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NAGARAJA PURANIK

KARARASHAH FAKRUDDIN KAMMAR

SHEELA DEVI

TÜBİTAK

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Anti-diabetic activity of Tinospora cordifolia (Willd.) in streptozotocin diabetic rats; does it act like sulfonylureas?

Nagaraja PURANIK[1], Kararashah Fakruddin KAMMAR[2], Sheela DEVI[1]

Aim: As an alternative medicine to treat diabetes mellitus, many herbal drugs are being studied throughout the world. In the present study, an attempt was made to investigate the anti-diabetic activity of *Tinospora cordifolia* (Willd.) (TC) stem extracts (both aqueous and alcoholic) in different dosages (200 and 400 mg/kg b.w.) in streptozotocin diabetic albino rats. The probable mechanism by which TC may act as an anti-hyperglycemic drug was also investigated.

Materials and methods: The drug was administered orally for 10 days and 30 days in different groups of animal, with each group containing 6 animals. The efficacy of this drug was compared with the Lante Zinc Insulin (6 U/kg b.w. daily, i.p.) treated diabetic rats. The serum insulin levels, histology of endocrine pancreas and activity of key enzymes of glucose metabolism, i.e. glycogen synthase and glycogen phosphorylase, were studied.

Results: Our 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 cause any increase in serum insulin levels or regeneration of pancreatic β cells but caused increased hepatic glycogen synthase and decreased glycogen phosphorylase activity.

Conclusion: The probable mechanism by which TC may act as an anti-hyperglycemic drug is not through insulin secretion like sulfonylureas. It may be through some peripheral mechanisms, such as increasing the glycogen storage in the liver or decreasing the glucose release from the liver.

Key words: *Tinospora cordifolia*, diabetes mellitus, anti-diabetic, hypoglycemia, streptozotocin

Introduction

Diabetes mellitus (DM) is a metabolic disorder that affects people of all age groups and from all walks of life. There are an estimated 150 million people worldwide suffering from diabetes (1), which is almost 5 times more than the estimated number 10 years ago.

Management of diabetes without any side effects is still a challenge in the medical field, as presently available drugs for diabetes have one or more adverse effects (2). Since the existing drugs for the treatment of DM do not satisfy our need completely, the search for new drugs continues. In recent years, herbal remedies for the unsolved medical problems have been gaining importance in the research field. Although many researchers have studied the anti-diabetic activity of *Tinospora cordifolia*, no satisfactory study was conducted to investigate its efficacy in streptozotocin induced diabetic rats or to explore how this drug acts as an anti-diabetic agent. Thus, this study was undertaken to explore the efficacy of anti-diabetic activity of *Tinospora cordifolia* in diabetic rats. The possible mechanism by which this drug may act is discussed in this study.

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[1] Department of Physiology, Dr. ALM. PG. Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai, Tamilnadu, SOUTH INDIA
[2] Department of Physiology, Karnataka Institute of Medical Sciences, Hubli, Karnataka, Pin code – 580 022, SOUTH INDIA
Correspondence: *Nagaraja PURANIK, Department of Physiology, Karnataka Institute of Medical Sciences, Hubli, Karnataka, Pin code – 580 022, SOUTH INDIA*
E-mail: puranik_nk@yahoo.com

Tinospora cordifolia (Willd.) Miers ex Hook. F. & Thoms. (TC) belongs to the family Menispermaceae and is known as Gulancha in English, Guduchi in Sanskrit, and Giloya in Hindi. It is a large, glabrous, deciduous climbing succulent shrub, commonly found in hedges. It has been known for long in the Ayurvedic literature (i.e. the system of traditional medicine native to India and practiced in other parts of the world as a form of alternative medicine) as a tonic and vitalizer and as a remedy for diabetes and other metabolic disorders (3,4).

Materials and methods

Plant material and extraction

Tinospora cordifolia stem was collected fresh from the forest areas in Udupi- district, Karnataka state, South India, and dried in the shade and then powdered. The plant was identified by Professor and Head of the Department of Botany, Mangala Gangothri, Mangalore University. A specimen (Voucher No. 31) was deposited in the botany department museum. The powdered materials were kept in an air-tight container in a refrigerator until the time of use.

Aqueous and alcoholic extracts of TC were prepared according to the standard extract procedure (5). The yield of extracts was approximately 8.5% and 7%, respectively.

Animals

Female albino rats of inbred Wistar strain (body wt. 180-210 g) were used in this study. Animal ethical committee clearance was obtained from Institutional Animal Ethics Committee (IACE No. 08/004/02). The animals were fed on a pellet diet (Hindustan Lever Ltd. Bangalore) and water ad libitum throughout the study period. All the experiments were carried out in between 8 and 10 A.M in order to avoid circadian rhythm induced changes.

