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EVALUATION OF ANTIDIABETIC POTENTIAL AND AMELIORATION OF BLOOD SERUM PARAMETERS OF THE ROOT OF *CLITORIA TERNATEA* ON ALBINO WISTAR RAT

Ranjit Mohapatra and Debananda Champatisingh*

Department of Pharmacology, University Department of Pharmaceutical Sciences, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India.

***Corresponding Author: Debananda Champatisingh**
Department of Pharmacology, University Department of Pharmaceutical Sciences, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India.

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ABSTRACT
Diabetes is an endocrine dysfunction that impairs immunity and has an impact on other bodily organ systems. It is a highly prevalent condition with potentially deadly consequences all around the world. In this study, rats with diabetes brought on by alloxan were treated with the hydroalcoholic extract of the root of *Clitoria ternatea* (HARCT). The goal was to boost the body weight and blood biochemical markers associated with diabetes. The diabetic rats were gavaged with glibenclamide (10 mg/kg) and HARCT (200, 250, and 300 mg/kg) at different doses for fifteen days in a row. The HARCT showed more significant ($P< 0.05$) reduction in blood sugar level, a significant gain in body weight, and a correction of serum protein parameters in alloxan induced diabetic albino wistar rats compared to control and glibenclamide (10 mg/kg). Important phytoconstituents such as alkaloids, flavonoids, steroids, coumarins, resins, and carbohydrates are found in the roots of *Clitoria ternatea L.* (Fabaceae). These results provide evidence for the first time that *Clitoria ternatea* root may have anti-diabetic and insulin-mimetic properties.

KEYWORDS: *Clitoria ternatea,* Antidiabetic, Alloxan monohydrate, Blood serum parameters.

INTRODUCTION

Diabetes is an illness related to metabolism that occurs when the body is unable to create insulin, a hormone needed for cells to use glucose as energy. Hyperglycaemia, the primary cause of diabetic complications such as nephropathy, retinopathy, and neuropathy, is a defining feature of diabetes mellitus, an endocrine condition.[1,2] Many strong and effective medications are derived from medicinal plants. A safer and healthier alternative to synthetic medications are those generated from plants.[3] Several pharmacological active components are extracted from medicinal plant parts- such as the flower, fruit, root, stem, bark, and seed. Secondary metabolites including glycosides, tannins, alkaloids, flavonoids, and terpenoids that are found in these plants are responsible for their medicinal qualities. Pharmacological agents, or lead molecules are separated from the extract for direct medication. Nowadays, herbal remedies are employed all over the world even in the absence of proof of their therapeutic efficacy, and the pharmacological analysis of the many plants utilized in the conventional medical system is a subject of little vital knowledge.[4] Many studies indicated that many of the plants have strong antidiabetic action, and that oral anti-hyperglycaemic medicines derived from plants can be employed in traditional medicine.[5, 6] The ethnobotanical material provides over 800 plants that may exhibit anti-diabetic potential, proving that plants are a possible source of anti-diabetic medications. Synthetic oral hypoglycaemic medications and insulin, are the standard diabetes treatment and are successful in reducing hyperglycaemia, but they come with a lot of side effects and don't change how diabetic problems progress. This is the primary cause of the growing number of patients seeking for alternative medicines with potentially less or non-existent side effects.[7]

Clitoria ternatea L. is a member of the Fabaceae family. It is a good-looking perennial herb. Commonly used in aphrodisiac tonic. The root of the plant has anti-inflammatory, analgesic, and antipyretic values with a bitter taste.[8,9] In India, this plant is referred to as "butterfly pea" and is said to have many therapeutic uses. It has several ornamental and therapeutic qualities in addition to untapped potential. The methanolic extract of leaves of this plant has immunomodulatory, diuretic, anti-pyretic, anti-inflammatory, and smooth muscle relaxant properties.[10] Using albino wistar rats that have

been given alloxan i.p injections, the current study intends to assess the antidiabetic potential of hydroalcoholic extract of the root of *C. ternatea*.

