

Hypoglycaemic Activity of Seed Extract of *Clitoria ternatea* Linn in Streptozotocin- Induced Diabetic Rats.

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ABSTRACT

Introduction: Traditional plant medicines are used throughout the world for a range of diabetic complication. The evaluation of phytochemical is the logical way of searching for the new drugs to treat diabetes. The leaves and flowers of *Clitoria ternatea* have been reported for antidiabetic activity, hence an attempt was made to evaluate the antidiabetic potential of seeds of *Clitoria ternatea*. **Methods:** Ethanol extract of seeds of *Clitoria ternatea* Linn was subjected to preliminary phytochemical investigations. The seed extracts at two dose levels like 200mg and 400mg/kg body weight were screened for hypoglycaemic activity in Streptozotocin induced diabetic rats (60mg/kg, i.p.). **Results:** Ethanol extract showed the presence of various phytoconstituents viz. sterols, alkaloids, glycosides, saponins, tannins, carbohydrates, proteins, phenolic compounds and flavonoids. The ethanol extract at 400mg/kg.b.wt dose showed significant decreased blood glucose ($p < 0.001$), cholesterol ($p < 0.05$), alkaline phosphatase ($p < 0.001$), aspartate amino transferase ($p < 0.001$) and alanine amino transferase ($p < 0.001$), when compared to diabetic control. **Conclusion:** Further study is required to isolate active phytoconstituents from ethanolic extract of seeds of *Clitoria ternatea* Linn.

Keywords: *Clitoria ternatea* Linn, Antidiabetic, Streptozotocin.

INTRODUCTION

Traditional plant medicines are used throughout the world for a range of diabetic complication. The study of such medicine offers a natural key to unlock a diabetologist pharmacy for the future. Plant based medicines have enormous therapeutic potential with simultaneous mitigation of many of the side effects that are often associated with synthetic antidiabetic drugs. So the evaluation of phytochemical is the logical way of searching for the new drugs to treat diabetes^[1].

Clitoria ternatea Linn is a plant species belonging to the Fabaceae family and it is native to tropical equatorial Asia. It is a perennial herbaceous plant. Its leaves are elliptic and obtuse. It grows as a vine or creeper, doing well in moist neutral soil. The most striking feature about this plant is its vivid deep blue flowers.

They are solitary, with light yellow markings. They are about 4 cm long by 3 cm wide. There are some varieties that yield white flowers. The fruits are 5-7 cm long, flat pods with 6 to 10 seeds in each pod.^[2]The roots of *Clitoria ternatea* have been reported for activities like Antidiarrhoeal^[3], antipyretic^[4], Antihelmintic.^[5] The leaves and flowers of *Clitoria ternatea* have been reported for antidiabetic activity^[6], Antimicrobial activity^[7]. Phytoconstituents Malonylated flavonol glycosides^[8], Anthocyanins^[9], ternatin^[10], Acylated anthocyanins^[11], Acylated delphinidin glycosides and flavonols^[12], Taraxerol^[13], Taraxerone^[14] were isolated from *Clitoria ternatea* flowers. Oil content and Fatty acid composition^[15,16] were studied in seeds of *Clitoria ternatea*.

Hence an attempt was made to evaluate the antidiabetic potential of seeds of *Clitoria ternatea*.

MATERIALS AND METHODS

Plant Collection

The seeds of the plant *Clitoria ternatea* were collected during the month of June 2009 from Rajahmundry, Andhra Pradesh, India and was identified and authenticated in Department of Botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

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Extraction procedure

The seeds of *Clitoria ternatea* was dried at room temperature, the dried seed material was powdered mechanically. Around 500gm of finely powdered seeds were extracted by cold maceration method with aqueous ethanol (30:70) for one week with occasional shaking. After extraction the solvent was distilled off and extract was concentrated on water bath to a dry residue and dried in a dessicator.

Phytochemical Screening

The ethanolic extract was subjected to qualitative phytochemical investigation for the identification of the phytoconstituents viz., sterols, alkaloids, glycosides, saponins, tannins, carbohydrates, proteins, phenolic compounds and flavonoids^[17].

Experimental Animals

Healthy adult Wistar rats weighing 150-220 g were used for the antidiabetic activity. The animals were housed in clean polypropylene cages and maintained in a well-ventilated temperature controlled animal house with a constant 12h light/dark schedule.

Acute toxicity studies

The acute toxicity test of the extract was evaluated in Wistar rats (150-200gm), at dose of 2000 mg/kg.b.wt (p.o.). The treated animals were monitored for 14 days, for mortality and general behaviour. No death was observed till the end of the study. The test samples were found to be safe up to the dose of 2000 mg/kg, and, from the results, 200 mg/kg and 400 mg/kg dose were chosen for further experimentation.

