Biomedical Research 2008; 19 (2): 92-96

Efficacy of *Tinospora cordifolia* (Willd.) extracts on blood lipid profile in streptozotocin diabetic rats. Is it beneficial to the heart?

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Keywords: *Tinospora cordifolia*, Diabetes mellitus, Streptozotocin, Metabolic derangements, Hyperlipidemia.

Accepted May 19 2008

Abstract

Efficacy of *Tinospora cordifolia* (Willd.) stem extracts (both aqueous and alcoholic) in different dosages (200 and 400 mg/ kg b.w) on blood lipid profile in streptozotocin induced diabetic albino rats was investigated in this study. The drug was administered orally for 10 days in 24 rats of 4 different groups treated with *Tinospora cordifolia*. Similarly, in another group of study consisting of 24 rats, the drug was administered orally for 30 days. Efficacy of *Tinospora cordifolia* in ameliorating the metabolic derangements in lipid metabolism caused by diabetes was compared with the Lante Zinc Insulin (6 Units / kg b.w. daily. i.p.) treated diabetic rats. Plasma total cholesterol, triglycerides, free fatty acids, phospholipids and lipoproteins like high density lipoprotein, low density lipoprotein and very low density lipoprotein -cholesterol levels were measured according to the standard biochemical methods. Drug treated diabetic animals showed a significant ($p < 0.05$) effect of *Tinospora cordifolia* on all these parameters compared to untreated animals. Treatment with insulin restored all these altered parameters to near normal levels in diabetic animals. Our results indicated that *Tinospora cordifolia* stem extract is able to ameliorate the derangements in lipid metabolism caused by diabetes mellitus in streptozotocin induced diabetic rats towards normal level. Hence, this study may reveal the usefulness and beneficial value of herbal drug *Tinospora cordifolia* in treating hyperlipidemia.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder affecting carbohydrate, fat, and protein metabolisms. It has a significant impact on health, quality of life, and life expectancy of patients as well as on the health care system [1].

One of the most common complications in DM is hyperlipidemia, which is common in about 40% of diabetics [2]. Accumulation of lipids in diabetic condition is mediated through variety of derangements in metabolic and regulatory processes, thereby rendering the diabetics more prone to hypercholesterolemia and hyperglyceridemia. Diabetes is usually associated with micro vascular and macro vascular complications, which are the major causes of morbidity and mortality in diabetic patients [3].

Management of diabetes without any side effect is still a challenge to the medical field. Currently available drugs for diabetes have number of limitations, such as adverse effects and high rates of secondary failure. People today, are more concerned about the side effect and the cost effectiveness of drugs and have began to rely more firmly upon herbs, which are comparatively less exploited for their medicinal qualities. As a complimentary or alternative approach, herbal drugs with anti-hyperglycemic activities are increasingly sought after by diabetic patients and healthcare professionals. Plants like *Momordica cymbalaria*, Neem seed kernel powder, *Averrhoa bilimbi* [4] have shown to have hypoglycemic properties and were able to correct the metabolic derangements caused by experimental diabetes.

Tinospora cordifolia (Willd.) Miers ex Hook. F. & Thoms. (TC) belongs to the Menispermaceae family and

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 Ayurvedic literature as a tonic, vitalizer and as a remedy for diabetes and other metabolic disorders [5].

However, the effects of TC stem extracts on the metabolic derangements caused by streptozotocin diabetes, especially on blood lipid profile and the efficacy of this effect compared to standard drug insulin have not been studied intensively so far, which forms the basis of the present study.

Material and methods

Plant material

TC stem was collected fresh from forest areas in Udupi-district, Karnataka state, South India and dried in shade and then powdered. The plant was identified by Professor and Head, 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.

Extraction

Aqueous and alcoholic extracts of TC were prepared according to the standard extract procedure [6]. The yield of extracts was approximately 8.5 % and 7 % respectively.