MATERIALS AND METHODS

Chemicals and reagents

Alloxan monohydrate was purchased from S.D. Fine Chemicals Ltd., Boisar. Glibenclamide tablets and other chemicals of high analytical grade were purchased for the study.

Animals

Albino wistar male rats weighing 150-200 g were used for the present study. All animals were maintained in the animal house of Dadhichi College of Pharmacy, Cuttack, Odisha, under controlled conditions of temperature 25 ± 2^0 C, relative humidity 55-60 %, and 12-h light-dark cycles for experimental purposes. They are grouped into experimental and control groups, kept in polypropylene cages with sterile paddy husk as bedding and free access to standard pellets to feed and water *ad libitum*. All the studies conducted were approved by the institutional animal ethical committee (IAEC) of Dadhichi College of Pharmacy, Sundargram, Cuttack, Odisha (Approval No. - 1200/PO/Re/S/08/CCSEA).

Collection of Plant parts and preparation of extract

The Chief Scientist, Dr. Nabin Kumar Dhal, IMMT, BBSR, Odisha, India, verified the authenticity of the *C. ternatea* plant, which was harvested in November 2022 from the college medicinal garden at U.D.P.S., Bhubaneswar (voucher number; IMMT-002/22). Cleaning, washing, and room temperature drying were done on the *C. ternatea* roots in the shade until all moisture was gone. The material was allowed to dry before being crushed and sieved through sizes 10 and 40 meshes. The finely ground substance was sieved and then kept in an airtight container until needed.

500 g of dried and coarsely powdered *C. ternatea* roots were extracted in a Soxhlet apparatus using a solvent of ethanol (70): water (30) ratio. The hydro-alcoholic extract of *Clitoria ternatea* roots (HARCT), 11.27% w/w was obtained by drying the extract in a water bath after it was dried in a rotary evaporator with decreased pressure.

Acute toxicity studies

An acute oral toxicity study as per OECD-423 guidelines (acute toxic class method) was done by taking rats (n=6) of either sex. The animals were kept fasting for 12h (night), with only access to water *ad libitum* before administration of test extract dose (300 mg/kg HARCT) body weight by intra-gastric tube and observed for 14 days. No mortality was seen and the same procedure was repeated for higher doses (250mg/kg and 300 mg/kg) body weight. Mortality was not observed.[11] Repeat this procedure and at a dose of 500 mg/kg, behavioral changes were observed in animals. So, 300 mg/kg was taken as a therapeutic dose.

Induction of diabetes

Alloxan monohydrate (120 mg/kg body weight) dissolved in normal saline and administered intra-peritoneally to overnight fast (12 hours), albino wistar rats (only access to water *ad libitum*) for developing diabetes. Then animals were accessed to a standard diet and water *ad libitum*. After 3 days, the blood glucose levels were measured by using glucometer, and the range above 200 mg/dl was kept for this study.[12]

Antidiabetic Study

Design of model[13]

The animals were divided into six groups and six animals constituted a group.

Group-I: Normal control administered with 0.9% normal saline.

Group II: Diabetic control administered with Alloxan monohydrate (120 mg/kg) i.p, in 0.9% normal saline.

Group III: Diabetic control was administered with the standard drug glibenclamide (10 mg/kg), served as standard.

Group IV: Diabetic control administered with test drug HARCT (200 mg/kg)

Group-V: Diabetic control administered with test drug HARCT (250 mg/kg)

Group-VI: Diabetic control administered with test drug HARCT (300 mg/kg)

Estimation of blood glucose

The treatment was started after induction of diabetes except for, the normal control group and diabetic control groups for 15 days, orally. During this period, the animals in all groups had free access to diet and water *ad libitum*. Blood glucose levels were measured on 1^{st}, 5^{th}, 10^{th}, and 15^{th} days of treatment. Blood was collected from the tail vein of the rats and glucose level was measured by using glucometer and strips, Sugar Check (Mumbai).