Experimental groups

All the experimental animals were divided into 7 groups with each group consisting of 6 animals as follows:

Group 1- Control**:** This group was used for studying the baseline values of the parameters studied. **Group 2**- Diabetic control**:** This group consisted of streptozotocin induced diabetic rats. **Group 3**- Diabetic rats treated with (200 mg/kg b.w.) aqueous extract of TC. **Group 4**- Diabetic rats treated with (400 mg/kg b.w.) aqueous extract of TC. **Group 5**- Diabetic rats treated with (200 mg/kg. b.w) alcoholic extract of TC. **Group 6**- Diabetic rats treated with (400 mg/kg. b.w.) alcoholic extract of TC. **Group 7**- Diabetic rats treated with insulin.

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). 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 °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) 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

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.

Results and discussion

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 β 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 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.

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 (Table 2) 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

Table 1. Effect of TC extracts on fasting blood glucose and comparison of efficacy of TC extracts with insulin (values expressed in % amelioration towards normal levels).

	Days	Control	Diabetic control	Diabetic +Aq.200	Diabetic +Aq.400	Diabetic +Al. 200	Diabetic +Al. 400	Diabetic +Insulin
Fasting blood	11th day	89.17 ± 5.1*	353.24 ± 4.5*^{a}	162.68 ± 12.7*^{a}	221.74 ± 5.2*^{a}	196.45 ± 5.6*^{a}	215.57 ± 4.4*	126.21 ± 2.8
glucose (mg/ dL)	31st day	89.60 ± 2.5*	346.97 ± 5.5*^{a}	190.69 ± 6.5*^{a}	236.87 ± 3.0*^{a}	228.90 ± 2.9*^{a}	214.27 ± 2.9*	138.20 ± 2.1
Efficacy (%)	11th day	-	-	72.16	49.79	59.37	52.13	85.97
	31st day	-	-	60.72	42.77	45.87	51.56	81.11

Data expressed as mean ± SD (n = 6) P < 0. 05

* Control vs. other groups

[a] Diabetic control vs. TC treated diabetic groups.

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 (Table 3) 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 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

Table 2. Effect of TC extracts on hepatic glycogen phosphorylase, glycogen synthase activities, and comparison of efficacy of TC extracts with insulin (values expressed in % amelioration towards normal levels).

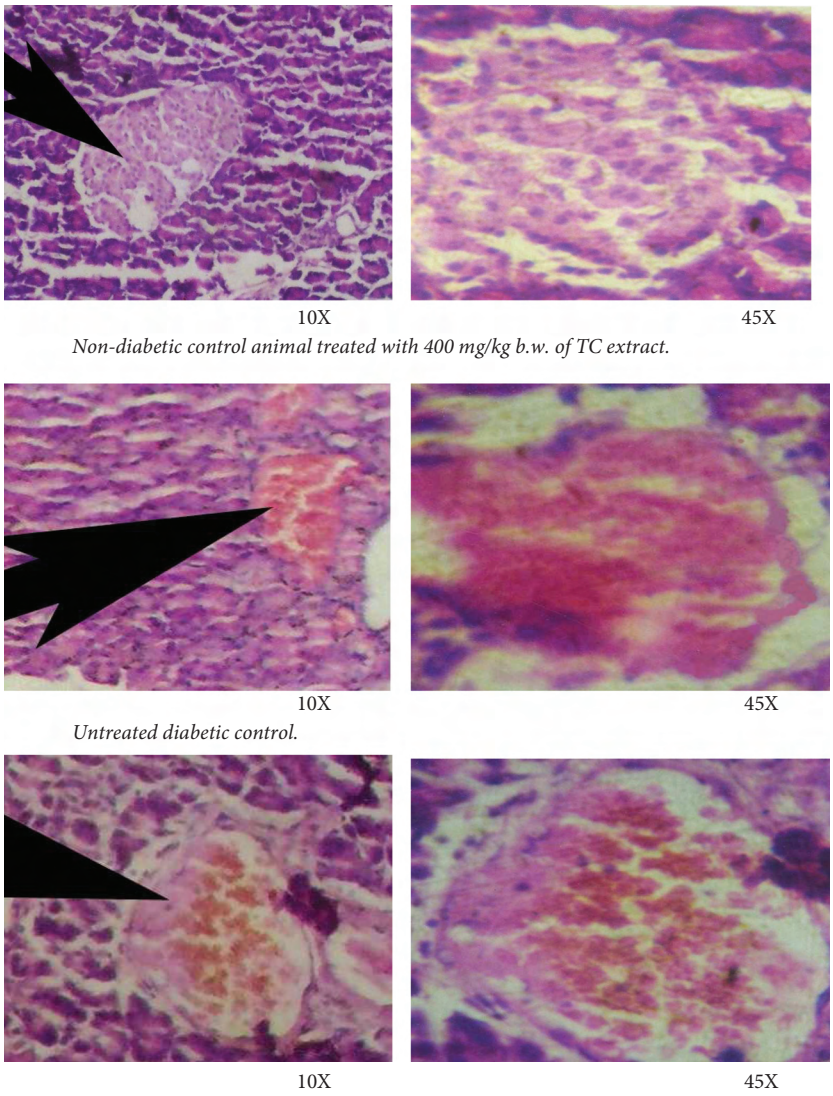
	Days	Control control	Diabetic +Aq.200	Diabetic +Aq.400	Diabetic +Al. 200	Diabetic +Al. 400	Diabetic +Insulin	Diabetic
Glycogen synthase activity (µmol of UDP formed/mg protein/h).	11th day	659.88 ± 6.08*	918.65 ± 4.08*[a]	735.82 ± 5.53*[a]	742.35 ± 4.89*[a]	741.78 ± 2.76*[a]	744.15 ± 3.71*	694.12 ± 2.86
	31st day	660.12 ± 3.99*	910.05 ± 4.16*[a]	730.15 ± 3.05*[a]	735.07 ± 3.19*[a]	742.90 ± 3.68*[a]	739.67 ± 3.36*	701.87 ± 2.46
Efficacy (%)	11th day	-	-	70.65	68.12	68.35	67.43	86.77
	31st day	-	-	71.97	70.01	66.87	68.17	83.29
Glycogen synthase activity (µmol of UDP formed/mg protein/h).	11th day	179.85 ± 4.9*	48.98 ± 3.7*[a]	93.93 ± 2.1*[a]	80.41 ± 2.4*[a]	90.43 ± 2.4*[a]	83.33 ± 1.8	177.03 ± 6.4
	31st day	177.08 ± 4.00*	54.56 ± 2.5*[a]	94.11 ± 2.7*[a]	80.56 ± 4.6*[a]	91.20 ± 3.9*[a]	87.40 ± 2.1	179.87 ± 4.2
Efficacy (%)	11th day	-	-	34.34	24.01	31.67	26.24	97.85
	31st day	-	-	32.28	21.22	29.90	26.80	98.77

