbonate (1.06 g in 100 ml water), 0.4 ml of 24 mM nitroblutetrazolin (NBT) and 0.2 ml of Ethylene diamine tetra acetic acid [EDTA (37 mg in 100 ml water)] was added, and 0 min reading was taken at 560 nm. Reaction was initiated by addition of 0.4 ml of 1 mM hydroxylamine hydrochloride, incubated at 25°C for 5 min and the reduction of NBT was measured at 560 nm. SOD level was calculated using the standard calibration curve, and expressed in µg/mg of protein.<sup>[33]</sup>

### Catalase levels

Catalase (CAT) activity was assayed by the method of Claiborne (1985). To 100 µl of supernatant, 1.9 ml of phosphate buffer (pH 7) was added and absorbance was read at 240 nm. The reading was taken again 1 min after adding 1 ml of 1 nM Hydrogen peroxide solution to the reaction mixture. One international unit of CAT utilized is the amount that catalyzes the decomposition of 1 nM H<sub>2</sub>O<sub>2</sub>/min/mg of protein at 37°C. CAT activity was calculated using the standard calibration curve, and expressed as µg/mg of protein.<sup>[34]</sup>

### Glutathione levels

1 ml of supernatant (10%) was precipitated with 1ml of sulphosalicylic acid (4%). The samples were kept at 4°C for at least 1 h and then subjected to centrifugation at 1200 g for 15 min at 4°C. The assay mixture contained 0.1 ml supernatant, 2.7 ml phosphate buffer (0.1 M, pH 7.4) and 0.2 ml Ellman's reagent [5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB], in a total volume of 3 ml. The yellow color developed was read immediately at 412 nm and expressed as µg/mg of protein. Glutathione (GSH) activity was calculated using the standard calibration curve, and expressed as µg/mg of protein.<sup>[35]</sup>

### Statistical analysis

The mean ± SEM values were calculated for each group. ANOVA followed by Dunnett's multiple comparison test was used for statistical analysis.  $P < 0.05$  was considered statistically significant.

## Results

### Preliminary phytochemical analysis

Total extract yield was 3.70% w/w. Preliminary phytochemical screening of extract indicated the presence of alkaloids, glycosides, steroids and flavonoids as major constituents, while tannins, triterpenoids, saponins, carbohydrates, proteins and amino acids were absent.

### High performance thin layer chromatogram analysis of ethanol extract of *Clitorea ternatea*

Optimized High Performance Thin Layer Chromatogram (HPTLC) of EECT at 650 nm [Figure 1] showed the presence

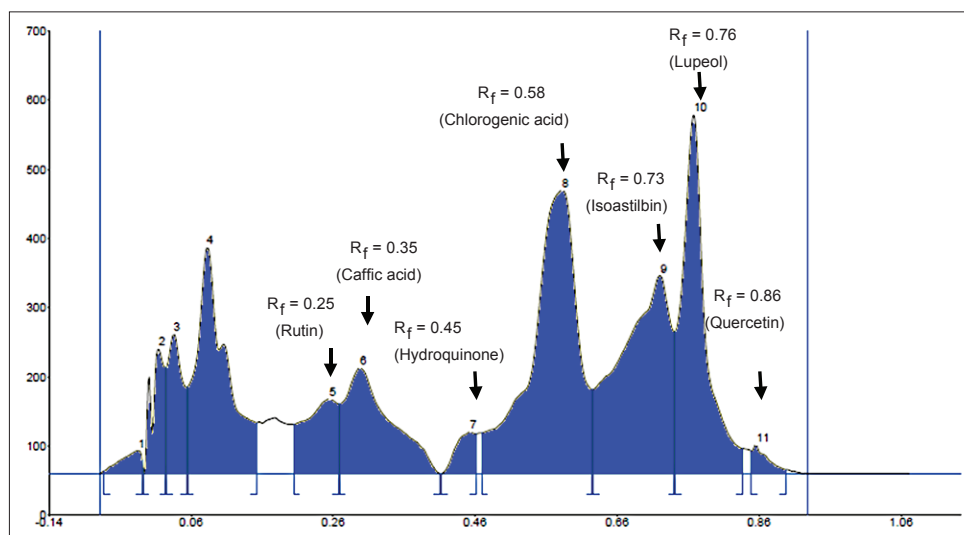


Figure 1: Optimized high performance thin layer chromatogram fingerprinting of ethanol extract of *C. ternatea* at 650 nm