Measurement of Bodyweight

During the treatment period, on, 5^{th}, 10^{th}, and 15^{th} days the body weight of all groups of animals was measured by using an electronic balance.

Estimation of biochemical parameters

Blood was collected from all groups of animals via the retro-orbital route on the last (15^{th}) day of the experiment. Before doing this experiment all the animals were fasted for 12 hours. Blood samples of rats were centrifuged at 2500 rpm for 10 minutes at 4^0 C and aliquoted for respective biochemical tests.

Statistical Analysis

Values were reported as mean ± SD. The differences were compared by using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests with $P< 0.05$ to notify the best treatment group.

RESULTS AND DISCUSSION

Acute toxicity study

In an acute toxicity study, the hydroalcoholic extract of the root of *Clitoria ternatea* did not show any mortality up to a dose of 3 g/kg body weight. After all, there were no behavioral changes seen at this dose.

Preliminary phytochemical test

Identification of phytoconstituents of hydroalcoholic extract of the root of *Clitoria ternatea* was carried out by using different screening methods. Hydroalcoholic extract of the root of *Clitoria ternatea* showed the presence of different primary and secondary metabolites; alkaloid, carbohydrate, flavonoid, coumarin, steroid, resin, and phenol.[14] The findings of phytochemical constituents are summarized in Table 1.

Table 1: Summary of Phytochemicals in *C. ternatea* Roots.

Test	Inference
Alkaloid, Carbohydrate, Flavonoid, Coumarin, Steroid, Resin, Phenol.	+
Tannin, Saponin, Glycoside, Protein.	-
(+) - present; (-) – negative	

Antidiabetic activity and Body weight[15]

It was demonstrated that the antidiabetic activities of the hydro-alcoholic extract of root of *C. ternatea* on alloxan induced diabetes in albono wistar rats, with a dose of 200 mg/kg body weight was not show significant activity. So higher doses with 250 mg/kgand 300 mg/kg body weight were selected for screening activity. The dose of 300 mg/kg body weight was shown highly significant ($P<0.05$) in reducing blood sugar levels as comparison with glibenclamide (10 mg/kg), the standard drug. The diabetic rats treated with the hydroalcoholic extract of the root of *C. ternatea* (300 mg/kg, body weight) for 15 days showed a decrease in blood glucose level from 393.16 ± 3.69 to 143.83 ± 1.72 mg/dl. Whereas the glibenclamide at a dose of 10 mg/kg, body weight for 15 days resulted from 393.16 ± 3.69 to 188.5 ± 2.88 mg/dl. The decreased blood glucose level proved the efficacy of the plant extract as compared with the standard drug. Glibenclamide stimulates the pancreatic islet cells to produce more insulin. According to this study, *C. ternatea* root extract may be able to stimulate the pancreas to release insulin and the peripheral uptake of glucose. (As seen in Table 2 and Figure 1).

In other terms along with the antidiabetic potential of the plant extract it also showed an improvement in body weight as compared to standard medication. The diabetic rats, the untreated group showed a decrease in body weight over the experiment period. Treating with the hydroalcoholic extract of the root of *C. ternatea* (300 mg/kg, body weight) showed improvement in body weight from 128.66 ± 1.06 to 155.83 ± 1.72 g in comparison with the glibenclamide (10 mg/kg, body weight) from 128.66 ± 1.06 to 154.16 ± 2.85 g. Glibenclamide showed significant improvement in body weight on day 15. All doses of root extract of *Clitoria ternatea* (200 mg /kg, 250 mg /kg, and 300 mg /kg) showed significant ($P<0.05$) improvement in body weight from day 1 to day 15 in comparison to the diabetic control group. (As seen in Table 3 and Figure 2).