Antidiabetic screening

For experiment overnight fasted Wistar rats was induced by a single intraperitoneal administration of Streptozotocin (60 mg/kg.b.wt) in 0.1 M citrate buffer, pH 4.5. Those animals with fasting blood glucose level more than 300 mg/dl after Streptozotocin administration were selected for the study and they were divided into four groups of six animals each.

Group I served as diabetic control and received 0.3% CMC,

Group II served as positive control and received glibenclamide (10 mg/kg.b.wt),

Groups III and IV received the ethanolic extract (200 mg/kg.b.wt and 400 mg/kg.b.wt respectively). The treatment was continued for fourteen days, orally, once daily. After

the treatment period the blood glucose level, cholesterol and triglyceride were estimated. Blood samples were collected by orbital sinus puncture under mild ether anaesthesia and blood glucose was estimated by electronic glucometer (Accu-Check active).

For the estimation of cholesterol, triglyceride, alkaline phosphatase (ALP), aspartate amino transferase (AST) and alanine amino transferase (ALT), the blood samples were collected in Eppendroff's tubes (1 ml) containing 50 µl of anticoagulant (10% trisodium citrate) and plasma was separated by centrifuging at 6000 rpm for 15 min and analyzed in Autoanalyzer Microlab 200 using Ecoline-kits (E merck).

Statistical analysis

Values are expressed as mean ± standard error mean (SEM) and analyzed using statistical package for social sciences (SPSS) version 7.5 using ANOVA followed by Dunnett's test. P values < 0.001 were considered significant.

RESULTS AND DISCUSSION

The qualitative phytochemical evaluation of Ethanolic extract showed the presence of sterols, alkaloids, glycosides, saponins, tannins, carbohydrates, proteins, phenolic compounds and flavonoids.

Streptozotocin is a nitrosourea compound produced by *streptomyces achrogenes* which induce DNA strand breakage in β-cells leads to insulin deficiency. Insulin deficiency leads to various metabolic disorders viz. increased blood glucose, cholesterol, alkaline phosphatase, aspartate amino transferase and alanine amino transferase^[18]. Oral treatment of 200mg/kg.b.wt and 400 mg/kg.b.wt. showed decreased blood glucose, cholesterol, alkaline phosphatase, aspartate amino transferase and alanine amino transferase. But 400mg/kg.b.wt dose showed significant decreased blood glucose (p < 0.001), cholesterol (p < 0.05), alkaline phosphatase (p < 0.001), aspartate amino transferase (p < 0.001) and alanine amino transferase (p < 0.001), when compared to diabetic control (Table-1 and 2).

CONCLUSION

From the results it indicates ethanolic seed extract of *Clitoria ternatea* showed potent antidiabetic activity but it is dose dependent. Flavonoids, proteins and saponins have been reported to possess significant anti-diabetic activity and antilipidemic activity.^[19,20] *Clitoria ternatea* Linn seed extract showed the presence of flavonoids, proteins and saponins; hence the activity of the plant may be due to this phytoconstituents. However, further study is required to isolate active phytoconstituents from Ethanolic extract.

Table 1: Effect of Ethanolic seed extract of *Clitoria ternatea* on blood glucose level.

Group	Dose	Glucose level		
		Normal	After Streptozotocin	After Treatment
Control	0.3% CMC	93.64 ± 4.25	312.16 ± 12.04	338.10 ± 10.12
Glibenclamide	10 mg/kg.b.wt	98.34 ± 6.08	302.05 ± 8.56	102.34 ± 8.34 ^a
Ethanolic extract of <i>Clitoria ternatea</i>	200mg/kg.b.wt	102.12 ± 10.12	308.46 ± 20.03	198.16 ± 15.08 ^b
Ethanolic extract of <i>Clitoria ternatea</i>	400mg/kg.b.wt	88.63 ± 1.02	304.24 ± 5.08	103.64 ± 3.14 ^a

Values are Mean ± SEM of Six Animals. Statistical Significance: a = p < 0.001 and b = p < 0.05 as compared to control

Table 2: Effect of Ethanolic seed extract of *Clitoria ternatea* on biochemical parameters.