Animals and time of experiment

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 pellet diet (Hindustan Lever Ltd. Bangalore) and water ad libitum through out the study period. All the experiments were carried out in between 8-10 A.M in order to avoid the circadian rhythm induced changes.

Experimental Induction of diabetes

To induce diabetes, the rats were fasted for 16 hours and injected with freshly prepared streptozotocin (**STZ**) (Sigma chemicals, USA) at the dose of 55 mg / kg b.w. intravenously in 0.1 M Citrate buffer of pH 4.5 [7]. Control animals were received citrate buffer alone. Diabetes status was confirmed by estimating the fasting blood glucose levels and urine glucose (Benedict's test) after 72 hours of STZ injection. Animals showing fasting blood glucose levels above 250 mg/dl were selected for this study.

Experimental protocol

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

Group 1- Control: This group was used for studying the base line values of the parameters studied.
Group 2- Diabetic control: This group consists 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.

Drug treatment

Single dosage of either aqueous extract (dissolved in normal saline) or alcoholic extract (dissolved in gum acacia) of TC was given orally [6] for 10 days and 30 days to specific group through oral intubations and the control animals received the vehicle alone. Lante zinc insulin (6 units /kg. b.w. i.p.) was given to specific group daily [8].

At the end of the experimental period, animals were anesthetized and fasting blood and plasma samples were collected.

Biochemical estimation

Plasma total cholesterol [9], Triglycerides [10], Free fatty acids [11], Phospholipids [12], Lipoproteins- HDL-Cholesterol [13], LDL &VLDL-Cholesterols [14] were measured by standard procedures.

Statistics analysis

Statistical significance between the different groups was determined by One way Analysis of Variance (**ANOVA**) followed by Tukey's multiple comparisons by fixing the P value as $p< 0.05$.

Results

Plasma total cholesterol, triglycerides, free fatty acids and phospholipids in untreated diabetic rats were elevated to high levels during this study period. TC treatment for 10 and 30 days in diabetic rats showed a significant reduction ($p< 0.05$) in all these lipids profile (Table.1) levels compared to untreated diabetic animals. Furthermore, in present study, 200 mg of aqueous extract showed better potency in lowering the elevated lipids profile. Efficacy of TC in ameliorating all these parameters was apprecia-

Table 1: Effect of TC extracts on plasma total cholesterol, triglycerides, free fatty acids and phospholipids levels (mg/dl) and comparison of efficacy of TC extracts with insulin [Values expressed in % amelioration towards normal levels].