Table 2: Effect of Hydro-alcoholic Root Extract of *C. ternatea* on Fasting Blood Glucose Level in Alloxan induced Diabetic Rats.

GROUP	TREATMENT	FASTING BLOOD GLUCOSE LEVEL (mg /dl)			
		Day 1	Day 5	Day 10	Day 15
I	Normal control	82.16 ± 2.22	82.83 ± 2.4	83.16 ± 1.32	81.83 ± 1.16
II	Diabetic control	383.83 ± 4.63	392.5 ± 6.02	395.66 ± 3.37	393.16 ± 3.69
III	Glibenclamide (10 mg/kg)	320.83 ± 4.11	306.33 ± 8.11	232.16 ± 6.21	188.5 ± 2.88*
IV	HARCT (200 mg/kg)	360.33 ± 4.67	355.16 ± 3.06	311.33 ± 6.94	276.83 ± 2.92
V	HARCT (250 mg/kg)	355.66 ± 4.76	345.5 ± 3.83	299.5 ± 9.13	210.83 ± 1.94*
VI	HARCT (300 mg/kg)	329.83 ± 5.41	325.5 ± 8.47	241.83 ± 4.11	143.83 ± 1.72**

Data are represented as mean ± S.D, (n=6), *P< 0.05 when compared with the control and standard group.

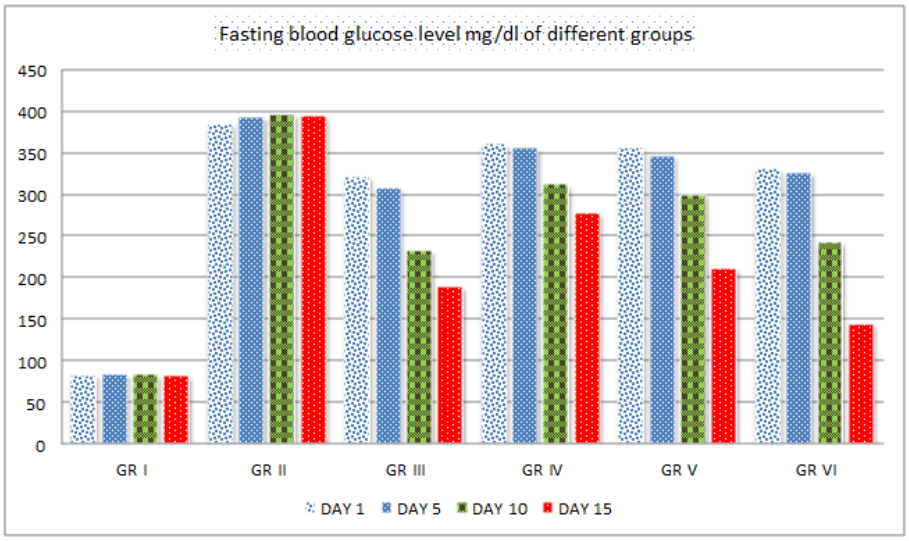

Fig. 1: Comparison of blood glucose (mg/dl) in different concentrations of test drug (HARCT) with standard drug (Glibenclamide) during the experimental period.

Table 3: Effect of Hydro-alcoholic Root Extract of *C. ternatea* on Body Weight in Alloxan induced Diabetic Rats.

GROUP	TREATMENT	BODY WEIGHT OF ANIMALS (g)			
		Day 1	Day 5	Day 10	Day 15
I	Normal control	159.83 ± 4.44	157.5 ± 4.03	155.66 ± 1.86	155.33 ± 1.86
II	Diabetic control	158.66 ± 4.62	145.5 ± 3.08	135.66 ± 3.03	128.66 ± 1.06
III	Glibenclamide (10 mg/kg)	160.16 ± 3.97	149.33 ± 3.82	152.66 ± 3.01	154.16 ± 2.85*
IV	HARCT (200 mg/kg)	162.5 ± 5.92	149.83 ± 3.71	150.16 ± 3.54	150.5 ± 3.01
V	HARCT (250 mg/kg)	163.16 ± 4.87	150.16 ± 4.11	150.16 ± 4.11	152.66 ± 2.58
VI	HARCT (300 mg/kg)	163.33 ± 5.98	152.16 ± 2.31	153.16 ± 2.22*	155.83 ± 1.72**

Data are represented as mean ± S.D, (n=6), *P< 0.05 when compared with the control and standard group.