of eleven components with their retardation factor ( $R_f$ ) value and concentration sequentially as  $R_f$  -0.02 (1.43%), 0.01 (7.77%), 0.03 (8.69%), 0.08 (14.09%), 0.25 (4.65%), 0.35 (6.59%), 0.45 (2.61%), 0.58 (17.66%), 0.73 (12.34%), 0.76 (22.37%), 0.86 (1.80%). Component number 10 at 0.76  $R_f$  showed maximum concentration. With reference to  $R_f$  values, if we look into literature of earlier studies in this regard they appear to be Rutin ( $R_f = 0.25$ ), Caffeic acid ( $R_f = 0.35$ ), Hydroquinone ( $R_f = 0.45$ ), Chlorogenic acid ( $R_f = 0.58$ ), Isoastilbin ( $R_f = 0.73$ ), Lupeol ( $R_f = 0.76$ ), Quercetin ( $R_f = 0.86$ ).<sup>[36,37]</sup>

### Acute toxicity study

EECT administered at a dose of 2000 mg/kg did not show any signs or symptoms of toxicity or mortality during the observation period. The starting dose was selected as 1/10<sup>th</sup> and 1/5<sup>th</sup> of 2000 mg/kg (i.e. 200 mg/kg and 400 mg/kg) for the antihyperglycemic and antioxidant evaluation.

### Antidiabetic study

#### Evaluation of single dose ethanol extract of *Clitorea ternatea* in normal fasted rats

Administration of EECT was found to produce hypoglycemia in normal fasted rats. EECT 200 and 400 mg/kg treated rats showed significant ( $P < 0.05$ ) decrease in serum glucose level

(6.76% and 12.17% respectively) after 4 h of treatment and this fall was found to be more pronounced ( $P < 0.01$ ) in EECT 400 mg/kg treated animals after 2 h [Table 1].

#### Evaluation of single dose ethanol extract of *Clitorea ternatea* in glucose fed hyperglycemic rats

Serum glucose level was found to be significantly raised in glucose (1.5 g/kg) fed rats. This raised glucose level was significantly decreased in animals treated with EECT 400 ( $P < 0.05$ ) and metformin 200 mg/kg ( $P < 0.001$ ) within 1/2 h by 19.49%, 35.71% respectively. At 1 h, both EECT groups ( $P < 0.01$ ,  $P < 0.001$ ) and metformin ( $P < 0.001$ ) showed 16.72%, 22.57% and 31.94% decrease in serum glucose level respectively whereas at 2 h significant decrease was observed only with EECT 400 and metformin by 22.40% and 30.52% respectively [Table 2].

#### Evaluation of ethanol extract of *Clitorea ternatea* in epinephrine induced hyperglycemic rats

Administration of epinephrine significantly increased serum glucose level at 1/2, 1 and 2 h. EECT 400 mg/kg ( $P < 0.01$ ,  $P < 0.05$ ) showed 25.24%, 20.77% and 31.58% decrease at 1/2, 1 and 2 h in serum glucose level respectively. However, metformin showed significant ( $P < 0.001$ ,  $P < 0.01$ ) decrease by 40.62%, 33.71% at 1/2 and 1 h respectively [Table 3].

#### Evaluation of ethanol extract of *Clitorea ternatea* in streptozotocin induced diabetic rats

Significant ( $P < 0.001$ ) increase in FSG was observed in STZ induced diabetic rats. EECT dose dependently decreased ( $P < 0.01$ ,  $P < 0.001$  respectively) FSG after 2 week treatment of diabetic rats; which was comparable to metformin ( $P < 0.001$ ) induced decrease in FSG [Table 4].