Treatment	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Alkaline Phosphatase (U/L)	Aspartate Amino Transferase (U/L)	Alanine Amino Transferase (U/L)
Control (0.3% CMC)	184.21 ± 4.08	172.46 ± 12.46	258.13 ± 16.01	134.67 ± 3.92	88.12 ± 5.86
Glibenclamide (10mg/kg.b.wt)	79.08 ± 6.20 ^a	94.16 ± 6.24 ^a	184.24 ± 8.12 ^a	88.13 ± 10.02 ^a	48.24 ± 3.93 ^a
Ethanolic extract of <i>Clitoria ternatea</i> (200mg/kg.b.wt)	134.24 ± 6.42 ^b	142.08 ± 12.21 ^b	208.12 ± 12.34 ^b	102.24 ± 8.12 ^b	64.12 ± 8.12 ^b
Ethanolic extract of <i>Clitoria ternatea</i> (400mg/kg.b.wt)	82.34 ± 3.04 ^a	95.23 ± 02.13 ^b	189.64 ± 8.32 ^a	92.12 ± 5.12 ^a	49.46 ± 2.96 ^a

Values are Mean ± SEM of Six Animals. Statistical Significance: a = p < 0.001 and b = p < 0.05 as compared to control

REFERENCES

- Tiwari AK, Rao JM. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: present status and future prospects. *Cur Sci* 2002;83:30-8.
- The Ayurvedic Pharmacopoeia of India (Part I Volume II). Ministry of Health and Family Welfare. p. 10-11.
- Nitinkumar U, Roshan P, Naheed W, Naveen Kumar M. Evaluation of Antidiarrhoeal Activity of the Root of *Clitoria Ternatea* Linn. *Int J Pharm Sci Rev Res* 2010;5:131-134.
- Parimaladevi B, Boominathan R, Subhash C. Evaluation of antipyretic potential of *Clitoria ternatea* L. extract in rats. *Int J Phytother Phytopharmacol*, 2004;1:121-124.
- Khadatkar SN, Manwar JV, Bhajipale NS. In-vitro anthelmintic activity of root of *Clitoria ternatea*. *Phcog Mag* 2008;4:148-150.
- Daisy P, Santosh K, Rajathi M. Antihyperglycemic and antihyperlipidemic effects of *Clitoria ternatea* Linn in alloxan- induced diabetic rats. *Afr J Microbiol Res* 2009;3:287-291
- Babu U, Kesani P, Sadayappan R. Phytochemical Analysis and Antimicrobial activity of *Clitoria ternatea* Linn against extended spectrum beta lactamase producing enteric and urinary pathogens. *Asian J Pharma Clin Res* 2009;2:94-96.
- Kazuma K. Malonylated flavonol glycosides from the petals of *Clitoria ternatea*. *Phytochemistry* 2003;62:229-37.
- Terahara, N.; Eight new anthocyanins, ternatins C1-C5 and D3 and preternatins A3 and C4 from young *Clitoria ternatea* flowers. *J Nat Prod* 1998;61:1361-1367.
- Terahara, N, Saito N, Honda T, Toki K, Osajima Y. Structure of ternatin A1 the largest ternatin in the major blue anthocyanin from *Clitoria ternatea* flowers. *Tetrahedron Lett* 1990;31:2921-2924.
- Terahara, N, Saito N, Honda T, Toki K, Osajima Y. Acylated anthocyanins of *Clitoria ternatea* flowers and their acyl moieties. *Phytochemistry* 1990;29:949-953.
- Saito N, Abe K, Honda T, Timberlake CF, Bridle P. Acylated delphinidin glycosides and flavonols from *Clitoria ternatea*. *Phytochemistry* 1985;4:1583-1586.
- Banerjee Sk, Chakravarti RN. Taraxerol from *Clitoria ternatea*. *Bull Calcutta Sch Trop Med* 1963;11:106-107.
- Banerjee Sk, Chakravarti RN. Taraxerone from *Clitoria ternatea*. *Bull Calcutta Sch Trop Med* 1964;12:23.
- Vianni R, Soute SM. Oil content and Fatty acid composition of *Clitoria ternatea* seeds. *Arquivos Da Universidade Federal Rural Do Rio De Janeiro* 1971;1:47-50.
- Joshi SS, Shrivastava RK, Shrivastava DK. Chemical examination of *Clitoria ternatea* seeds. *J Am Oil Chem Soc* 1981;55:714-715.
- Kokate CK, Purohit AP, Gokhale SB. Text Book of Pharmacognosy. 7th ed. Nirali Prakashan (Publ); 1997. p.105-144.
- Shanmugasundaram KR, Panneerselvam C, Samudram P, Shanmugasundaram ER. Enzymes changes and glucose utilisation in diabetic rabbits, the *Gymnema sylvestrae*. *J Ethnopharmacol* 1983;7:205-234.
- Rajnarayana K, Sripal Reddy M, Chaluvadi MR, Krishna DR. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. *Indian J Pharmacol* 2001;33:2.
- J.S.Choi, O.H.Yokozawa; Hypolipidemic effect of *Prunus dadidian*. *J Nat Prod* 1991;54:218.