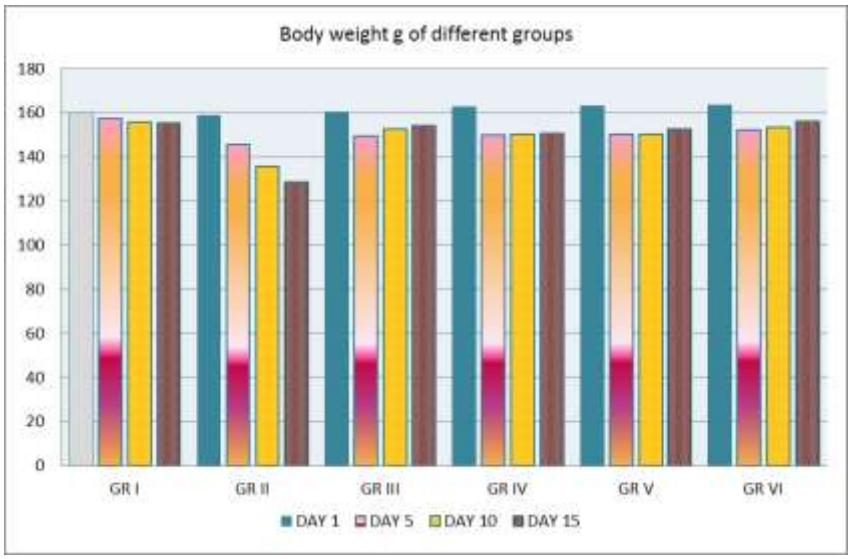

Fig. 2: Comparison of body weight (g) in different concentrations of test drug (HARCT) with standard drug (Glibenclamide) during the experimental period.

Biochemical parameters

The diabetic rats were reported to have higher serum creatinine 1.39±0.05 and urea 38.61±0.23 mg/dl. The hydro-alcoholic extract of the root of *C. ternatea* (300 mg/kg) significantly decreased the serum creatinine level to 0.94±0.06 mg/dl and the serum urea to 26.34±0.21 mg/dl, while Glibenclamide successfully lowered the serum creatinine level to 0.93±0.05 mg/dl and the serum urea 22.21±0.6 mg/dl, respectively. Since the kidney constantly removes creatinine and urea, the two main

nitrogenous waste products, from the blood through urine, the levels of these substances are usually low. When the kidneys are unable to filter out nitrogenous waste products because of renal failure or high blood glucose, the waste products might accumulate in the blood and cause insomnia and even death.[16] The reduction of these nitrogenous wastes throughout therapy suggests that the plant extract has the potential to cure kidney damage caused by diabetes.

In the diabetic rats, there was a significant rise in the triglycerides level of 69.14±0.21 mg/dl and total cholesterol level of 168.33±1.52 mg/dl. The hydro-alcoholic extract of the root of *C. ternatea* (300 mg/kg) significantly decreased the serum triglyceride level to 61.77±0.21 mg/dl and the serum cholesterol level to 99.02±1.53 mg/dl, respectively. Whereas Glibenclamide successfully lowered the serum triglyceride level to 61.82±1.05 mg/dl and the serum cholesterol level to 122.67±2.41 mg/dl. Similarly, the hydro-alcoholic extract of the root of *C. ternatea* (300 mg/kg) and glibenclamide reduced the level of LDL to 60.01±0.39 mg/dl and 54.72±0.71 mg/dl, respectively. The level of HDL was increased to 35.22±0.41 mg/dl by the hydro-alcoholic extract of the root of *C. ternatea* (300 mg/kg) and 34.81±0.73 mg /dl by glibenclamide, respectively.[17]

