

# An Evaluation of antidiabetic activity of *Tinospora cordifolia*

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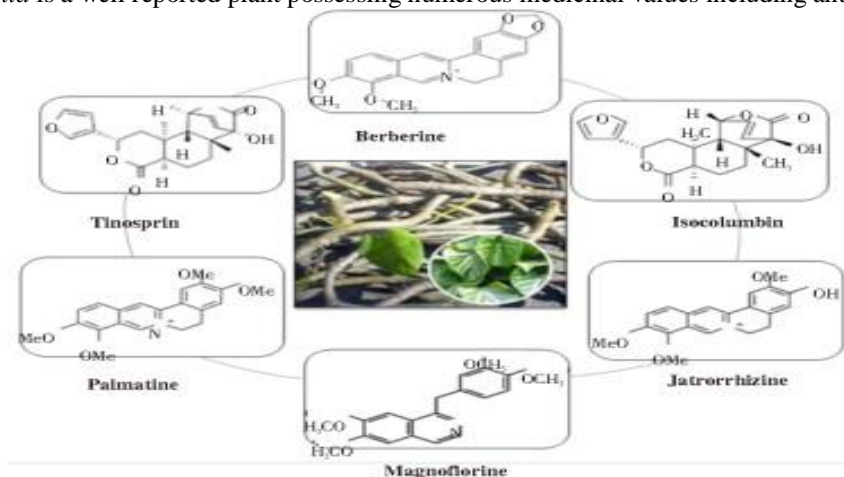
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**ABSTRACT:** Currently, available conventional options for diabetes mellitus have certain limitations of their own, and options from medicinal plants with antihyperglycemic activities are being searched to meet the need. Antidiabetic properties of *Tinospora cordifolia* are highly appreciated in Ayurveda and even in recent modern researches. Several studies on its extracts (*viz.* immune- modulatory, anti-hyperglycemic, antioxidant, adaptogenic, hepatoprotective, hormone regulator *etc.*) and isolated phytoconstituents (like tinosporin, berberine, jatrorrhizine *etc.*) have reported that it is a preventive and curative antidiabetic herb, which are substantiated by clinical trials. Scattered information pertaining to antidiabetic potential of *Tinospora* is reported. Present review encompasses (i) in-depth information of reported antidiabetic activities of the plant in light of available experimental and clinical studies, and (ii) understanding on the possible mechanism of its action in combating the complex pathology of diabetes.

**KEYWORDS:** *Tinospora cordifolia*, antidiabetic, ayurvedic, diabetes, hormone, medicinal plant, antioxidant

## I. INTRODUCTION

*Tinospora cordifolia* is a well reported plant possessing numerous medicinal values including anti-diabetic property.



*Tinospora cordifolia* (antidiabetic claims)

