BIO ACTIVITY OF *TINOSPORA CORDIFOLIA* CRUDE METHANOLIC EXTRACT IN EXPIREMENTAL DIABETES

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Summary

The traditional system of the medicine remains the major source of the health care. It is safe alternative, lesser cost and better tolerance and its complications. The major defense against free radicals found in medicinal plants is in the form of natural antioxidants. The present investigation is an attempt to asses the bioactivity of daily oral administration of methanolic extract of *Tinospora cordifolia* stem (T.C.S) (500mg/kg body weight) for 6weeks in normal and alloxan induced diabetic rats. A significant decrease in blood glucose, glycosylated haemoglobin, cholesterol ($p<0.05$), and increase in body weight, protein ($p<0.01$) were observed in diabetic rats on treatment with T.C.S. methanolic extract when compared to normal. The activity of the hepatic enzyme hexokinase was significantly increased where as glucose 6-phosphatase and fructose 1, 6- bisphosphatase were significantly decreased ($p<0.01$) by the oral administration of T.C.S. methanolic extract in diabetic rats when compared to normal. The six weeks treatment of T.C.S. methanolic extract was proved natural antioxidant present in the plant, because the activity of erythrocytes lipid peroxide and catalase (CAT) was significantly ($p<0.01$) increased whereas superoxide dismutase (SOD) and reduced glutathione (GHS- Px**)** were significantly ($p<0.01$) decreased when compared to normal rats.

Key words: *Tinospora cordifolia*, Blood glucose, Cholesterol, Antioxidants, Alloxan diabetes, Methanolic extract.

Introduction

Diabetes mellitus is a fast growing metabolic disorder affecting approximately 171 million of the world's population suffer from diabetes in the year 2000 and this is projected to increase to 366 million by 2030 (1). Diabetes is a condition primarily defined by the level of hyperglycemia giving rise to risk of micro vascular damage (retinopathy, nephropathy and neuropathy), significant morbidity due to specific diabetes related macro vascular complications. (Ischemia heart disease, stroke and peripheral vascular disease) and diminished quality of life (2). In spite of the introduction of hypoglycemic agents, diabetes and related complications continue to be a major medicinal problem. Since time immemorial, patients with non-insulin requiring diabetes have been treated orally in folk medicine with a variety of plant extracts. The treatment aimed not only decreasing the blood sugar level to normal limits, but also at correcting the metabolic defects. In India a number of plants are mentioned in ancient literature for the cure of diabetes conditions and its related complications (3).

The oxidative stress is produced by free radicals, predominately reactive oxygen species (ROS), which cause tissue damage from oxidative stress. Free radicals are largely responsible for diverse diseases and disorders such as diabetes and related problems of ageing. The major defense against free radicals can be found in medicinal plants in the form of natural antioxidants (4). Antioxidants play an important role in neutralizing such free radicals. Natural antioxidants have consumer's acceptability as they are considered to be safe. Plants and natural compounds from medicinal plants having antioxidant activities have potential as therapeutic agents (4).

Tinospora cordifolia is a large, glabrous, deciduous climbing succulent shrub on large trees and belongs to the family Menispermaceae (5). Previous studies showed that the plant stem possesses hypoglycemic (6) and antipyretic activities (7). Accordingly the present investigation is an attempt to assess the bioactivity of methanolic extract of *Tinospora cordifolia* stem in experimental diabetes in rats.

Materials and methods

Plant materials

The stem plant material of *Tinospora cordifolia* was collected fresh from Vellore District area in Tamilnadu. The plant stem was authentified by the Herbarium of Botany Directorate in National Institute of Herbal Science, Plant Anatomy Research Center, in Chennai. A voucher specimen (No: TC08) was deposited in the Center.

Animals

Healthy adult cross breed of male Wistar albino rats (weighing 180 – 210g) were used in the experiments. Animals were housed in polypropylene cages at 22 ± 2^{o}C with relative humidity of 45 – 55 % under 12 hour's light and dark cycle. They were fed with standard laboratory animal feed (Hindustan lever Ltd., India) and water ad libitum. The ethical clearance was obtained from the Institutional Animal Ethical Committee (Approval No.115/ac/07/CPCSEA).

