



EVALUATION OF HYPOGLYCEMIC EFFECT OF ETHANOLIC EXTRACTS OF LEAF AND FRUIT OF *T.DIOICA* AND LEAF OF *C.TERNATEA* IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Trichosanthes dioica and *Clitoria ternatea* were traditional plants that were used for the treatment of diabetes mellitus, stress and depressant. The present work was undertaken to evaluate the antihyperglycemic effect of individual and combined ethanolic extracts of these two different herbs to study the blood glucose level and the activities of some carbohydrate metabolizing enzymes in liver of normal and STZ-induced diabetic rats. The extracts were administered orally to the diabetic rats for 28 days, resulted in significant decrease of blood glucose and decrease the activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase and increase the activities of glucokinase, pyruvate kinase and glucose-6-phosphate dehydrogenase. From the study it was revealed that combined plant extract was more effective for treating diabetes mellitus.

KEYWORDS : *Trichosanthes dioica*, *Clitoria ternatea*, antihyperglycemic, glucokinase



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INTRODUCTION

Diabetes mellitus is a multifactorial disease that has no cure, is a group of disorders as a result of high levels of blood glucose resulting from defects in insulin secretion, insulin action or both¹. It is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism². Alterations in glucose metabolism in diabetes are accompanied by changes in the activities of the enzymes that control glycolysis and gluconeogenesis in liver and muscle³. At least 382 million people worldwide in between the age group of 20-79 years suffered from diabetes in 2013. The number is expected to grow to 592 million by 2035. Each year another 7 million people develop diabetes⁴. In modern medicine, no drug is available with satisfactory and effective therapy for the management of diabetes mellitus⁵. Insulin therapy and oral hypoglycemic agents are available for treatment of diabetic patients. But they are unable to lower glucose concentration to within normal range and use of these therapies is restricted by their pharmacokinetic properties and associated with serious side effects^{6,7}. People with diabetes may need to take these medicines for the rest of their lives⁸. Hence there is a need to search for newer hypoglycemic agents with high therapeutic efficacy and fewer side effects. This may be fulfilled by treating diabetes with plant derived antidiabetic agents. The lesions in the pathophysiology of diabetes are multiple and therefore it would require more than a single drug agent to reverse all or majority of the aspects of the disease. Polyherbal therapy is considered as the preferred therapeutic approach in the management of diabetes⁹. In the traditional system of Indian medicinal plant formulations and several cases, combined extracts of plants are used as drug of choice rather than individual¹⁰. *Trichosanthes dioica* Roxb. and *Clitoria ternatea* L. are being used as popular remedy for the treatment of diabetes mellitus in Ayurveda and Siddha medicine. *Trichosanthes dioica* (family: Cucurbitaceae) is a dioecious perennial plant, grown throughout India and it is known as the pointed gourd. The leaves of the plant have

been used for constipation, fever, skin infection and cancer like conditions. The fruits are used as a remedy for spermatorrhoea and also used for improving appetite and digestion¹¹ and haemagglutinating activities¹². *Clitoria ternatea* L. is a perennial twining herbaceous plant, found in South and Central America, East and West Indies, China, Bangladesh and India. It is commonly called Shankpushpi. It could serve as therapeutic agent for various ailments¹³. In traditional Ayurvedic medicine, it has been used as a memory enhancer, anti-stress, anxiolytic, anti-depressant, anti-convulsant, sedative agent^{14,15}, anticancer activity¹⁶ and neurological disorders¹⁷. In the present study, the above mentioned plant materials are used individually and in combination to evaluate the hypoglycemic effectiveness and regulation of carbohydrate metabolizing enzymes in liver of normal and STZ-induced diabetic rats and compared to the effect of standard drug glibenclamide.

MATERIALS AND METHODS

(i) Collection and authentication of plant materials

Fresh unripe fruit and leaf of *Trichosanthes dioica* Roxb. (*T.dioica*) and the leaf of *Clitoria ternatea* L. (*C.ternatea*) were collected from SKM Herbal Research Centre, Erode, Tamil Nadu, India. The plants were identified and authenticated by Dr. V.R.Mohan, Associate Professor, Department of Botany, V.O.Chidambaram College, Tuticorin. A voucher specimen (No. VOCB 2307 and VOCB 2453) was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