	Days	Control	Dia. control	Dia.+ Aq.200	Dia.+ Aq.400	Dia.+ Al.200	Dia.+ Al.400	Dia.+ Insulin
Total cholesterol (mg/dl)	11thday	86.02 ± 6.1	*185.64 ±10.8	*^{a} 119.66 ± 9.6	*a 143.13 ± 2.8	*^{a} 130.40 ± 2.2	*^{a}137.81 ±4.9	*102.78 ±3.4
	31stday	87.21 ±5.4	*180.96 ±2.5	*^{a}126.35 ±2.0	*a 146.36 ± 4.2	*^{a} 137.05 ± 2.4	*^{a} 131.38 ±2.3	94.18 ±5.3
Efficacy (%)	11thday	-	-	66.23	42.67	55.45	48.01	83.17
	31stday	-	-	58.25	36.89	46.84	52.88	92.56
Triglycerides (mg/dl)	11thday	64.35 ±4.4	*255.29 ±9.7	*a149.28 ±7.5	*a 179.40 ± 6.6	*^{a} 161.29 ±4.6	*^{a}183.01 ±4.1	*79.21 ±4.2
	1stday	60.75 ±4.2	*260.42 ±3.7	*^{a}158.20 ±3.7	*a 184.21 ± 3.1	*^{a}176.45 ±2.4	*^{a}170.46 ±3.9	* 80.24 ±4.4
Efficacy (%)	11thday	-	-	55.52	39.75	49.26	37.86	92.21
	31stday	-	-	51.19	38.17	42.05	45.05	90.23
Free fatty acids (mg/dl)	1thday	26.91 ±3.6	*65.88 ±3.9	*^{a}43.94 ±4.5	*^{a}44.51 ± 3.7	*^{a}44.67 ±1.2	*^{a}43.99 ±0.6	26.45 ±2.2
	1stday	26.53 ± 3.6	*67.31 ±2.3	*^{a}44.37 ±2.0	*a 51.66 ± 3.1	*^{a} 48.95 ± 3.2	*^{a} 46.61 ±2.5	30.24 ±1.4
Efficacy (%)	11thday	-	-	56.29	54.83	54.42	56.17	100
	31stday	-	-	56.25	38.37	45.02	51.49	90.90
Phospholipids (mg/dl)	1thday	99.38 ± 4.8	*153.48 ±8.9	*^{a}124.87 ± 5.8	*^{a} 139.55 ± 4.7	*^{a} 128.20 ± 3.7	*^{a}133.57 ±1.4	99.52 ±1.2
	1stday	102.04 ± 4.4	*144.18 ±2.3	*^{a}127.00 ± 1.0	*^{a} 136.38 ± 1.8	*^{a} 130.25 ± 1.0	*^{a}128.89 ±1.0	105.48 ±3.7
Efficacy (%)	11thday	-	-	52.86	25.74	46.70	36.80	99.63
	31stday	-	-	40.77	18.49	33.06	36.26	91.83

Data expressed as Mean ± SD. (n=6) p< 0.05.
**Control Vs other groups*
[a]*Diabetic control Vs TC treated diabetic group.*

bly good compared to insulin. The HDL cholesterol levels were decreased and LDL and VLDL cholesterol levels were increased significantly in untreated diabetic rats compared to control during study period. Significant effect of TC on plasma HDL cholesterol levels was observed in these TC treated rats after 10 and 30 days of treatment (Table.2). Additionally, treatment with TC could reduce the elevated levels of LDL and VLDL cholesterol effectively in these treated diabetic rats. However, their levels were not normalized even after 30 days of TC treatment. Treatment with insulin could normalize these altered lipids profile in insulin treated diabetic rats.

Table 2. Effect of TC extracts on plasma HDL cholesterol, LDL cholesterol, VLDL cholesterol levels (mg/dl) and comparison of efficacy of TC extracts with insulin [Values expressed in % amelioration towards normal levels].

	Days	Control	Dia. control	Dia.+ Aq.200	Dia.+ Aq.400	Dia.+ Al.200	Dia.+ Al.400	Dia.+ Insulin
HDL cholesterol (mg/dl)	11th day	25.01± 2.7	*18.20± 0.8	*[a]21.74 ±1.7	*20.38± 0.4	*20.84± 0.6	a22.83± 0.5	24.06± 0.7
	31st day	25.06± 2.2	*17.35± 1.6	*20.16 ±1.2	*18.44± 0.4	*19.21± 0.31	*19.66± 0.3	23.76± 0.8
Efficacy (%)	11th day	-	-	51.98	32.01	38.76	67.98	86.04
	31st day	-	-	36.44	14.13	24.12	29.96	83.13
LDL cholesterol (mg/dl)	11th day	46.75± 4.4	* 107.23± 6.6	*a75.77 ±4.1	*a 99.06± 3.6	*a79.17± 1.4	*a 79.33± 3.7	47.17± 4.0
	31st day	47.39± 5.5	*101.79± 2.7	*a72.47 ±2.5	*[a]93.50 ±3.4	*a79.31 ±2.5	*a75.26± 2.8	49.86± 3.8
Efficacy (%)	11th day	-	-	52.01	13.52	46.39	46.13	99.30
	31st day	-	-	53.88	15.22	41.31	48.75	95.45
VLDL cholesterol (mg/dl)	11th day	13.50± 2.1	*51.05± 1.9	*a29.49± 1.1	*a 35.87± 1.3	*a 31.54± 1.0	*a 36.56± 2.6	13.79± 1.1
	31st day	12.81± 1.7	*52.62± 2.7	*a27.89± 1.1	*a37.16± 1.1	*a35.79± 1.5	*a30.73± 1.0	15.11± 0.8
Efficacy (%)	11th day	-	-	57.41	40.42	51.95	38.58	99.22
	31st day	-	-	62.12	38.83	42.27	54.98	94.22