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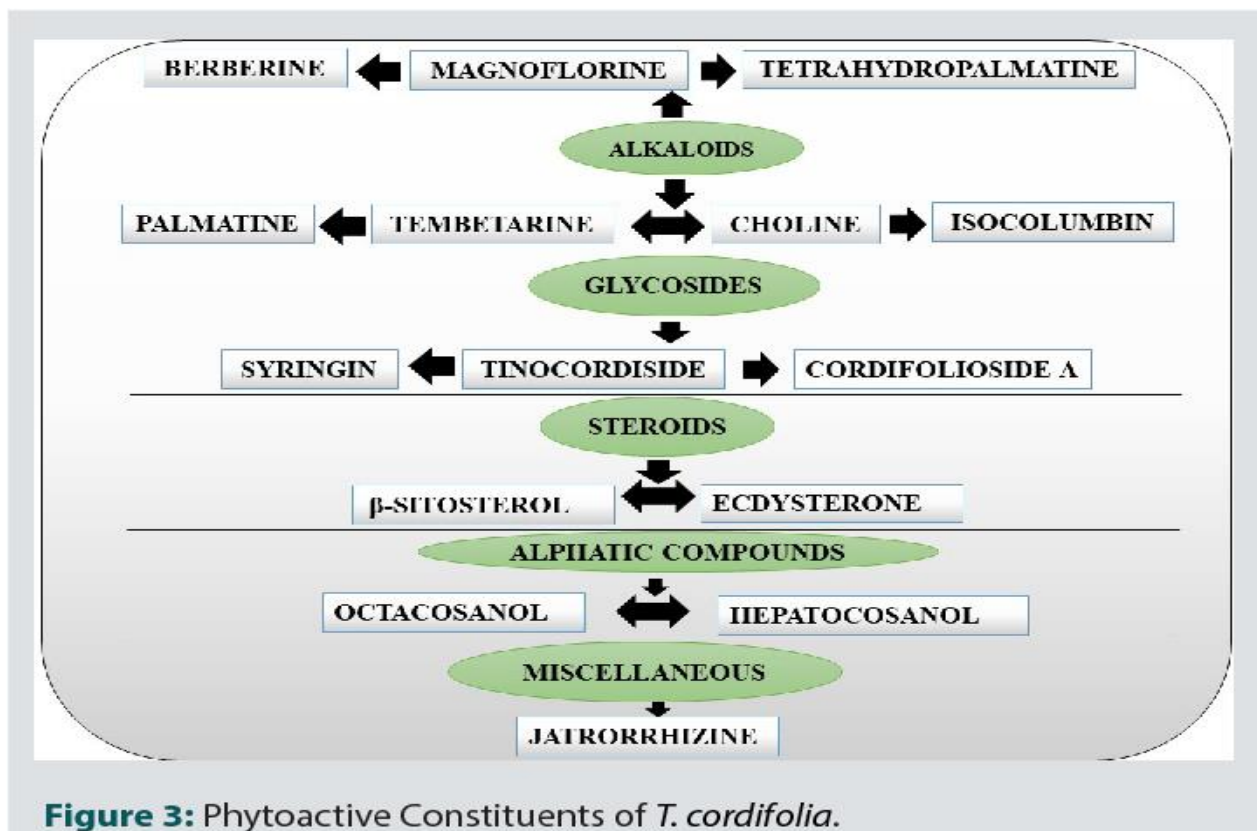
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Aim of the present study is to study the mechanism of action of *Tinospora cordifolia* and its active compound in differentiated myocytes, L6 cells. Key marker of diabetes in cells is the insulin dependent glucose transporter-4 (Glut-4) which also responds to exogenous chemicals, and is over expressed up to 5- and 4-fold, by *Tinospora cordifolia* and palmatine.

Next to Glut-4, the predominant protein influencing glucose metabolism is PPAR $\alpha$  and  $\gamma$  whose expressions were also positively modulated. Further, the inhibitors of insulin pathway prevented glucose uptake mediated by *Tinospora cordifolia* and palmatine which shows that the activity is majorly mediated through insulin pathway. *Guduchi* (*Tinospora cordifolia* [Willd.] Miers) is a flagship rejuvenating herb of Ayurveda with reported anti-diabetic potential. In the present study, different dosage forms of *Guduchi* stem (growing on neem tree) were developed by adopting Ayurvedic pharmaceutical process of *Bhavana* (levigation). *Guduchi Churna* (GC) was subjected to 07 times *Bhavana* separately with its own extracted juice, decoction and potable water, and dosage forms namely *Svarasa Bhavita Guduchi Churna* (SBGC), *Kwatha Bhavita Guduchi Churna* (KBGC), and *Jala Bhavita Guduchi Churna* (JBGC) were prepared.