(ii) Preparation of the plant extracts

Freshly collected leaf and fruit of *T.dioica* and leaf of *C.ternatea* were washed with distilled water and the fruits were cut into small pieces. Both fruits and leaves were dried under shade for two weeks. The shade dried leaves and fruits were coarsely powdered separately. The powdered materials were kept in airtight containers to use. About 500

gm of dried coarse powdered samples was weighed and subjected to 1250 ml of ethanol in a Soxhlet extractor for 24 hrs. All the extracts were filtered through Whatmann No.41 filter paper separately and the extracts were concentrated in vacuum at 60°C using a rotary evaporator. To evaporate the remaining solvent, the extracts were kept in an oven at a temperature of 40-50°C for 8 hrs. The hypoglycemic effects were evaluated by oral administration of the extracts to STZ-induced diabetic rats.

(iii) Collection of experimental animals

Healthy male adult albino rats of Wistar strain approximately of same age, weighing around 160-180 gm were procured from Nandha College of Pharmacy. The entire process was approved by the Institutional Animal Ethics Committee (IAEC) which is certified by the Committee for the Purpose of Control and Supervision of Experiments on Animals, (CPCSEA), India (Proposal number: NCP/IAEC/PHD/01/2007-2008), Nandha College of Pharmacy, Erode, Tamil Nadu .

(iv) Experimental induction of diabetes by streptozotocin in rats

Diabetes was induced by single dose intraperitoneal administration of streptozotocin at a dose of 60 mg/kg body weight in 0.1 M citrate buffer (pH 4.5) and then injected into the tail of the sixty rats. The injection volume was prepared to contain 1 ml/kg bw¹⁸. After 72 hrs of STZ administration, the blood glucose content was measured. The animals with blood glucose levels \geq 250 mg/dl were considered to be diabetic and used for the experiment¹⁹.

(v) Experimental grouping of animals

In the present investigation, a total of 66 rats (60 diabetic surviving rats and 6 normal rats) were taken and divided into eleven groups of 6 rats each to determine the antidiabetic activity of ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea*. The actions of the extracts were compared with that of the standard oral hypoglycemic agent, glibenclamide.

- Group I** : Rats are provided with normal saline for 28 days orally by using an intragastric catheter tube (IGC).
- Group II** : Diabetic rats are provided with normal saline for 28 days orally by IGC.
- Group III** : Diabetic rats treated with ethanolic leaf extract of *T.dioica* at the dose of 200 mg/kg bw, orally for 28 days consecutively by IGC.
- Group IV** : Diabetic rats treated with ethanolic leaf extract of *T.dioica* at the dose of 400 mg/kg bw, orally for 28 days consecutively by IGC.
- Group V** : Diabetic rats treated with ethanolic fruit extract of *T.dioica* at the dose of 200 mg/kg bw, orally for 28 days consecutively by IGC.
- Group VI** : Diabetic rats treated with ethanolic fruit extract of *T.dioica* at the dose of 400 mg/kg bw, orally for 28 days consecutively by IGC.
- Group VII** : Diabetic rats treated with ethanolic leaf extract of *C.ternatea* at the dose of 200 mg/kg bw, orally for 28 days consecutively by IGC.
- Group VIII** : Diabetic rats treated with ethanolic leaf extract of *C.ternatea* at the dose of 400 mg/kg bw, orally for 28 days consecutively by IGC.
- Group IX** : Diabetic rats treated with combined ethanolic leaf extract of *T.dioica* at the dose of 200 mg/kg bw and leaf extract of *C.ternatea* at the dose of 200 mg/kg bw, orally for 28 days consecutively by IGC.
- Group X** : Diabetic rats treated with combined ethanolic fruit extract of *T.dioica* at the dose of 200 mg/kg bw and leaf extract of *C.ternatea* at the dose of 200 mg/kg bw, orally for 28 days consecutively by IGC.
- Group XI** : Diabetic rats treated with standard drug glibenclamide at the dose of 600 µg/kg bw, orally for 28 days consecutively by IGC.

(vi) Collection of blood and preparation of tissue homogenate

At the end of the treatment, all rats were sacrificed by cervical dislocation. Blood was collected from the experimental animals by direct cardiac puncture. Serum and plasma were separated by

centrifugation at 2500 rpm for 10 min and stored at -20°C until used for the enzyme and biochemical assays. Liver of the sacrificed animals was excised immediately and thoroughly washed with ice cold physiological saline and kept in deep freezer at -20°C till used. The homogenate was filtered and then centrifuged at 10,000 rpm for 20 min at 4°C . The supernatant obtained was used for the estimation of biochemical parameters in liver.