### Antioxidant study

#### Effects of ethanol extract of *Clitorea ternatea* on lipid peroxide and total nitric oxide levels

Significant ( $P < 0.001$ ) increase was observed in Thiobarbituric acid reactive substances TBARS and total nitric oxide levels

**Table 1: Effect of single dose ethanol extract of *Clitorea ternatea* on serum glucose level in normal fasted rats**

Treatment (mg/kg, p.o.)	Serum glucose level (mg/dl)			
	0 h	2 h	4 h	6 h
Control	84.48±4.78	83.31±1.81	78.91±1.31	74.11±1.69
Standard	82.75±1.94	80.39±2.27	73.20±2.52	70.21±0.74
EECT (200)	77.24±1.23	84.08±1.63	73.57±1.71*	74.55±2.36
EECT (400)	78.76±3.34	73.79±1.75**	69.30±3.05*	69.89±0.52*

n=6 values are mean±SEM, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , Compared to normal control group, EECT: Ethanol extract of *Clitorea ternatea*

**Table 2: Effect of single dose ethanol extract of *Clitorea ternatea* on serum glucose level in glucose fed hyperglycemic rats**

Treatment (mg/kg, p.o.)	Serum glucose level (mg/dl)			
	0 h	1/2 h	1 h	2 h
Control	85.88±1.71	180.07±4.08	157.75±3.12	120.53±6.40
Standard	83.81±2.81	115.76±2.53***	107.36±6.86***	83.74±4.79**
EECT (200)	85.27±4.50	176.40±2.64	131.37±5.83**	109.68±5.12
EECT (400)	84.22±2.08	164.46±4.46*	122.14±3.23***	93.52±7.47*

n=6 values are mean±SEM, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , compared to normal control group, EECT: Ethanol extract of *Clitorea ternatea*

**Table 3: Effect of single dose ethanol extract of *Clitorea ternatea* on serum glucose level in epinephrine-induced hyperglycemic rats**

Treatment (mg/kg, p.o.)	Serum glucose level (mg/dl)			
	0 h	1/2 h	1 h	2 h
Control	85.84±2.14	159.89±8.35	146.97±11.47	127.58±10.97
Standard	82.87±3.74	94.94±6.65***	97.42±8.46**	110.81±9.99
EECT (200)	82.40±3.44	150.42±8.48	140.10±6.51	132.33±5.23
EECT (400)	86.93±4.02	119.53±8.47**	114.43±9.28*	87.28±5.21*

n=6 values are mean±SEM, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , Compared to normal control group, EECT: Ethanol extract of *Clitorea ternatea*

**Table 4: Effect of 2 weeks ethanol extract of *Clitorea ternatea* treatment on serum glucose level in streptozotocin induced diabetic rats**

Treatment (mg/kg, p.o.)	Fasting serum glucose level (mg/dl)		
	Day 1 (single dose)	1 week (daily dose)	2 week (daily dose)
Normal control	88.98±9.01	79.91±4.94	94.20±6.96
Diabetic control	†290.3±12.13**	†285.5±19.85**	†280.6±18.11**
Diabetic+EECT (200)	284.7±11.43	290.3±12.13	‡153.6±22.59*
Diabetic+EECT (400)	280.6±18.11	282.9±14.31	‡125.2±19.26**
Standard	285.5±19.85	‡146.97±11.47**	‡80.88±7.53**

n=6 values are mean±SEM, \*P<0.01, \*\*P<0.001, †: Compared to normal control group, ‡: Compared to diabetic controls, EECT: Ethanol extract of *Clitorea ternatea*

in diabetic controls compared to normal controls. Two weeks repeated treatment with EECT (200 and 400 mg/kg) and metformin (200 mg/kg) showed significant ( $P < 0.001$ ) reduction in TBARS and total nitric oxide in diabetic animals [Table 5].

#### Effects of ethanol extract of *Clitorea ternatea* on superoxide dismutase, catalase and reduced glutathione levels

The results showed significant ( $P < 0.001$ ) decrease in superoxide dismutase (SOD), CAT and reduced glutathione levels in diabetic controls compared to normal controls. Two weeks repeated treatment with EECT (400 mg/kg) showed significant improvement in SOD ( $P < 0.001$ ), CAT ( $P < 0.01$ ) and reduced glutathione ( $P < 0.001$ ) levels, whereas, EECT (200 mg/kg) caused significant increase in CAT ( $P < 0.01$ ) and reduced glutathione ( $P < 0.001$ ) levels with no significant increase in SOD levels. Metformin (200 mg/kg) however, showed significant increase in SOD ( $P < 0.001$ ) and CAT ( $P < 0.01$ ) and reduced glutathione ( $P < 0.001$ ) levels [Table 6].