Preparation of extract from *Tinospora cordifolia*

The dried powdered stem of *Tinospora cordifolia* was allowed to pass through ss sieve (20 meshes). It was defatted by treating with petroleum ether (60-80^{o}C) and then extracted to exhaustion (soxhlet) with methanol. The solvent was removed under vacuum to get solid mass. The extract was dissolved in physiological saline solution and given orally to diabetic and normal (control) groups of at a dose of 500mg/kg of body weight daily once up to six weeks (8).

Induction of diabetes mellitus

The experimental animal in this model is the male, adult Wistar albino rats, weighing 180–210g. After a 12-hour fast, the rats were weighed and a solution of 2% alloxan monohydrate (S.D. Fine Chemicals, Mumbai) diluted in saline (0.9%) corresponding to 80 mg of alloxan per kg body weight was administered intraperitoneally in a single dose. Food and water were given to the rats after 30 minutes of drug administration (9). After two week rats blood glucose levels of 200-260mg/100ml were used for the study. Blood was taken from eyes (Venous pool) and glucose was estimated by Sasaki method (10). All the biochemical and chemicals used in the experiment were of analytical grade.

Experimental designs

The 24 rats were divided into four groups each group in six rats. Group 1: Normal rats received 0.5 ml of physiological saline. Group 2: Normal rats were given methanol extract of TCS in 500 mg /kg of body weight once every day up to 6 weeks. Group 3: Alloxan induced diabetic rats. Group 4: Alloxan induced diabetic rats were given methanol extract of TCS in 500 mg /kg of body weight once every day up to 6 weeks.

Collection of samples

During the second, fourth, and sixth week of treatment, the body weight, urine sugar and blood glucose of all the rats were determined. At end of the 6th week the animals were deprived of food overnight and sacrificed by decapitation. Fasting blood sample was collected in fresh vials. Liver is dissected out and washed in ice-cold saline immediately.

Evaluation of effect on biochemical variables

Fasting blood glucose (10), protein (11), urea (12), cholesterol (13) were estimated and glycosylated haemoglobin (14) was estimated by using the hemolysate obtained during the isolation of erythrocyte membrane. The liver supernatant was extracted and used for the assay of hexokinase (15), fructose -1, 6- bisphosphatase (16), and glucose -6- phosphatase (17).

The isolation of erythrocytes membrane by Dodge (18) method with a change in buffer according to Quest (19) method, blood collected with EDTA as an anticoagulant and centrifuged at 1500 x g for 15 min. The supernatant plasma was discarded. The packed cells was washed three times with 0.9 % saline, the cells was lyses by suspending than in hypotonic buffer for one hour and centrifuged at 15000 x g for 30 min. the supernatant red fluid containing the membrane, was washed with hypotonic buffer until it became colorless or pale yellow. The membrane solution was used for analyses.

Superoxide dismutase was assayed according to the method of Misra and Fridovich (20), catalase was assayed according to the method of Bergmeyer (21), glutathione peroxidase was assayed according to the method of Nuchelse (22), lipid peroxides concentrates was determined by thiobarbituric acid reaction as described by Ohkawa (23). Biochemical determinations were carried out using Shimadzu Spectrophotometer.

Statistical analysis

The results are expressed as mean ± SD. The data were analyzed by one way ANOVA followed by Dun net's test at level of significance was expressed as P<0.05 and P<0.01.

Results

Table 1 show that the blood glucose and urine glucose level were significantly higher and animal body weight was decreased in diabetic untreated rats as compared to normal rats. The administration of methanolic T.C.S. extract decreased the blood glucose level and urine sugar was nil. The body weight return to normal as compared to diabetic rats

Table 1: Effect of T.C stem extract on Blood glucose, Body weight and Urine sugars in Normal, T.C.S. extract treated control, Diabetes, and T.C.S. extract treated diabetes rats.