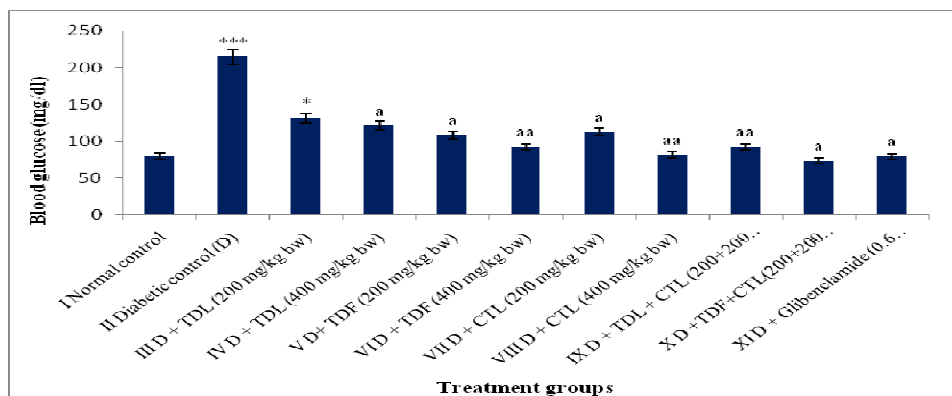
Estimation of biochemical parameters

Blood glucose was estimated by the method²⁰, glucokinase²¹, pyruvate kinase²², glucose-6-phosphatase²³, glucose-6-phosphate dehydrogenase²⁴ and Fructose-1,6-bisphosphatase²⁵.

RESULTS

The levels of blood glucose in normal and experimental rats were shown in Figure 1. The diabetic rats (Group II) showed a significant increase in blood glucose ($p < 0.001$), compared with normal control rats (Group I). The ethanolic extracts of individual, combined extracts and known drug treated groups showed a significant ($p < 0.05$; $p < 0.01$) decrease in the blood glucose level in diabetic treated rats.

Figure - 1
Effect of ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea* on blood glucose in control and experimental rats



TDL – *T. dioica* leaf, TDF – *T. dioica* fruit, CTL – *C.ternatea* leaf Values are reported as mean \pm SD for six animals in each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significance between normal control vs diabetic control and drug treated groups; ^a $p < 0.05$, ^{aa} $p < 0.01$ significance between diabetic control vs drug treated groups; NS: Not significant

Carbohydrate metabolizing enzymes in liver

The carbohydrate metabolizing enzymes activity in liver of control and experimental groups of rats were depicted in Table 1 and 2. Table 1 showed the assay of glucokinase and pyruvate kinase enzymes in liver of control and experimental rats. There was a significant decrease in the activities of glucokinase (hexokinase D) ($p < 0.001$) and pyruvate kinase ($p < 0.01$) in diabetic rats (Group II) when compared to normal control rats (Group I). The plant extracts and glibenclamide treated diabetic rats demonstrated increase in the activities except low dose of plant extracts treated groups for pyruvate kinase. Higher dose of ethanolic

extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea* treated groups showed significant ($p < 0.01$) increase in glucokinase level. Pyruvate kinase activity was significantly ($p < 0.01$) increased in diabetic rats treated with high dose of *T.dioica* fruit extract (Group VI), combined plant extracts (Group IX and X) and reference drug (Group XI). Table 2 showed the assay of glucose-6-phosphatase, fructose-1,6-bisphosphatase and glucose-6-phosphate dehydrogenase enzymes in liver of control and experimental rats. There was a significant ($p < 0.001$) increase in the activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase and highly significant ($p < 0.001$) decrease in glucose-6-phosphate dehydrogenase in diabetic control

rats (Group II) compared to normal control rats (Group I). The plant extracts and glibenclamide treated diabetic rats established a significant ($p < 0.01$; $p < 0.05$) decrease in the activities of glucose-6-

phosphatase and fructose-1,6-bisphosphatase and significant ($p < 0.01$; $p < 0.05$) increase in the activity of glucose-6-phosphate dehydrogenase which were found to be dose dependent.