## Discussion

STZ selectively destroys pancreatic  $\beta$ -cells, inhibits the synthesis and secretion of hormone insulin, and causes diabetes.<sup>[38,39]</sup> The intraperitoneal injection of STZ in the present study effectively induced diabetes in rats after 48 h; which was confirmed by the elevation in the FSG.

The administration of EECT significantly reduced the FSG level in various animal models studied in this study; which indicates its antihyperglycemic efficacy. Several researchers<sup>[19,20]</sup> have already reported the antidiabetic efficacy of the different parts of *C. ternatea*; and our findings are in agreement with these reports.

Antioxidant and its protective efficacy against oxidative stress induced organ damage have also been demonstrated by few researchers.<sup>[40,41]</sup> However, its antioxidant potential in brain has not been studied in detail. Therefore authors of this study have designed to study the antihyperglycemic as well as antioxidant activity in brain of STZ induced diabetic rats. In the present study, lipid peroxide and nitric oxide levels were significantly increased ( $P < 0.001$ ) whereas glutathione, SOD and CAT levels were markedly reduced in the brain of diabetic controls. Treatment with EECT significantly reduced the levels of lipid peroxides, whereas glutathione, SOD and CAT levels were increased. From the results of this study it

**Table 5: Effects of ethanol extract of *Clitorea ternatea* on lipid peroxide and total nitric oxide levels in streptozotocin induced diabetic rats**

Treatment (mg/kg, p.o.)	TBARS (nmol of MDA/mg of protein)	Total nitric oxide (ng/mg of protein)
Normal control	23.83±0.57	116.4±0.36
Diabetic control	†52.70±2.36**	†238.3±1.07**
Diabetic+EECT (200)	‡35.50±2.09**	‡204.1±0.98**
Diabetic+EECT (400)	‡25.14±0.27**	‡151.5±1.15**
Standard	‡39.88±0.38*	‡161.3±0.40**

n=6 values are mean±SEM, \*P<0.01, \*\*P<0.001, †: Compared to normal control group, ‡: Compared to diabetic control group, EECT: Ethanol extract of *Clitorea ternatea*, TBARS: Thiobarbituric acid reactive substances

becomes evident that EECT has antioxidant potential not only in liver, kidney<sup>[40,41]</sup> but also in brain of diabetic animals. Hence antihyperglycemic-antioxidant activity may effectively protect from diabetes and its complications like nephropathy, cardiovascular complications and memory impairment associated with oxidative stress induced organ damage.

The preliminary phytochemical screening of EECT leaves showed presence of alkaloids, glycosides, flavonoids, steroids and HPTLC analysis revealed presence of flavonoids, anthocyanins ( $R_f = 0.25-0.86$ ) and alkaloids ( $R_f = -0.02-0.08$ ). Various phytochemicals like flavonoids, anthocyanin glycosides, pentacyclic triterpenoids, and phytosterols have been reported from this plant<sup>[14]</sup> and our results have also demonstrated the presence of these phytochemicals except triterpenoids which were found to be absent in our screening. Thus, EECT exhibited antihyperglycemic-antioxidant activity can be attributed to presence of rich flavonoids, anthocyanins and alkaloids content in the extract. However, detailed studies to isolate and characterize the phytochemicals; understand its molecular mechanism of action are needed to be carried out.

## Conclusion

From the results, it becomes evident that EECT has potential antihyperglycemic and antioxidant activity that may prove beneficial to attenuate the diabetes and its complications. This activity is said to be attributed to flavonoids, anthocyanins and alkaloids found in the EECT.