**Figure 3:** Phytoactive Constituents of *T. cordifolia*.

The present study was aimed to evaluate the role of *Bhavana* on the potentiation of therapeutic properties

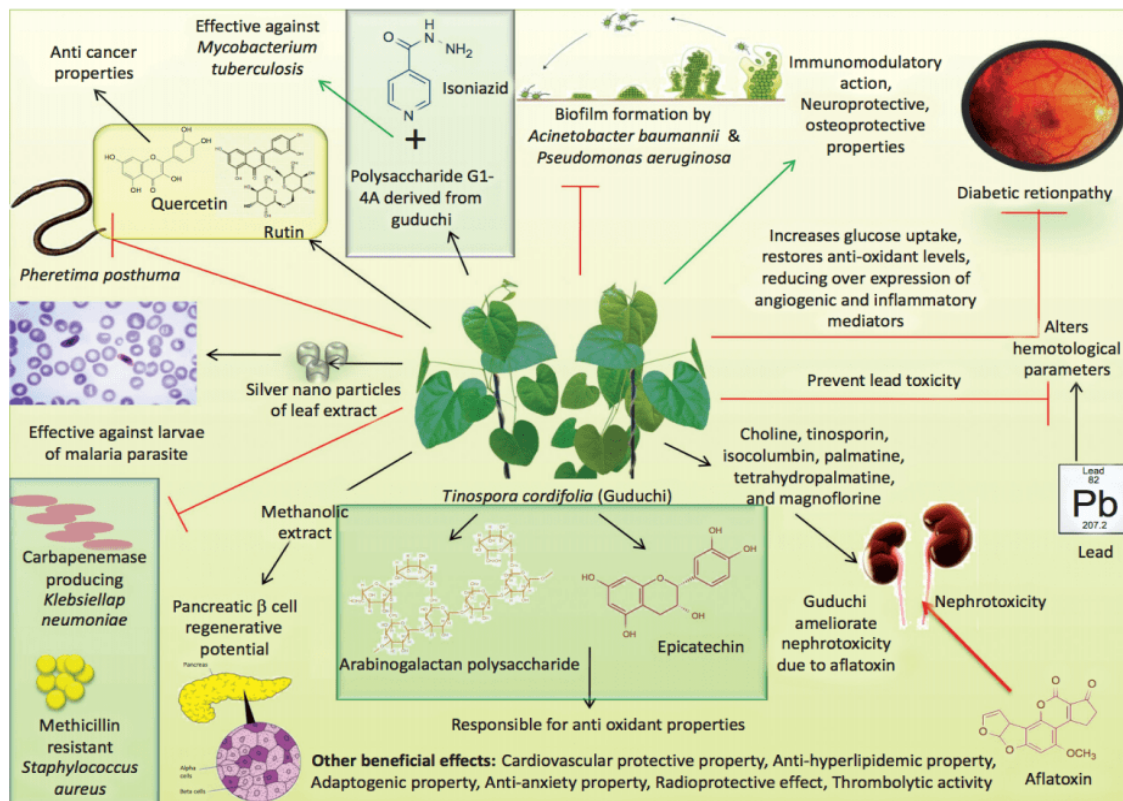
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of *Guduchi*. Sequential solvent extracts (5, 10, 15 and 25%) of GC, SBGC, KBGC and JBGC were prepared in different solvents [phosphate buffer, hexane, dichloromethane (DCM), chloroform] and screened for the  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity. The results revealed that phosphate buffer and DCM extracts of SBGC exhibited strong  $\alpha$ -amylase inhibitory potential (>80% inhibition at 25% concentration) followed by KBGC, JBGC and GC with reference to the standard acarbose. In  $\alpha$ -glucosidase inhibitory activity, maximum inhibition was observed in DCM and chloroform extracts of SBGC (>85% inhibition at 25% concentration), followed by KBGC (>80% inhibition at 25% concentration), JBGC and GC. *In vivo* anti-hyperglycemic studies were carried out by oral glucose tolerance test in Swiss albino mice. Test drugs (JBGC, KBGC, SBGC) treated groups showed marginal decrease of blood glucose levels in normo glycaemic mice. However, the blood glucose level in test drug JBGC, KBGC and SBGC treated groups was still within normal range in overnight fasted mice. In oral glucose tolerance test, among all dosage forms SBGC (51.08%) produced pronounced anti-hyperglycemic effect followed by KBGC (42.57%) at a dose of 520 mg/kg. The GC, JBGC, KBGC and SBGC samples were also standardized using berberine (a well established anti-diabetic compound) as a marker compound by HPTLC fingerprint analysis. Findings of the present study indicate that SBGC and KBGC can be used in the treatment of diabetes mellitus and gives supporting evidence to Ayurvedic claims that the *Bhavana* process has pharmaceutico-therapeutic significance in Ayurvedic drug development(1,2)