Table – 1
Effect of ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea* on the activities of carbohydrate metabolizing enzymes in liver of control and experimental groups of rats

Treatment groups	Dose (mg/kg bw)	Glucokinase [#]	Pyruvate kinase ^{##}
I Normal control	Normal saline	274.95±18.36	11.28±0.51
II Diabetic control	Normal saline	113.27±17.86 ^{***}	4.91±0.73 ^{**}
III Diabetic + <i>T.dioica</i> leaf	200	194.86±12.65 ^a	8.24±0.94 ^{NS}
IV Diabetic + <i>T.dioica</i> leaf	400	269.05±14.33 ^{aa}	10.93±0.26 ^a
V Diabetic + <i>T.dioica</i> fruit	200	246.17±15.28 ^{NS}	9.84±0.34 ^{NS}
VI Diabetic + <i>T.dioica</i> fruit	400	286.93±17.92 ^{aa}	13.84±0.77 ^{aa}
VII Diabetic + <i>C.ternatea</i> leaf	200	251.38±15.38 ^a	5.84±0.21
VIII Diabetic + <i>C.ternatea</i> leaf	400	296.22±18.89 ^{aa}	10.93±0.56 ^a
IX Diabetic + <i>T.dioica</i> leaf + <i>C.ternatea</i> leaf	200 +200	283.24±14.93 ^a	13.45±0.14 ^{aa}
X Diabetic + <i>T.dioica</i> fruit + <i>C.ternatea</i> leaf	200 +200	269.54±16.28 ^a	15.16±0.93 ^{aa}
XI Diabetic + Glibenclamide	0.6	256.12±18.25 ^a	12.93±0.67 ^{aa}

Values are reported as mean ± SD for six animals in each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significance between normal control vs diabetic control and drug treated groups; ^a $p < 0.05$, ^{aa} $p < 0.01$ significance between diabetic control vs drug treated groups; NS: Not significant. The enzyme activities are expressed as: [#]μmoles of glucose-6-phosphate formed/ hr/ mg protein. ^{##}μmoles of pyruvate formed/ min/ mg protein.

Table – 2
Effect of ethanolic extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea* on the activities of carbohydrate metabolizing enzymes in liver of control and experimental groups of rats

Treatment Groups	Dose (mg/kg bw)	Glucose-6-phosphatase [#]	Fructose-1,6-bisphosphatase [#]	Glucose-6-phosphate dehydrogenase ^{##}
I Normal control	Normal saline	985.67±29.28	414.34±21.67	9.81±0.64
II Diabetic control	Normal saline	1846.76±48.27 ^{***}	1073.19±48.27 ^{***}	2.69±0.15 ^{***}
III Diabetic + <i>T.dioica</i> leaf	200	1236.53±40.51 ^{a*}	639.96±30.91 ^{*aa}	5.91±0.26
IV Diabetic + <i>T.dioica</i> leaf	400	1013.28±39.46 ^{aa}	432.16±33.96 ^{aa}	8.46±0.13 ^a
V Diabetic + <i>T.dioica</i> fruit	200	1124.93±28.56 ^a	516.83±20.62 ^a	7.84±0.21 ^a
VI Diabetic + <i>T.dioica</i> fruit	400	971.26±30.36 ^{aa}	405.19±19.11 ^{aa}	10.11±0.35 ^{aa}
VII Diabetic + <i>C.ternatea</i> leaf	200	1183.92±46.93 ^a	584.39±16.51 ^{aa}	6.36±0.83 ^a
VIII Diabetic + <i>C.ternatea</i> leaf	400	1018.16±36.95 ^{aa}	436.13±18.39 ^{aa}	8.16±0.14 ^a
IX Diabetic + <i>T.dioica</i> leaf + <i>C.ternatea</i> leaf	200 +200	926.84±26.13 ^{aa}	475.28±15.92 ^{aa}	11.94±0.26 ^{aa}
X Diabetic + <i>T.dioica</i> fruit + <i>C.ternatea</i> leaf	200 +200	975.16±29.67 ^{aa}	398.13±10.54 ^{aa}	10.26±0.13 ^a
XI Diabetic + Glibenclamide	0.6	1004.86±34.63 ^a	424.68±28.16 ^{aa}	9.14±0.24 ^a

Values are reported as mean ± SD for six animals in each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significance between normal control vs diabetic control and drug treated groups; ^a $p < 0.05$, ^{aa} $p < 0.01$ significance between diabetic control vs drug treated groups; NS: Not significant. The enzyme activities are expressed as: [#]μmoles of Pi liberated/ hr/ mg protein. ^{##}μmoles of NADPH formed/ min/ gm tissue.

DISCUSSION

In the present study, the STZ-induced diabetic rats (Group II) elicited significant rise in blood glucose to a level of 62.92% compared to normal control rats (Group I).