**Table 6: Effects of ethanol extract of *Clitoria ternatea* on superoxide dismutase, catalase and reduced glutathione levels in streptozotocin induced diabetic rats**

Treatment (mg/kg, p.o.)	Superoxide dismutase ( $\mu\text{g}/\text{mg}$ of protein)	Catalase ( $\mu\text{g}/\text{mg}$ of protein)	Reduced glutathione (ng/mg of protein)
Normal control	108.40 $\pm$ 0.37	4.33 $\pm$ 0.05	14.97 $\pm$ 0.73
Diabetic control	<sup>†</sup> 47.85 $\pm$ 2.98**	<sup>†</sup> 2.19 $\pm$ 0.26**	<sup>†</sup> 3.30 $\pm$ 0.01**
Diabetic+EECT (200)	<sup>‡</sup> 52.35 $\pm$ 7.48	<sup>‡</sup> 3.87 $\pm$ 0.65*	<sup>‡</sup> 7.27 $\pm$ 0.09**
Diabetic+EECT (400)	<sup>‡</sup> 86.61 $\pm$ 1.09**	<sup>‡</sup> 3.46 $\pm$ 0.28*	<sup>‡</sup> 12.42 $\pm$ 1.4**
Standard	<sup>‡</sup> 83.99 $\pm$ 1.05**	<sup>‡</sup> 3.72 $\pm$ 0.05*	11.84 $\pm$ 0.54**

n=6 values are mean $\pm$ SEM, \*P<0.01, \*\*P<0.001, <sup>†</sup>: Compared to normal control group, <sup>‡</sup>: Compared to diabetic control group, EECT: Ethanol extract of *clitoria ternatea*

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## हिन्दी सारांश

# शंखपुष्पी वनस्पती (*Clitorea ternatea* Linn.) के उच्चशर्करा तथा लिपिड ऑक्सीडेशन गतिविधी प्रतिबंधीत गुणधर्म का स्ट्रैप्टोजोटोसिन प्रेरित मधुमेही चूहों में अध्ययन

करुणा ए. तलपते, उमा ए. भोसले, मंदार आर. झांबरे, राहुल सोमाणी

इस प्रयोग में शंखपुष्पी नामक वनस्पती के इथेनॉल में बने द्रव का परिणाम सामान्य और स्ट्रैप्टोजोटोसिन प्रेरित मधुमेही चूहों के रक्तशर्करा तथा ऑक्सीडेशन प्रतिबंधीत गतिविधी के उपर किया गया। इस प्रयोग में रक्तशर्करा प्रतिबंधित परिणाम अध्ययन हेतु GOD/POD एन्झाइम विधी का उपयोग किया गया है, जिसमे सामान्य उपवासित, शर्करा खिलाये और एपीनेफ्रिन प्रेरित उच्चशर्करा वाले चूहों के रक्तशर्करा का मूल्यांकन किया गया है। शंखपुष्पी द्रव का परिणाम ऑक्सीडेशन प्रतिबंधीत गतिविधी लीपिड पैरॉक्सीडेशन/टीबीएआरएस (TBARS) प्रतिक्रियाशील पदार्थ, सुपरऑक्साइड डीसम्युटेस (SOD), कुल नाइट्रीक एसीड, कॅटलेज (CAT) और ग्लुटाथीओन स्तर के मधुमेही चूहों में करने की क्रियाव्दारा अध्ययन किया गया है। शंखपुष्पी द्रव (२०० व ४०० मिलीग्राम प्रति कि.ग्रा.) शंखपुष्पी निचोड परिणामकारक सिद्ध हुआ है। शंखपुष्पी (२०० व ४०० मिलीग्राम प्रति कि.ग्रा.) निचोड के सेवन से मधुमेही चूहों के टीबीएआरएस और नाइट्रीक एसीड स्तर में महत्वपूर्ण ( $P<0.001$ ) कमी तथा सुपरऑक्साइड डीसम्युटेस ( $P<0.001$ ), कॅटलेज ( $P<0.01$ ) और ग्लुटाथीओन ( $P<0.001$ ) स्तर में महत्वपूर्ण वृद्धि देखी गई। जबकी ये वृद्धि मधुमेह नियंत्रण पशुसमुह में नहीं देखी गयी। इस प्रयोग के परिणामों से ये सिद्ध होता है कि शंखपुष्पी मधुमेही चूहों के उच्चशर्करा तथा ऑक्सीडेटिव तनाव पर प्रभावशाली है।