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To the best of our knowledge, to date, there is no scientific evidence on the inhibitory effects of any dosage form or formulation of *Guduchi* on carbohydrate hydrolyzing enzymes. Also, no reports are available exploring anti-hyperglycemic potential of *Bhavita* dosage form of *Guduchi*. Considering all these, the present study was planned to validate the classical uses of *Guduchi* and its preparations in diabetes and understand the role of *Bhavana* in herbal drug potentiation, especially whether *Bhavana* process could augment the therapeutic potential of *Guduchi Churna* (GC) to manage glycemic levels. GC was subjected to 07 times *Bhavana* separately with its own extracted juice, decoction and potable water, and the dosage forms namely *Svarasa Bhavita Guduchi Churna* (SBGC), *Kwatha Bhavita Guduchi Churna* (KBGC), and *Jala Bhavita Guduchi Churna* (JBGC) were prepared. The third trial preparation JBGC was prepared (by subjecting *Bhavana* to GC with potable water) to obtain the same particle size reduction as in first two groups and to understand the effect of *Bhavana*. These preparations were comparatively evaluated for *in-vitro*  $\alpha$ -amylase,  $\alpha$ -glucosidase inhibitory activities and *in-vivo* anti-hyperglycemic potential with High Performance Thin Layer Chromatography (HPTLC) profiling.(3,4)

## II. DISCUSSION

Experimental induction of diabetes To induce diabetes, the rats were fasted for 16 h and injected with freshly prepared streptozotocin (STZ) (Sigma Chemicals, USA) at the dose of 55 mg/kg b.w. intravenously (6) in 0.1 M citrate buffer of pH 4.5 (7).



### *Guduchi (Tinospora cordifolia)*

Control animals received citrate buffer alone. Diabetes status was confirmed by estimating the fasting blood glucose levels and urine glucose (Benedict's test) after 72 h of STZ injection. Animals showing fasting blood glucose levels above 250 mg/dL were selected for this study. Drug treatment Single dosage of either aqueous extract (dissolved in normal saline) or alcoholic extract (dissolved in gum acacia) (5) was given orally for 10 days and 30 days to specific groups through oral intubations and the control animals received the vehicle with TC. Lante zinc insulin (6 units/kg. b.w. i.p) was given to the specific group daily (8). Biochemical and histological studies At the end of the experimental period, the animals were killed by cervical decapitation. Fasting blood samples and serum were collected. Serum was also collected 1 h after giving the drug in the specific group. The liver was removed and kept in a deep freeze ( $-20^{\circ}\text{C}$ ) and later used for the estimation of enzymes. The pancreas was removed and was fixed in 10% buffered formalin for the histological studies. Fasting blood glucose was estimated by the glucose oxidase method described by Trinder (9). Glycogen synthase (EC 3.1.3.42) and glycogen phosphorylase (EC 2.4.1.1)

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activities in the liver were estimated as described by Leloir and Goldenberg (10) and Cornblath et al. (11), respectively. Histology studies of pancreas were carried out according to the procedure reported by Culling (12). Serum insulin levels were assayed using a standard Mercodia Rat.(5)



**A.**



**B.**



**C.**



**D.**



**E.**



**F.**

Insulin ELISA enzyme immunoassay kit from Mercodia, Sweden (cat. no-10-1124-01). Statistical analysis Statistical significance between the different groups was determined using one way analysis of variance (ANOVA) followed by Tukey's multiple comparisons by fixing the P value as  $< 0.05$