Data expressed as Mean ± SD. (n=6) p< 0.05.
** Control Vs other groups*
[a] Diabetic control Vs TC treated diabetic group.

Discussion

Diabetes mellitus is a metabolic disorder, showing significant impact on lipid metabolism with alterations in blood lipids and lipoproteins profile. Absence or deficiency of insulin alters the entire metabolism in the body including the lipid metabolism. The abnormal high levels of blood lipids in diabetes is mainly due to the increase in mobilization of free fatty acids from the peripheral depots (increased lipolysis) as hormone sensitive lipase is not inhibited in diabetes due to insulin lack. The marked hyperlipemia, which characterizes the diabetic state, may therefore be considered as a consequence of the uninhibited actions of lipolytic hormones on the fat depots [15].

In the present study, the plasma total cholesterol, triglycerides, free fatty acids and phospholipids in untreated dia betic rats were elevated to high levels during the study period, which is in well agreement with earlier reports [8, 16]. TC treatment for 10 and 30 days in diabetic rats showed a significant reduction in all these lipids profile levels compared to untreated diabetic animals. This study also showed that the hypolipidemic effect of TC is not exclusively depending on the dosage of drug, but on the effectiveness of TC in controlling the blood glucose, since glycemic control was better with lower dosage (data not shown).

High density lipoprotein (HDL) cholesterol is produced in both liver and intestine. HDL constituents are also derived from chylomicron and VLDL catabolism [17]. HDL serves as an acceptor of lipids, especially free cholesterol from various extra hepatic cells to the liver for the ultimate excretion in the bile [18]. Coronary heart disease is inversely related to the levels of HDL cholesterol in the plasma [19]. Low density lipoprotein (LDL) cholesterol is the major cholesterol carrying the lipoproteins in the plasma. Most of the LDL cholesterols are derived from the catabolism of VLDL, but some are synthesized directly. Very low density lipoprotein (VLDL) cholesterol is synthesized in the liver and is regulated by diet and hormones. The present study observations indicated that TC is very much beneficial in enhancing HDL cholesterol levels and lowering the LDL and VLDL cholesterol levels, thereby reveals its usefulness and therapeutic values.
There was a correlation exists between the blood glucose levels and blood lipid profile (data not shown). When we collectively look into the blood glucose levels and lipid profiles after the TC treatment in diabetic animals, it is evident that the anti- hyperglycemic activity of TC is responsible for the controlling and correcting the altered

lipids profile. Thus, the glucose lowering activity of TC may be one of the reasons for correcting the altered lipid profile in diabetic animals. However, an extensive case-control study is required to document its therapeutic application in human beings. Since this study has got some limitations in using large number of sample size and study period, further study is essential in exploring the enigma behind this effect.

Conclusion

This study concludes that, both the aqueous and alcoholic extracts of *Tinospora cordifolia* could ameliorate the metabolic derangements in lipid metabolism caused by STZ induced diabetes in rats. Efficacy of this activity is appreciably good when compared to standard drug insulin. Use of TC seems to be beneficial to the heart, as it has got the positive effect on HDL cholesterol. Our observations in this study favor the usefulness of TC as a supportive drug and its therapeutic values. However, an extensive clinical case-control study is required to support this aspect.

Acknowledgement

The authors gratefully acknowledge Late Prof. A. Namasivayam, Professor of Physiology, PGIBMS, Chennai for the suggestions given by him to design this study.

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