Data expressed as mean ± SD (n = 6) P < 0. 05
* Control vs. other groups
[a] Diabetic control vs. TC treated diabetic groups.

Table 3. Effect of TC extract on serum insulin levels (pmol/L).

	Control	Diabetic control	Diabetic +Aq.200	Diabetic +Aq.400	Diabetic +Al. 200	Diabetic +Al. 400
Fasting	473.11 ± 12.6	135.98 ± 13.08*	49.63 ± 10.20	147.5 ± 12.38	151.95 ± 6.68*	150.48 ± 10.68
1 h after giving TC	470.56 ± 16.78*	142.83 ± 6.7*	145.16 ± 12.9*	150 ± 15.6*	146.16 ± 8.8*	148.33 ± 12.7
On 11th day	473.32 ± 13.2*	135.98 ± 10.40*	149.63 ± 8.66*	147.50 ± 10.70*	151.95 ± 12.91*	150.48 ± 7.00
On 31st day	460.08 ± 9.66*	147.50 ± 7.89*	160.74 ± 8.08*	163.83 ± 12.81*	160.89 ± 12.02*	159.65 ± 9.54

Data expressed as mean ± SD (n = 6) P < 0. 05
* Control vs. other groups
[a] Diabetic control vs. TC treated diabetic groups.



10X 45X

Non-diabetic control animal treated with 400 mg/kg b.w. of TC extract.

10X 45X

Untreated diabetic control.

10X 45X

Diabetic animal treated with TC

Figure. Effect of TC on endocrine pancreas.

there was no regeneration of beta cells after the TC treatment; therefore, it appears that the anti-hyperglycemic 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.

Conclusion

Tinospora cordifolia is an effective anti-hyperglycemic drug that can be used in the treatment of DM. Although its activity is feeble compared to insulin, it can be used as a supportive drug in the treatment of DM. The anti-diabetic activity of TC is not through the insulin secretion by pancreatic beta cells. It may be due to the increased entry of glucose into the peripheral tissues and organs like the liver. Since TC increased the activity of glycogen synthase in the liver, it may increase the storage of glucose in hepatocytes. It also decreased the activity of phosphorylase in the liver; thereby it may prevent the

release of glucose into the blood. These observations strongly suggest that TC may not act like sulfonylureas, but like other oral anti-hyperglycemic drugs. This study indicates that treatment with TC may be an alternative to some of the presently available drugs, which have some adverse effects.

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