The present study clearly showed that TC has significant ( $P < 0.05$ ) anti-diabetic activity in diabetic animals and has an efficacy of 40% to 80% compared to insulin. TC administration in diabetic animals did not show any increase in serum insulin levels or regeneration of pancreatic  $\beta$  cells but showed increased hepatic glycogen synthase and decreased glycogen phosphorylase activity. Fasting hyperglycemia is a hallmark of DM. It has been postulated but is still debated that the fasting hyperglycemia in non-insulin dependent DM arises from the hepatic overproduction of glucose (13). However, studies by Jae et al. (14) suggest that the post-absorptive hyperglycemia in STZ diabetic rats is largely due to decreased peripheral glucose clearance, while increased hepatic glucose output might also be a contributing factor at a very high STZ dose. In the present study, the streptozotocin induced diabetic animals showed elevated fasting blood glucose levels (Table 1). Insulin treatment in these diabetic rats could not return the blood glucose to normal levels during the study period although blood glucose was decreased notably. The reason for this

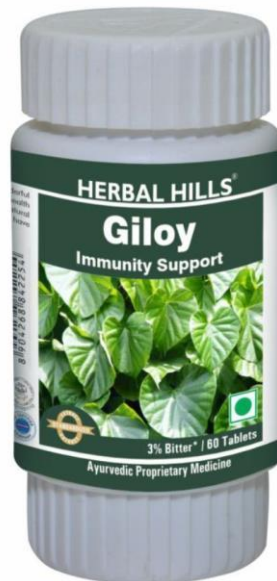
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observation may be that the insulin was injected almost 24 h before the blood sample was taken and during this gap period the insulin injected (single dose) might have been inactivated or the dosage used might have been insufficient. Treatment with TC in diabetic rats could significantly reduce the fasting blood glucose levels after 10 and 30 days of treatment. However, blood glucose in these treated groups was at elevated levels compared to insulin treated diabetic rats and controls. The efficacy of this anti-diabetic activity of TC was fairly good compared to insulin. (8,9)



The importance of the liver in the regulation of carbohydrate metabolism is recognized by its ability to store carbohydrates in the form of glycogen (glycogenesis) and to release them in the form of glucose (glycogenolysis) when needed. These processes are regulated by 2 key enzymes: glycogen synthase and glycogen phosphorylase. It is reported that in diabetic animals the glycogen synthase activity was decreased whereas phosphorylase activity increased (15). In the present study, the liver glycogen synthase activity decreased and phosphorylase activity increased substantially in untreated diabetic rats during the study period. The treatment with TC as well as insulin showed effects on the glycogen synthase and phosphorylase activity in these animals. These increased glycogen synthase activities in the liver of TC treated diabetic animals may indicate that the TC decreases blood sugar by increasing the glycogen storage in the liver. It seems that, since the TC treatment could not normalize the phosphorylase activity in the liver, the blood glucose was at elevated levels in TC treated diabetic rats in spite of the enhanced glycogen synthase activity. STZ is well known for its selective beta cell cytotoxicity. It is reported that in STZ induced diabetic rats the beta cell count/islet and the serum insulin levels decrease considerably (7). In the present study, the serum insulin levels also decreased drastically in untreated diabetic rats. Treatment with TC by administration of a single dosage of extract or for 10 and 30 days did not increase the serum insulin levels in diabetic animals. In addition, the histological examination of endocrine pancreas in TC treated diabetic rats (Figure) did not