Groups	Blood glucose (mg/dl)	Body weight (g)		Urine sugar
		Initial weight (g)	Final weight (g)	
Normal	82.4±3.3	182.3±0.4	194.6±0.2	Nil
T.C.S. extract treated control	78.2±1.3**	184.6±0.8	198.0±0.6**	Nil
Diabetes	226.4±6.9	181.4±0.5	142.7±0.8	+ + +
T.C.S. extract treated diabetes	102.7±8.3**	184.8±0.5	191.6±0.2**	Nil

+ + +, indicates more than 2% sugar.
Values are expressed as mean ± SD for six animals in each group.
* P < 0.05 and ** P < 0.01 significantly different compared with control

Table 2 shows the protein level was decreased and the cholesterol and glycosylated haemoglobine levels were significantly higher in untreated diabetic rats. The administration of methanolic T.C.S. extract in diabetic rats increased protein level and the cholesterol, glycosylated haemoglobine levels were significantly decreased as compared to diabetic rats.

Tables 2: Effect of T.C.S. stem extract on protein, cholesterol and Glycosylated Haehoglobine in Normal, T.C.S. extract treated control, Diabetes, and T.C.S. extract treated diabetes rats.

Groups	Protein (g/dl)	Cholesterol (mg/dl)	Glycosylated Haehoglobine(mg/g Hb)
Normal	7.3±0.23	162.0±1.44	0.246±0.04
T.C.S. extract treated control	7.6±0.38 **	168.0±1.72 **	0.237±0.03 **
Diabetes	6.2±0.54	243.4±0.82	0.723±0.03
T.C.S. extract treated diabetes	6.8±3.24 **	176.3±2.24*	0.432±0.02*

Values are expressed as mean ± SD for six animals in each group.
* P < 0.05 and ** P < 0.01 significantly different compared with control

Table 3 shows the level of haexokinase was decreased and glucose -6-phosphatase and fructose 1, 6 bi phosphatase were higher in untreated diabetic rats as compared to normal group. The administrations of methanolic T.C.S. extract to diabetic rats increased the haexokinase and decreased glucose -6-phosphatase, fructose 1, 6 - bi phosphatase as compared to diabetic rats.

Table 4 shows the level of erythrocytes lipid peroxide, CAT, were significantly higher and SOD, GSH-Px was decreased in untreated diabetic rats as compared to normal rats. The treatment of methanolic T.C.S. extract in diabetic rats, the lower in lipid peroxide, CAT and increased in SOD, GSH-Px levels as compared to diabetic rats.

Table 3: Changes in T.C.S. extract on Hexokinase, Glucose -6- phosphatase and Fructose 1.6 bi phosphatase in Normal, T.C.S. extract treated control, Diabetes, and T.C.S. extract treated diabetes rats.

Groups	Hexokinase [a]	Glucose-6-phosphatase [b]	Fructose 1.6 bi phosphatase [b]
Normal	264.68±0.83	1032.4±0.42	474.14±1.63
T.C.S. extract treated control	272.63±2.84**	963.3±1.18	456.64±2.32**
Diabetes	115.43±3.46	1236±3.22	757.42±3.82
T.C.S. extract treated diabetes	238.82±2.83**	1123±4.32**	563.56±3.87**

Values are expressed as mean ± SD for six animals in each group.
* P < 0.05 and ** P < 0.01 significantly different compared with control
a. μ – moles of glucose - 6 – phosphate formed/h/mg protein
b. n moles of phosphorous liberated/h/mg protein

Table 4: Changes in effect of T.C.S. extract on erythrocyte membrane – Lipid peroxide, CAT. SOD and GHS-Px in Normal, T.C.S. extract treated control, Diabetes, and T.C.S. extract treated diabetes rats.