The serum protein level rose from 4.64±0.05 mg/dl to 7.16±0.06 mg/dl by the hydroalcoholic extract of the root of *C. ternatea* (300 mg/kg) and from 4.64±0.05 mg/dl to 6.31±0.16 mg/dl by glibenclamide, respectively. Similarly, the plant extract improved albumin level from 3.11±0.07 mg/dl to 3.98±0.04 mg/dl and globulin level from 1.91±0.06 mg/dl to 2.91±0.06 mg/dl, whereas glibenclamide (10 mg/kg) improved albumin level from 3.11±0.07 mg/dl to 3.91±0.07 mg/dl and globulin level from 1.91±0.06 mg/dl to 3.41±0.14 mg/dl in diabetic rats.[18] (As seen in Table 4).

Table 4: Effect of Hydro-alcoholic Root Extract of *C. ternatea* on Biochemical Parameters in Alloxan induced Diabetic Rats.

Biochemical parameters	Day 1	Day 15 Treatment (Mean ± SD)					
		Gr. I	Gr. II	Gr. III	Gr. IV	Gr. V	Gr. VI
Creatinine	0.86±0.05	0.82±0.02	1.3 9±0.05	0.93±0.05	1.3±0.02	1.11±0.03	0.94±0.06
Urea	26.5±0.68	26.22±0.26	38.61±0.23	22.21±0.6	30.16±1.28	29.08±0.15	26.34±0.21*
Total Cholesterol	167.9±2.08	97.01±2.66	168.33±1.52	122.67±2.41*	131.29±1.73	99.66±1.51	99.02±1.53**
Triglycerides	61.96±1.00	41.66±1.53	69.14±0.21	61.82±1.05	72.01±1.01	65.84±0.75	61.77±0.21
LDL	64.02±1.7	51.64±0.9	64.59±0.51	54.72±0.71*	64.02±0.31	61.21±0.4	60.01±0.39*
HDL	33.66±1.52	34.03±0.08	27.51±1.51	34.81±0.73	34.48±0.21	34.51±0.42	35.22±0.41*
Albumin	3.78±0.03	3.87±0.05	3.11±0.07	3.91±0.07	3.17±0.03	3.39±0.08	3.98±0.04*
Globulin	2.66±0.06	2.68±0.07	1.91±0.06	3.41±0.14	2.53±0.04	2.61±0.05	2.91±0.06
Serum Protein	6.83±0.5	6.34±0.11	4.64±0.05	6.31±0.16*	5.15±0.12	5.96±0.12	7.16±0.06*

Data are represented as mean ± S.D, (n=6), *P< 0.05 when compared with the control and standard group.

CONCLUSION

This study manifested that treatment with a hydroalcoholic root extract of *Clitoria ternatea* delays the progression of diabetes and ameliorates the biochemical parameters of blood in diabetic animals. This may be due to the adjacency of flavonoids and coumarin phytoconstituents in the root of the *Clitoria* plant. The other phytoconstituents in the root of the plant, like steroids and resins, may be responsible for increasing the body weight of the animals. As the plant source is the cheapest and most effective way to get a desirable blood glucose profile in comparison with the conventional medication system, it is better to use the plant product as a supplement with lifestyle modification. On the other hand, the antidiabetic potential of the hydroalcoholic root extract of *C. ternatea* relating to the release of insulin from pancreatic islets and utilization of peripheral blood glucose may be the hypothetical active mechanism of action. So the study ascertains the therapeutic value of the plant in Ayurveda, which could be of considerable interest for the development of new drugs in the future. Further study may justify the potential of the extract for the regeneration of β-cells in the pancreas.

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