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reveal any evidence of regeneration of beta cells of islets of Langerhans. The histology section of the pancreas of the drug treated control rats showed the normal architecture of the islets of Langerhans with the granulated beta cells appearing dark. The histology of the pancreas in diabetic rats showed small and shrunken islets of Langerhans. Destruction of beta cells was observed in this section. The histology of the pancreas in TC treated diabetic rats showed a similar architecture to that of diabetic rats. There was no considerable change in the architecture of the islets of Langerhans after the TC treatment. It appears that there was no regeneration of beta cells after the TC treatment; therefore, it appears that the antihyperglycemic activity of TC is not through the insulin secretion and is independent of insulin secretion by pancreatic beta cells. Moreover, in the present study, treatment with 400 mg of extracts of TC in control rats for 30 days did not show any adverse effects on the normal histology of the pancreas. Since the present study has some limitations by having limited experimental sample size and methodology, this observation may be suggestive of a need for further research in the future to examine the adverse effects of this drug.(10,11)

### III. RESULTS

Now a day Diabetes mellitus is one of the most popular diseases in research field. Diabetes is metabolic disease which is also the cause of various lives threatening disease. Medical science is very much advanced now but still we have no medicine without insulin for procurement of this disease. The medicines which are mainly using against diabetes have more side effect than that of efficiency. That's why we are going for natural or traditional medicine.



*Tinospora cordifolia* has significant anti-diabetic activity in diabetic animals and has an efficacy of 50% to 58% compared to insulin. The alkaloid rich fraction of *Tinospora* including palmatine has been reported for insulin-mimicking activity which ultimately helps to release insulin from corresponding cell in vitro and in vivo. Different animal model study has been done for reviewing the antidiabetic activity of *Tinospora* plant. In a study diabetes is induced by injecting alloxan monohydrate (180 mg/kg bw) intraperitoneally and *Tinospora cordifolia* extract (20ml/kg bw) was administered orally twice a day. The whole plant extract of *Tinospora* very significantly ( $p < 0.001$ ) reduces the blood glucose level. In another study rats were injected with streptozotocin of 55 mg/kg b.w. intravenously to make them diabetic and the extract of *Tinospora* was administered in different concentration. The extract of *Tinospora cordifolia* has significant ( $P < 0.05$ ) anti-diabetic activity in diabetic animals and has an efficacy of 40% to 80% compared to insulin. In another study it has been found that the extract inhibits alpha glucosidase which ultimately show anti-diabetic properties. *Tinospora cordifolia* has various other biological

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activities but we have been focused on its anti-diabetic activity. The future prospect is to elucidate the structure of the chemical constituents which are mainly responsible for anti-diabetic activity. In future we may also modify the activity of those constituents by QSAR, pharmacophore study.(12,13)

## IV. CONCLUSIONS

Antidiabetic activity of plant and callus extracts of *T. cordifolia* were investigated by estimating degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically at 520nm. Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. 1 ml each of above solution was mixed. 25, 50 and 100µg/ml of native plant and callus extracts concentrations were added to above mixture. Mixture was incubated in dark at room temperature for 72 hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520nm. Metformin was used as a standard drug for assay. % inhibition was calculated Bailey and Day, 1989 reported that the human bodies possess enzymatic and non-enzymatic antioxidativemechanisms which minimize the generation of reactiveoxygen species, responsible for many degenerativediseases including diabetes. Increased concentration of glucose in the blood leads to its binding to hemoglobinwhich may result in the formation of the reactive oxygenspecies. Plant extracts play an important role theinhibition of the glycosylation end products. An increasein the glycosylation was observed on incubation of hemoglobin with the increasing concentration of the glucose over a period of 72hrs (Table 1). However, the plant extracts significantly inhibited the haemoglobin glycosylation which is indicated by the presence of increasing concentration of haemoglobin. The leaf callus extracts exhibited higher inhibition of glycosylation (75% in 100µg/ml) as compared with the standard drug 88% in 100µg/ml). The plant extracts also displayed the inhibition of haemoglobinglycosylation at different physiological concentrations of the glucose over the period of 72 hrs, indicating that the plant extracts decreases the formation of theglucose- haemoglobin complex and thus amount of free haemoglobin increases.(14,15)

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