Groups	Lipid peroxide[a]	CAT [b]	SOD [c]	GHS-Px [d]
Normal	0.34±0.23	0.165±0.25	3.19±0.23	48.43±0.41
T.C.S. extract treated control	0.30±0.25**	0.160±0.02**	3.43±0.32**	49.48±0.52**
Diabetes	0.83±0.42	0.348±0.32	2.10±0.38	30.11±0.73
T.C.S. extract treated diabetes	0.46±0.22*	0.221±0.30*	2.94±0.44**	45.24±0.63**

Values are expressed as mean ± SD for six animals in each group.
* P < 0.05 and ** P < 0.01 significantly different compared with control

a. Lipid peroxide – no of moles MDA/ mg/ Protein.
b. CAT activity is expressed as moles of H_2O_2 decomposed /min/ mg protein.
c. One unit of SOD activity was the amount of protein of protein required to given 50% inhibition of adrenaline autoxidation.
d. Glutothione peroxidase – no of moles of GSH oxidized/min/m protein.

Discussion

The present investigation was to confirm the antidiabetic and antioxidant activity of T.C.S stem methanolic extract in alloxan induced diabetic rats. The untreated diabetic rats showed the elevation in blood glucose level, conforming the abnormalities of glucose metabolism and also causes a massive reduction of the β- cells of the islets of langerhans and induce hyperglycemia (24) and change the level of antioxidants are absorbed (25).The treatment of T.C.S.methanolic extract (500mg/Kg of body weight for 6 weeks) significantly ($P < 0.01$) decreased the blood glucose level (Table 1) in diabetic rats.

Dehydration and loss of body weight have been associated with diabetic rats (26), the decreased body weight and protein; this indicates the polyphagia condition and loss of body weight due to excessive break down of tissue protein and protein wasting due to unavailability of carbohydrates as an energy source. Administration of T.C.S methanolic extract to significantly ($P < 0.01$) improved the body weight and protein levels in diabetic rats.

Cholesterol and glycosylated haemoglobine levels were increased in uncontrolled diabetes (Table 2). Glycosylated haemoglobine (HBA1c) was found to be increased in patients with diabetes. During diabetes the excess glucose present in blood reacts with hemoglobin. There four, the total haemoglobine level is decreased in alloxan induced diabetic rats (27). Administration of T.C .S methanolic extract in six weeks prevented significantly ($P<0.01$) elevation in glycosylated haemoglobine and cholesterol levels in diabetic rats which could be due to the result of improved glycemic control proved by *Tinospora cordifolia.*

The activity of hexokinase enzyme decreased in the liver of alloxan induced diabetic rats (Table 3). Our present study was showed the administration of T.C. methanolic stem extract in to alloxan induced rats resulted in an increased ($P<0.01$) activity of liver hexokinase. The increased activity of hexokinase leads to increased glycolysis and increased utilization of glucose for energy production (28).T.C. methanolic stem extract was found to reduce the level of glucose ($P < 0.01$) in the blood (Table 1).The activity of fructose 1,6 biphosphatase and glucose 6 phosphatase wear found to be increased in untreated diabetic rats liver (29) , in the present study, (Table 3) fructose 1,6 biphosphatase and glucose 6 phosphatase activity was brought to normal ($P<0.01$) on treatment with T.C. methanolic stem extract.

In hyperglycemic generates oxidative stress in reactive oxygen species which in turn cause lipid peroxidation and membrane damage (30). The result of present study shows (Table 4) increased erythrocytes lipid peroxide, CAT, in diabetic group. Several studies have shows increased lipid peroxide in clinical and experimental diabetes. Our results shows (Table 4) that treatment of methanol extract of T.C stem reduce the free radicals levels in diabetic rats because the lipid perexidase , CAT levels wear brought back to near normal ($P<0.05$) as compared to normal rats.

Glutathione is an important inhibitor of free radical mediated lipid peroxidation. The decreased level of GSH-Px and SOD in diabetes may be due to increased utilization in trapping the oxyradicals (31). The administration of T.C.methanolic stem extracted the level of GSH-Px and SOD wear brought back to near to normal ($P<0.01$) at six week of the treatment.

Conclusion

In conclusion, the methanol extract of *Tinospora cordifolia* stem was found to exhibit a signifying hypoglycemic and antioxidant activity in alloxan induced diabetic rats. Further studies are needed to isolate and characterize the bioactivity compounds of antidiabetic and antioxidant principles from *Tinospora cordifolia* medicinal plant.

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