On the contrary, diabetic rats treated with ethanolic extracts of individual and combined extracts of leaf and fruit of *T.dioica* and leaf of *C.ternatea* and standard drug glibenclamide

for 28 days, exhibited decrease in blood glucose level. It was observed that ethanolic extracts reversed this effect in diabetic animals. STZ-induced diabetes exhibit most of the diabetic complications mediated through oxidative stress involving in pancreatic cell destruction^{26,27}. The ethanolic extracts contain phytochemicals such as flavonoids, total phenolics, tannins, glycosides and alkaloids which might have played an important role in reduction of oxidative stress of pancreatic β -cells. This might have lead to increased glucose metabolism. Another possible mechanism may be by potentiation of the insulin from β -cells of islets of Langerhans or its responsiveness²⁸. Hexokinase is universally present in cells of all types. Hepatocytes contain a form of hexokinase called hexokinase D or glucokinase that is more specific for glucose and differ from other forms of hexokinase in kinetic and regulatory properties²⁹. Glucokinase is the rate limiting enzyme catalyses the phosphorylation of glucose to glucose-6-phosphate in glycolysis and plays a central role in regulation of hepatic glucose storage and disposal³⁰. Following a carbohydrate rich meal, hepatic glucokinase clears a significant amount of glucose from the blood circulation and facilitates its conversion into glycogen and fatty acids^{31,32}. Thus, hepatic glucokinase play a significant role in the prevention of postprandial hyperglycemia. In the present study, the decreased glucokinase activity in diabetic rats revealed the impairment of the enzyme. Being an insulin-dependent enzyme, the hepatic glucokinase activity of diabetic rats is almost entirely inhibited or inactivated due to the absence of insulin³³. Impairment of glucokinase activity suggests the impaired oxidation of glucose *via.*, glycolysis, weakens peripheral glucose utilization and augmented hepatic glucose production, leading to its accumulation, resulting in hyperglycemia³⁴. The increased activity of glucokinase in plant extracts and known drug treated groups suggested a shift towards carbohydrate metabolism and it enhanced the utilization of glucose at peripheral sites. Pyruvate kinase is regulated at the mRNA levels in insulin dependent diabetes³⁵. The leaf and fruit of *T.dioica* and leaf of *C.ternatea* and

glibenclamide treated diabetic rats showed increased activity of pyruvate kinase that may increase the utilization of glucose. The finding suggested that the leaf and fruit of *T.dioica* and leaf of *C.ternatea* were improvable to the glucose metabolism by increased utilization of glucose. Glucose-6-phosphatase, a crucial gluconeogenic enzyme, is mainly found as an integral protein in the lumen of the endoplasmic reticulum of hepatocytes that catalyzes the dephosphorylation of glucose-6-phosphate to glucose in the liver³⁶. Fructose-1,6-bisphosphatase is another gluconeogenic enzyme that catalyzes one of the irreversible steps in gluconeogenesis and serves as a site for the regulation of gluconeogenesis³⁷. In the present study, activities of these enzymes were increased significantly in diabetic rats which might be due to the activation or increased synthesis of these enzymes contributing to the increased glucose production during diabetes³⁸. It could also be due to insulin deficiency. Oral administration of ethanolic extracts of leaf and fruit of *T.dioica*, leaf of *C.ternatea* and combined extracts of *T.dioica* leaf + *C.ternatea* leaf and *T.dioica* fruit + *C.ternatea* leaf reversed the glucose-6-phosphatase and fructose-1,6-bisphosphatase activities in STZ-induced diabetic rats which illustrate improved glycemic control. Glucose-6-phosphate dehydrogenase is a key enzyme which catalyses the first and rate limiting step of the hexose monophosphate (HMP) shunt. A decrease in the activity of glucose-6-phosphate dehydrogenase has been observed in diabetic rats. Treatment with ethanolic extracts of plants increased the activity of the enzyme, *via.*, increased secretion of insulin which increases the influxes of glucose into pentose monophosphate shunt in an attempt to reduce high blood glucose levels. This results in an increased production of the reducing agent, NADPH, with concomitant decrease in oxidative stress³⁹.

CONCLUSION

Based on the results obtained in the present investigation, it may be concluded that the leaf and fruit of *T.dioica* and leaf of

C.ternatea contains bioactive compounds that may serve as antidiabetic therapy due to

its antihyperglycemic property.

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